HAND-OUT MATAKULIAH

BIOLOGI SEL

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MACROMOLECULE:
PROTEIN

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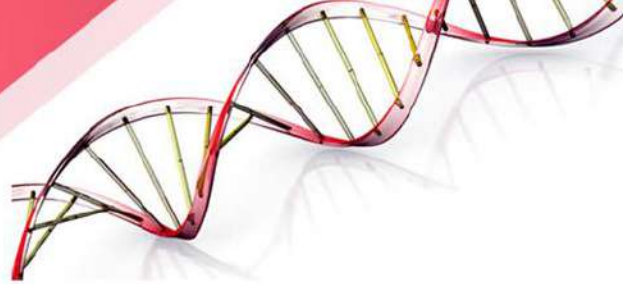
Empat kelompok makromolekul



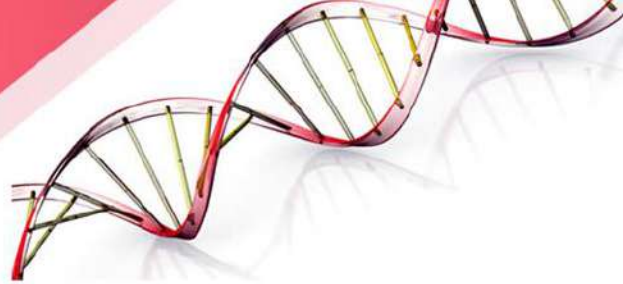
- Semua makhluk hidup tersusun dari 4 kelompok molekul biologis berukuran besar:
  - Karbohidrat
  - Lipid
  - Protein
  - Asam nukleat

- **Makromolekul** → molekul berukuran besar yang tersusun dari ribuan atom yang saling berikatan kovalen

- Struktur molekulernya → menentukan fungsinya

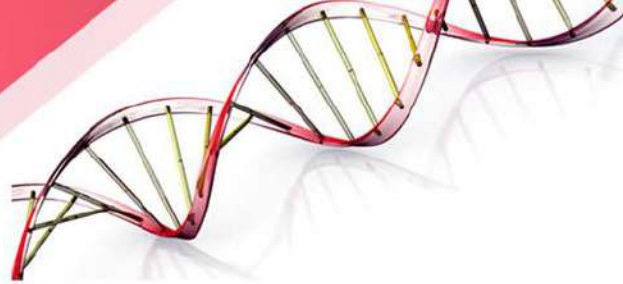
→ Beda struktur → beda fungsi biologisnya

Perbandingan antarmakromolekul



	Monomers or Components	Polymer or larger molecule	Type of linkage
Carbohydrates	Monosaccharides	Polysaccharides	Glycosidic linkages
Lipids	Fatty acids	Triacylglycerols	Ester linkages
Proteins	Amino acids	Polypeptides	Peptide bonds
Nucleic acids	Nucleotides	Polynucleotides	Phosphodiester linkages

Beragam jenis
Protein



- Protein meliputi beragam jenis struktur → beragam fungsi
- Protein menyusun lebih dari 50% massa kering dari sebagian besar sel
- Protein berfungsi sebagai katalis, penyangga struktural, penyimpan, transpor, komunikasi antarsel, pergerakan, dan pertahanan dari serangan benda asing.

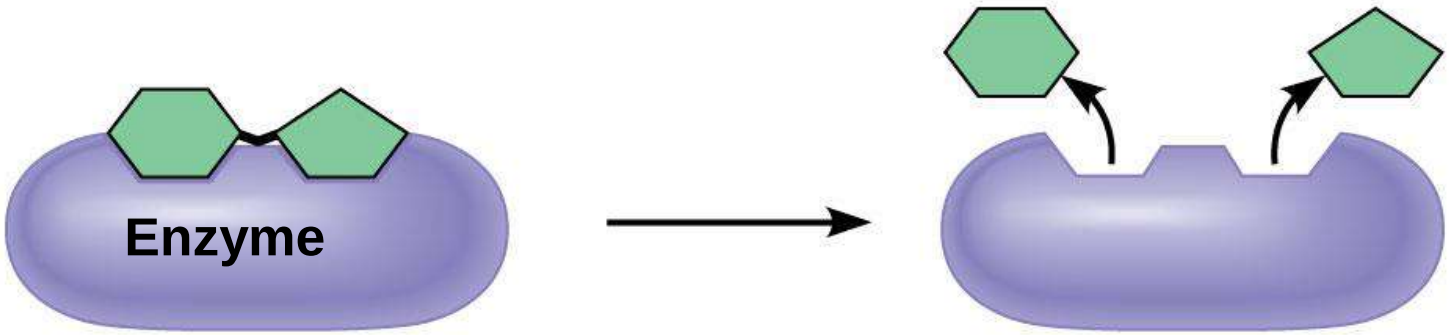
Fungsi katalisis →

Enzim

Enzymatic proteins

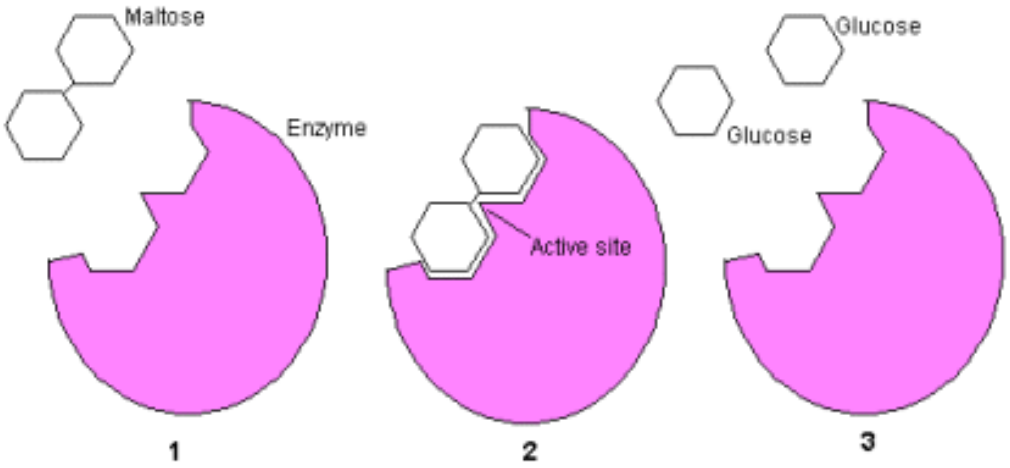
Function: Selective acceleration of chemical reactions

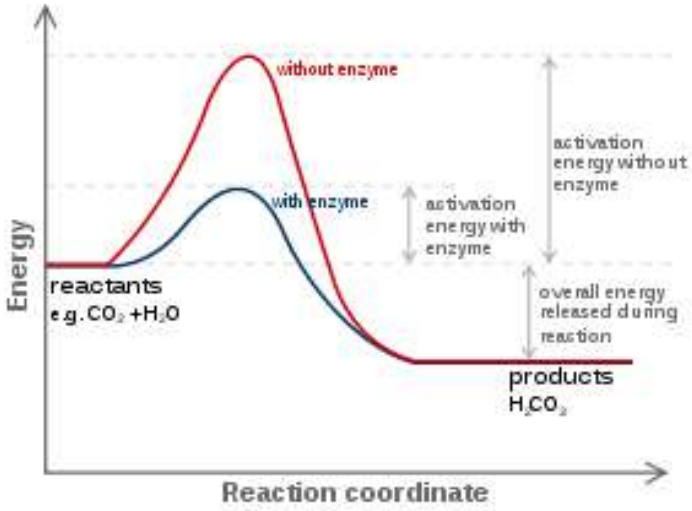
Example: Digestive enzymes catalyze the hydrolysis of bonds in food molecules.



More About Enzymes

- **Enzim →** jenis protein yang berfungsi sebagai **katalis** untuk mempercepat reaksi biokimiawi
- Enzim dapat melakukan fungsinya secara berulang





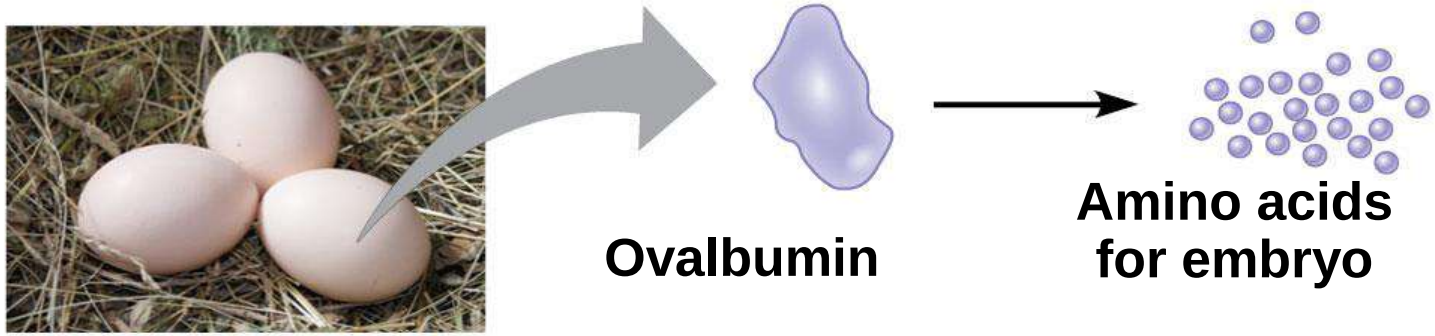
Fungsi penyimpanan

Storage proteins

Function: Storage of amino acids

Examples:

1. **Casein, the protein of milk, is the major source of amino acids for baby mammals.**
2. **Plants have storage proteins in their seeds.**
3. **Ovalbumin is the protein of egg white, used as an amino acid source for the developing embryo.**



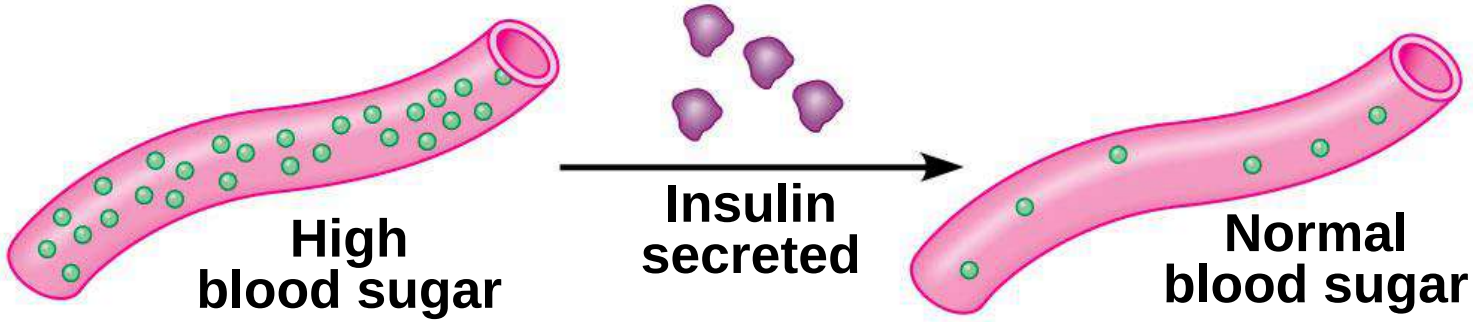
Komunikasi/koordinasi antarorgan →

Protein Hormonal

Hormonal proteins

Function: Coordination of an organism's activities

Example: Insulin, a hormone secreted by the pancreas, causes other tissues to take up glucose, thus regulating blood sugar concentration

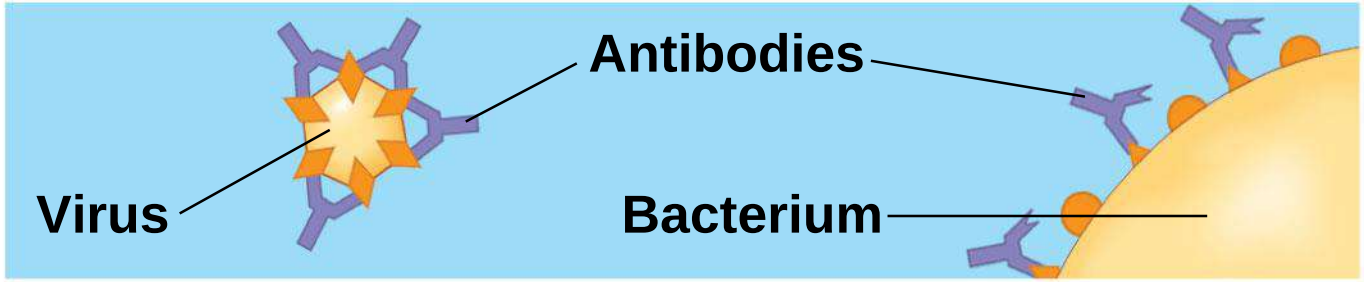


Mekanisme pertahanan

Defensive proteins

Function: Protection against disease

Example: Antibodies inactivate and help destroy viruses and bacteria.

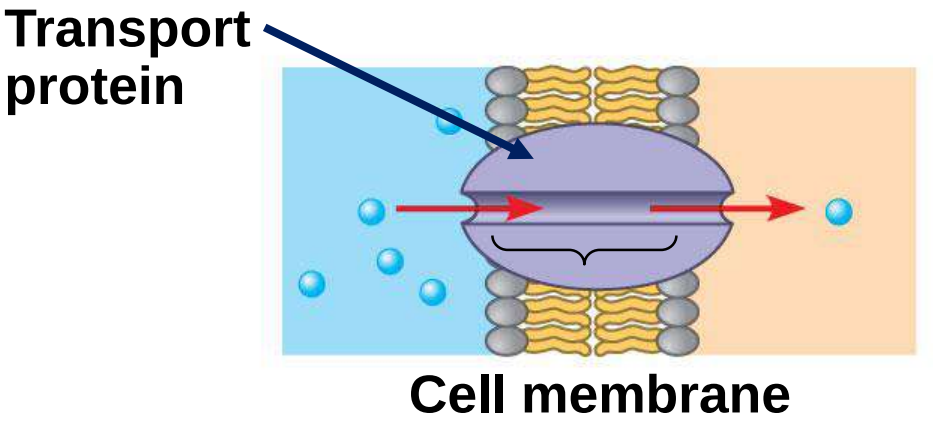


Transportasi

Transport proteins

Function: Transport of substances

Examples: Hemoglobin, the iron-containing protein of vertebrate blood, transports oxygen from the lungs to other parts of the body. Other proteins transport molecules across cell membranes.

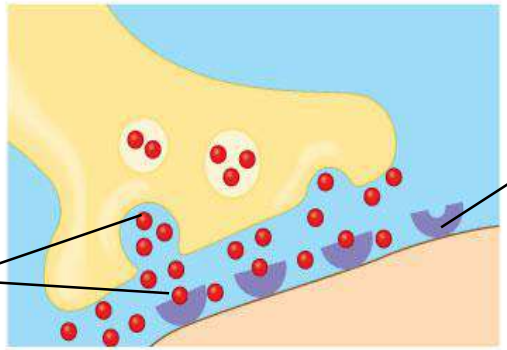


Protein Reseptor

Receptor proteins

Function: Response of cell to chemical stimuli

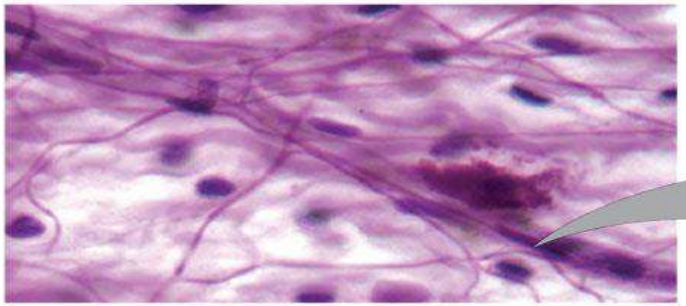
Example: Receptors built into the membrane of a nerve cell detect signaling molecules released by other nerve cells.



Protein Struktur

Structural proteins

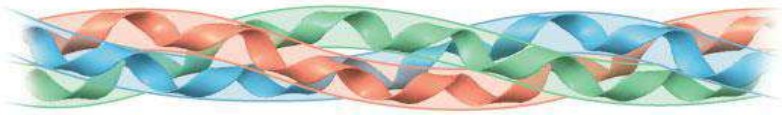
Function: Support
Examples:
1. Keratin is the protein of hair, horns, feathers, and other skin appendages.
2. silk fibers → insects and spiders use them to make their cocoons and webs, respectively.
3. Collagen and elastin proteins provide a fibrous framework in animal connective tissues.



Connective tissue

60 μm

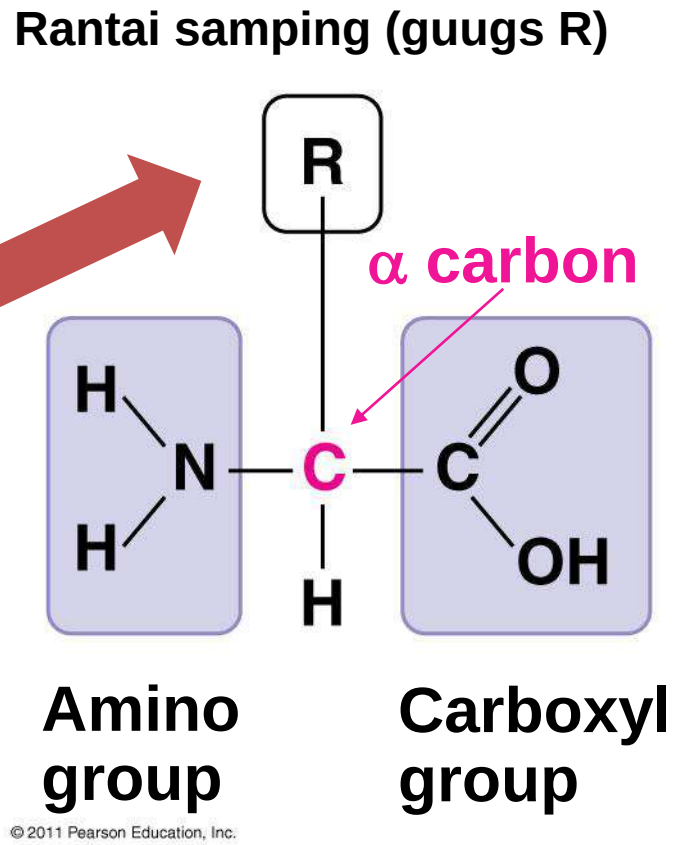
Collagen



Protein → susunan monomer Asam Amino

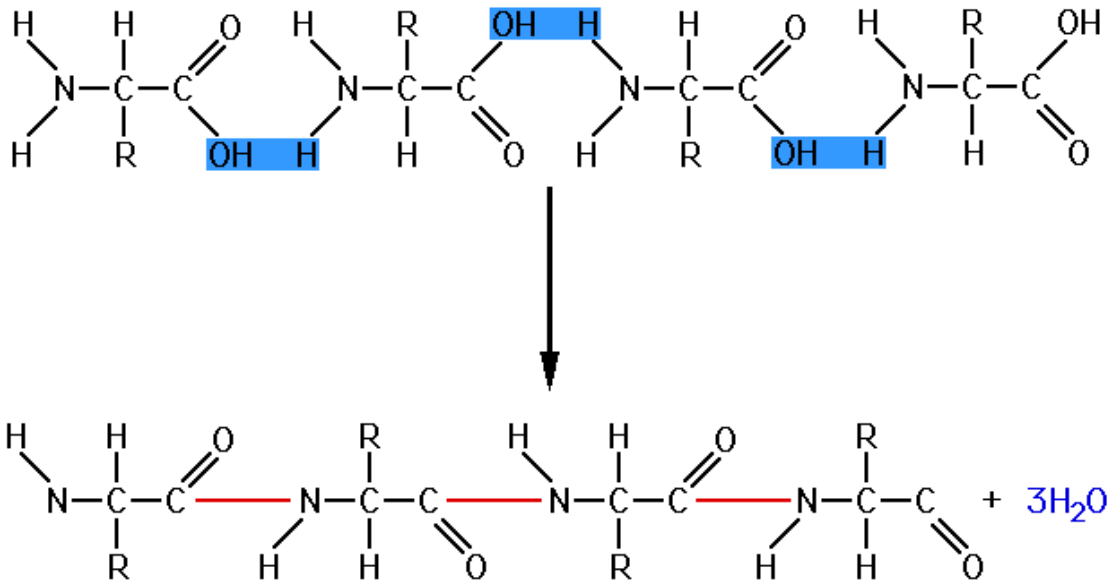
- **Asam amino →** molekul organik yang tersusun dari **gugus karboksil & gugus amino**

- Asam amino berbeda sifat berdasarkan variasi rantai samping → disebut gugus R

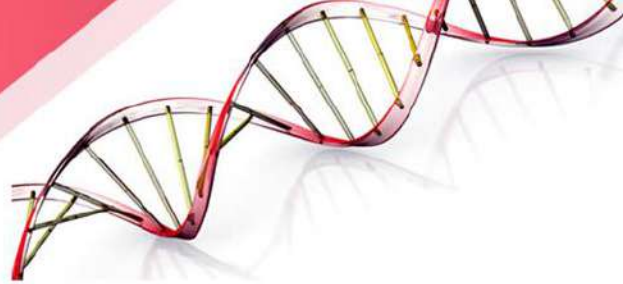


Polipeptida

- **Polipeptida → polimer tidak bercabang** yang disusun dari sekitar 20 asam amino
- **protein** merupakan molekul dengan fungsi biologis yang terdiri dari 1 atau beberapa polipeptida

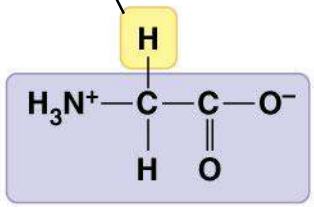


Asam amino Hidrofobik: Tidak larut air!

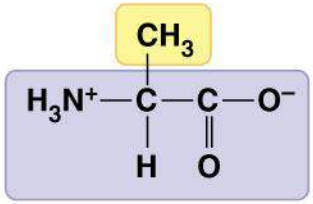


Nonpolar side chains; hydrophobic

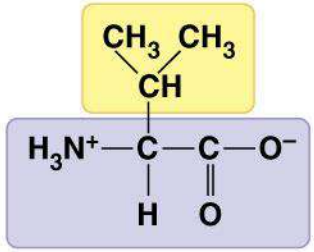
Side chain



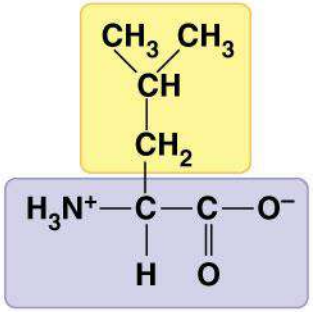
Glycine (Gly or G)



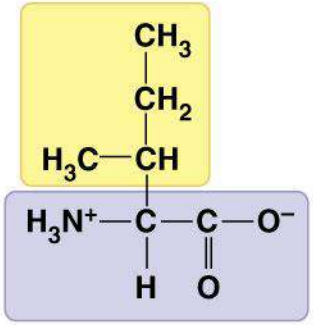
Alanine (Ala or A)



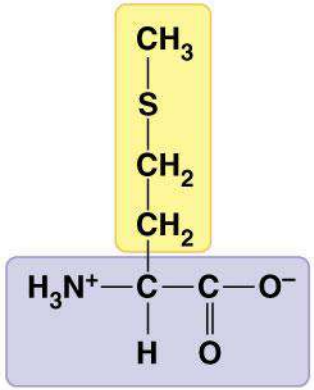
Valine (Val or V)



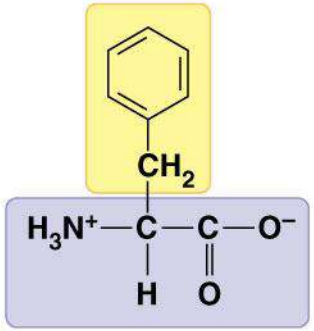
Leucine (Leu or L)



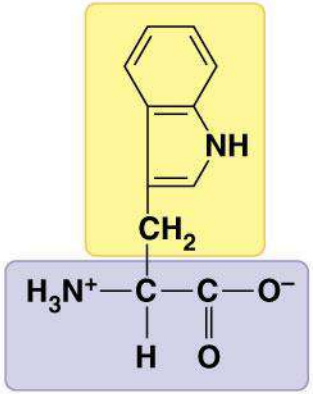
Isoleucine (Ile or I)



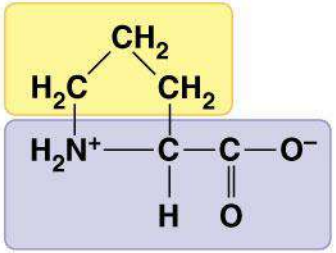
Methionine (Met or M)



Phenylalanine (Phe or F)

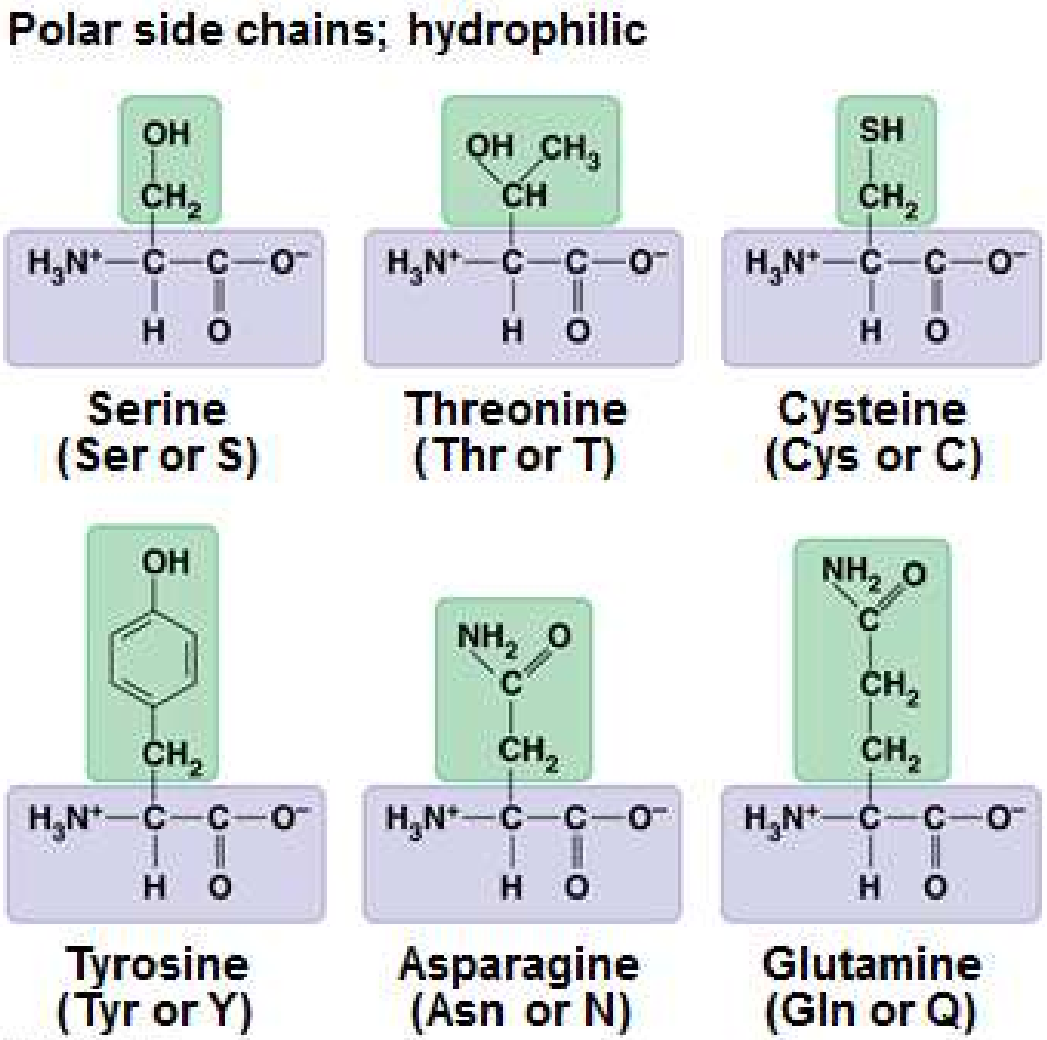


Tryptophan (Trp or W)

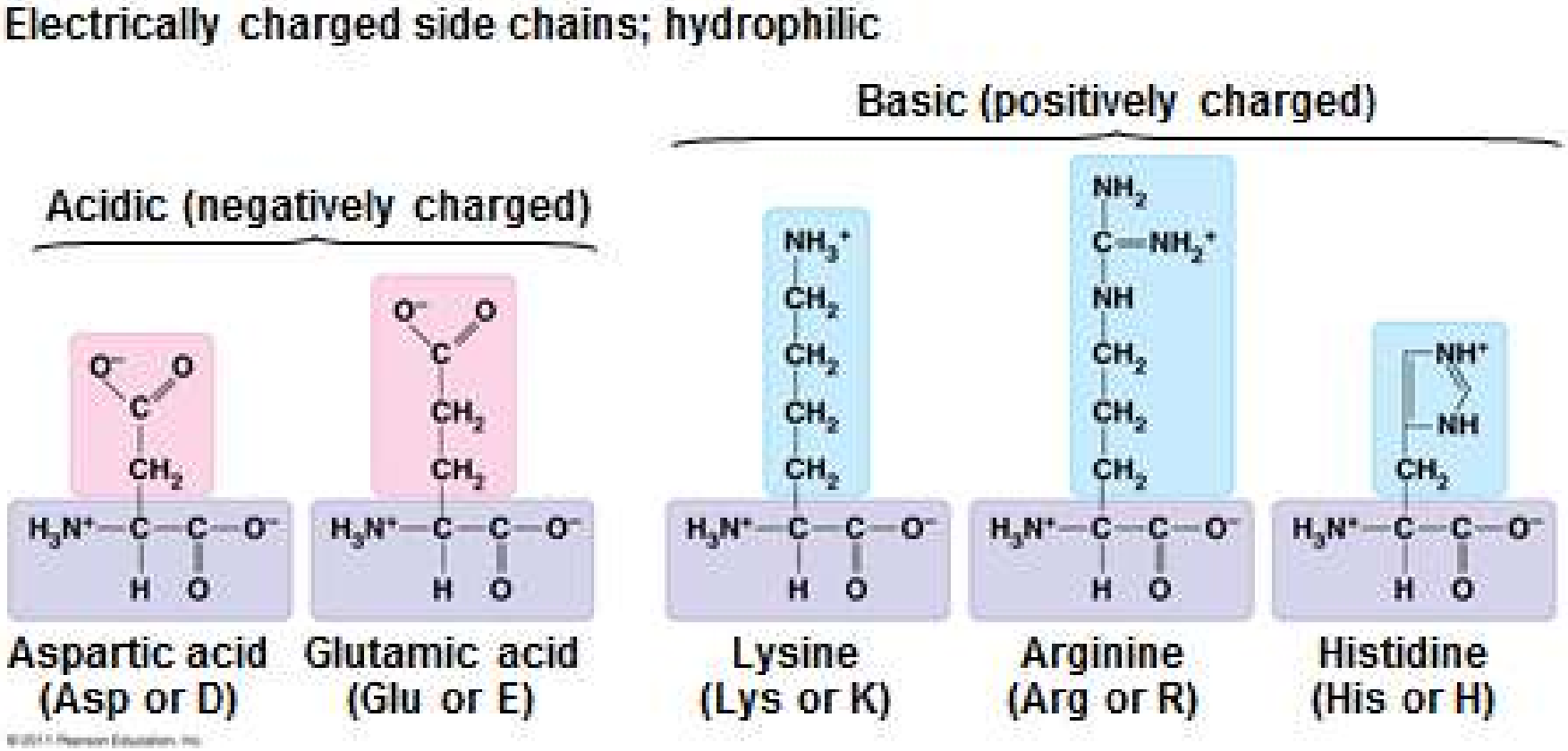


Proline (Pro or P)

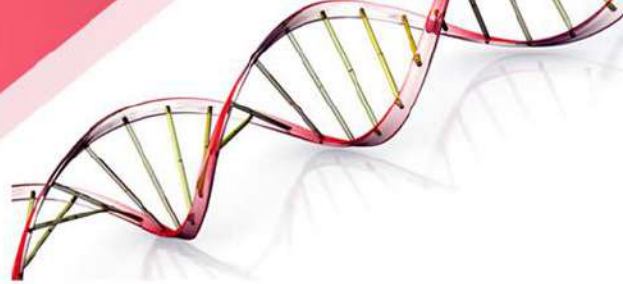
Hidrofilik: larut air



Hidrofilik **bermuatan**!

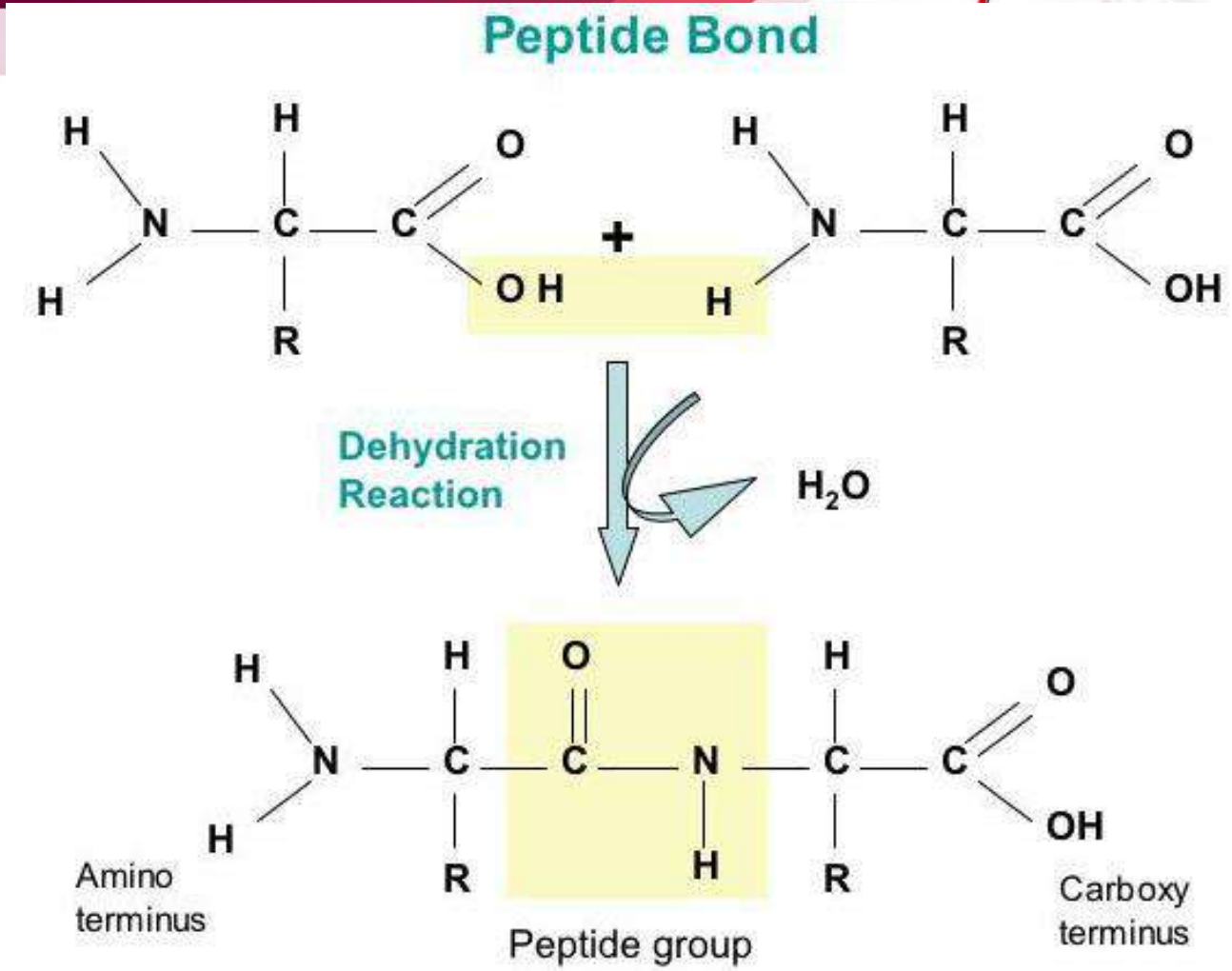


Ikatan Peptida

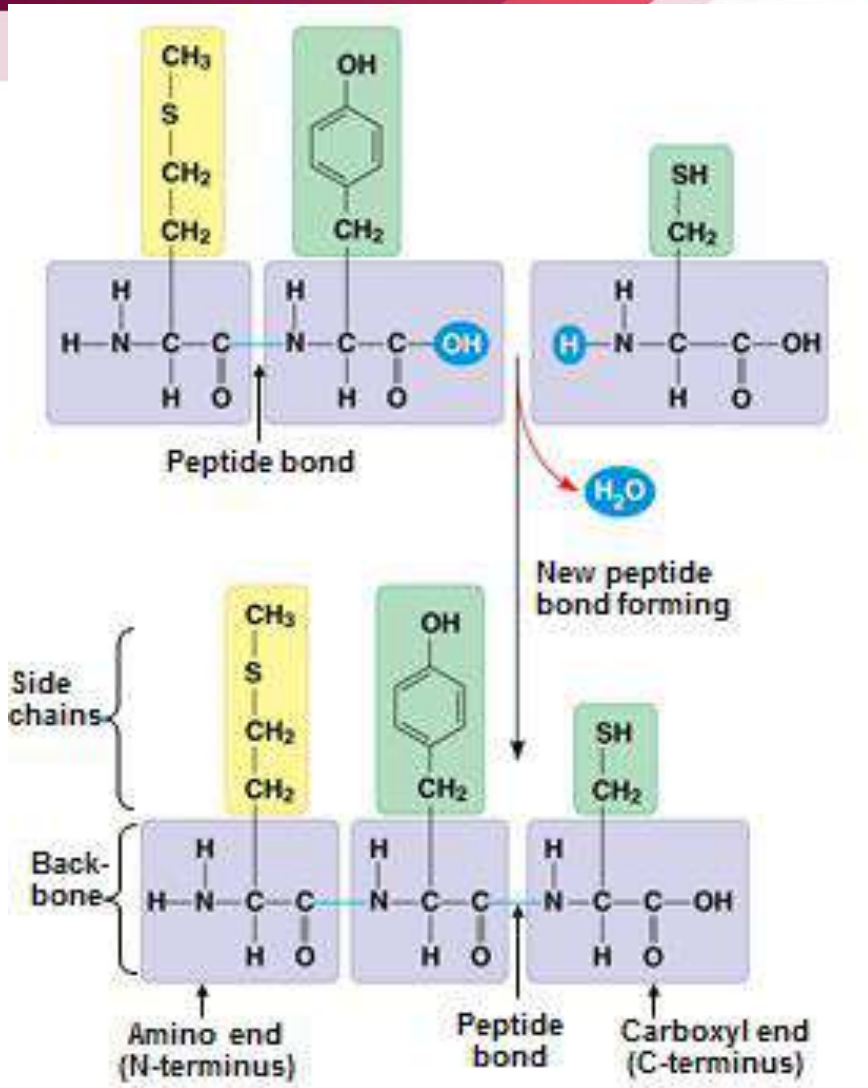


- Asam amino dihubungkan oleh ikata **peptide**
- 1 rantai polipeptida merupakan rantai asam amino yang bisa saja tersusun dari ribuan monomer
- Masing-masing polipeptida memiliki sekuen/urutan asam amino linier yang unik, dengan ujung karboksil (carboxyl end/C-terminus) dan ujung amino (amino end/N-terminus)

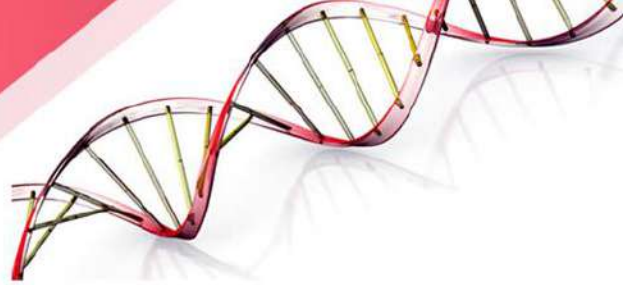
Pembentukan ikatan Peptida



Pembentukan ikatan Peptida



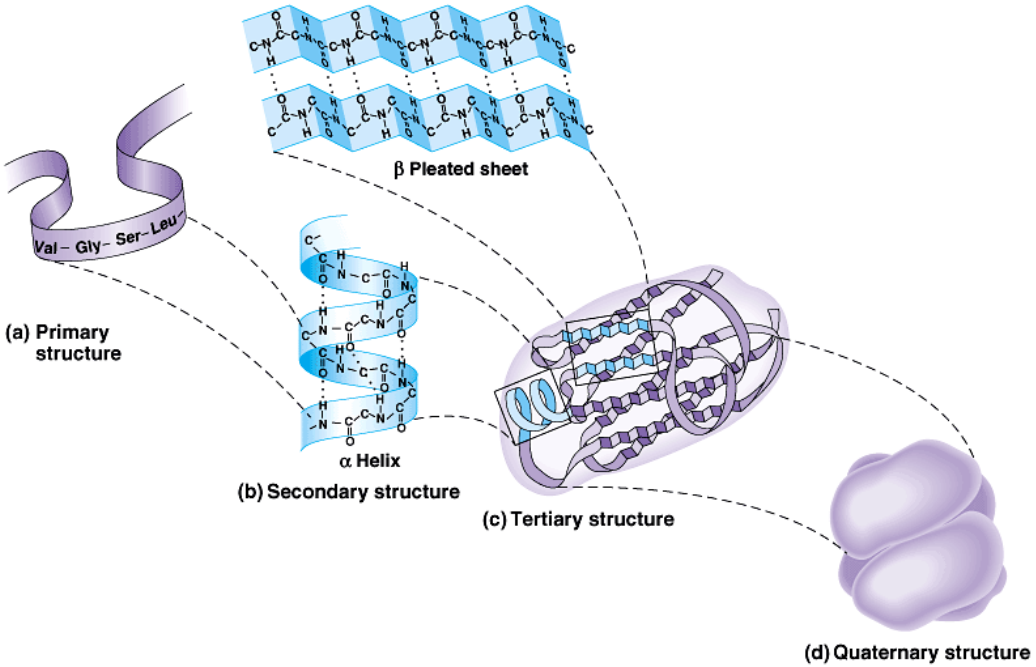
Struktur dan Fungsi Protein



- At first, all we have is a string of AA's bound with peptide bonds.
- Once the string of AA's interacts with itself and its environment (often aqueous), then we have a functional protein that consists of one or more polypeptides precisely twisted, folded, and coiled into a unique shape
- The sequence of amino acids determines a protein's three-dimensional structure
- A protein's structure determines its function

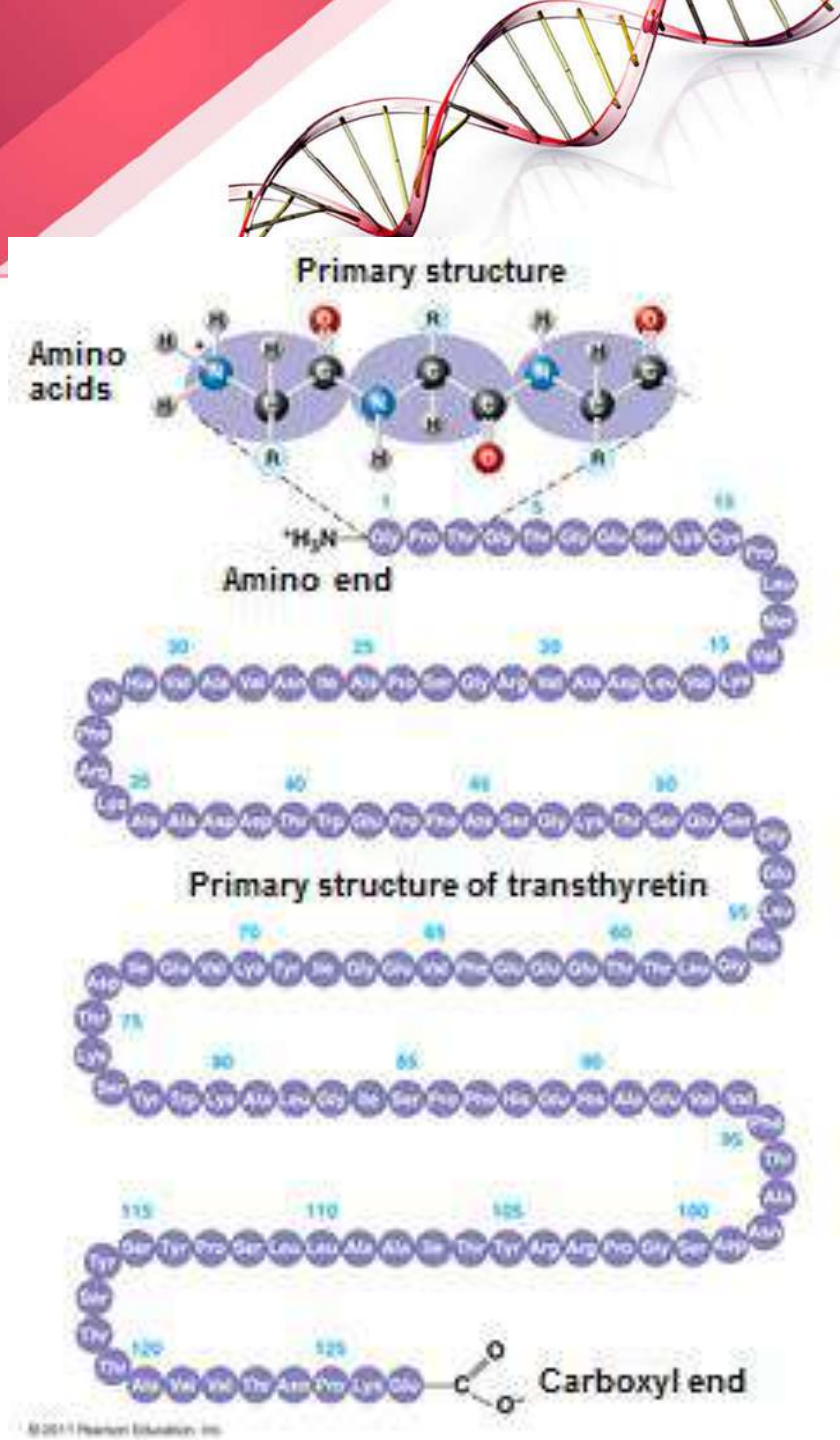
4 level Struktur Protein

- Primary structure → consists of its unique sequence of amino acids
- *Secondary* structure, found in most proteins, consists of coils and folds in the polypeptide chain
- *Tertiary* structure is determined by interactions among various side chains (R groups)
- *Quaternary* structure results when a protein consists of multiple polypeptide chains



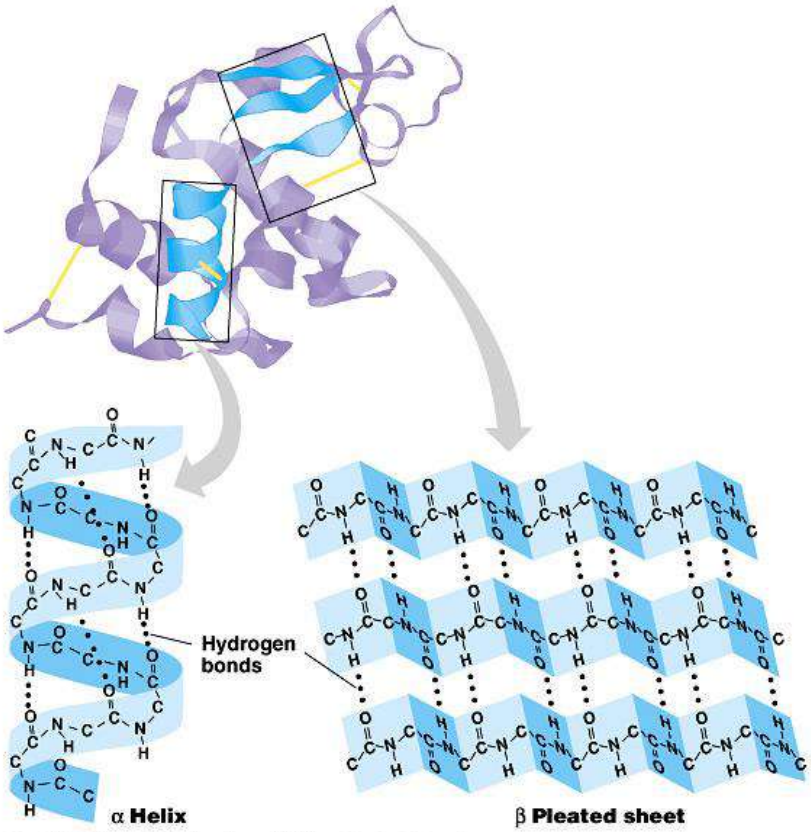
Primary Structure

- **Primary structure**, the sequence of amino acids in a protein, is like the order of letters in a long word
- Primary structure is determined by inherited genetic information



Secondary Structure

- The coils and folds of **secondary structure** result from hydrogen bonds between repeating constituents of the polypeptide backbone
- Typical secondary structures are a coil called an α **helix** and a folded structure called a β **pleated sheet**



Secondary Structure

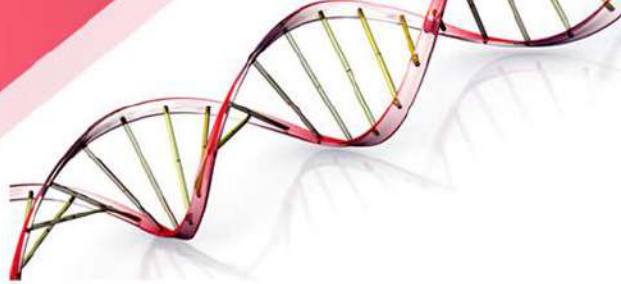
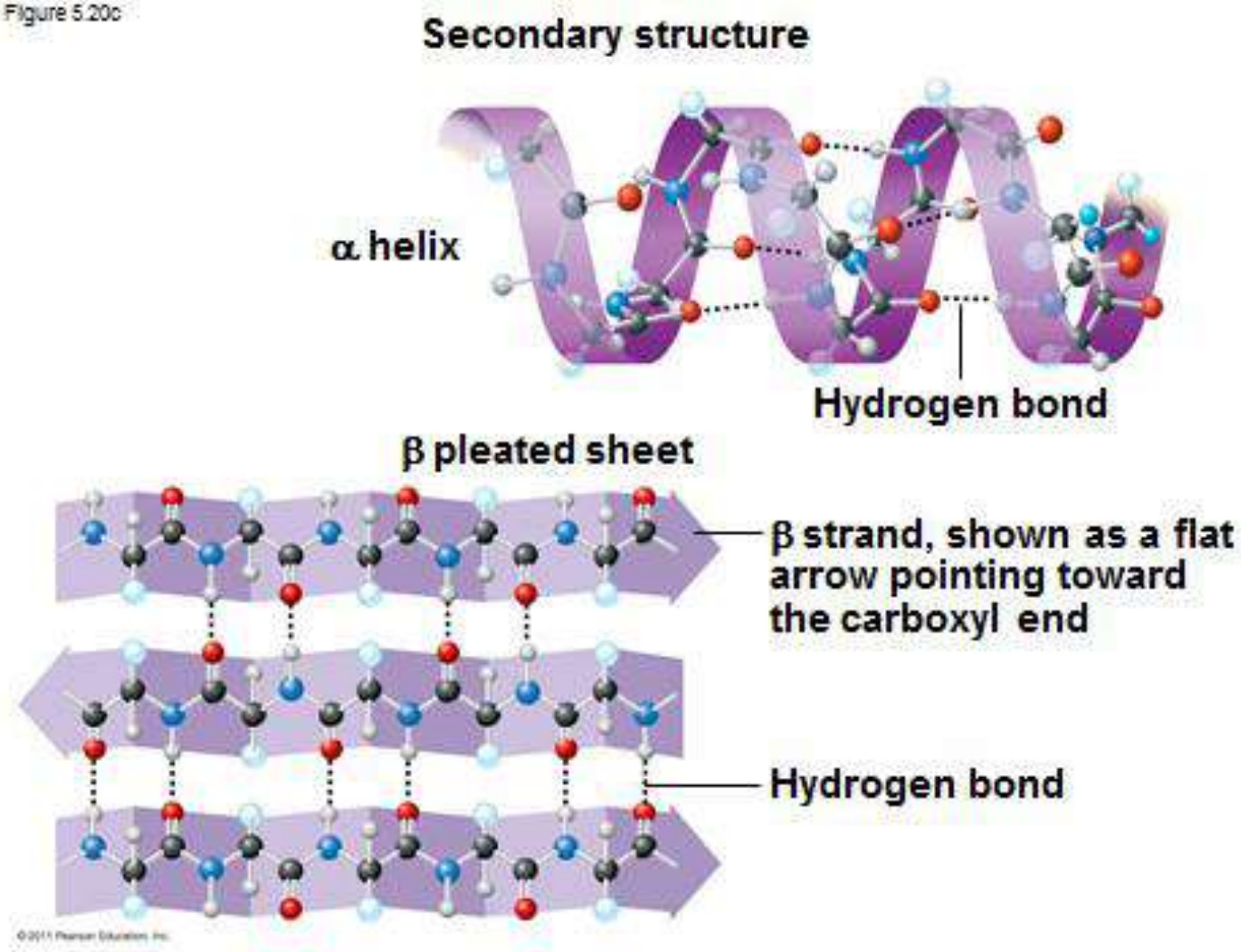
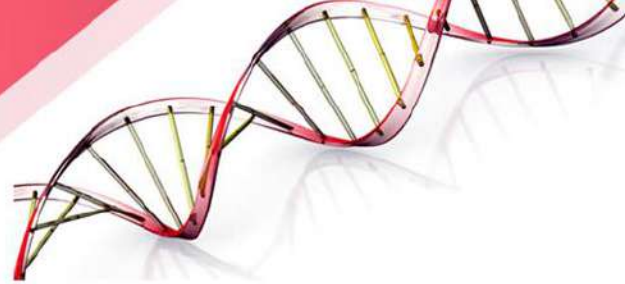


Figure 5.20c

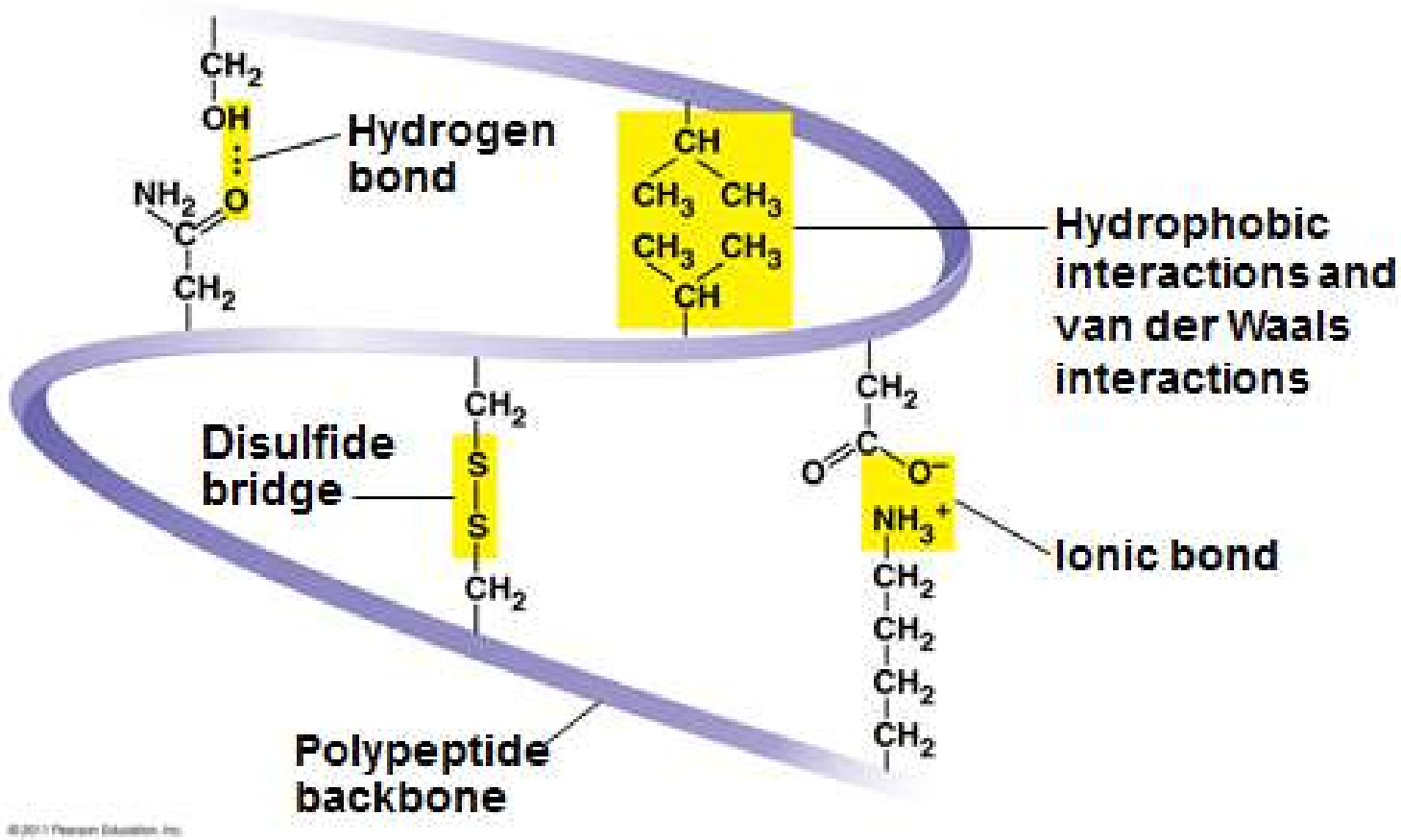


Tertiary Structure



- **Tertiary structure** is determined by interactions between R groups, rather than interactions between backbone constituents
- These interactions between R groups include actual *ionic bonds and* strong *covalent bonds* called **disulfide bridges** which may *reinforce* the protein's structure.
- **IMFs** such as London dispersion forces (LDFs a.k.a. and van der Waals interactions), hydrogen bonds (IMFs), and **hydrophobic interactions** (IMFs) may *affect* the protein's structure

Tertiary Structure



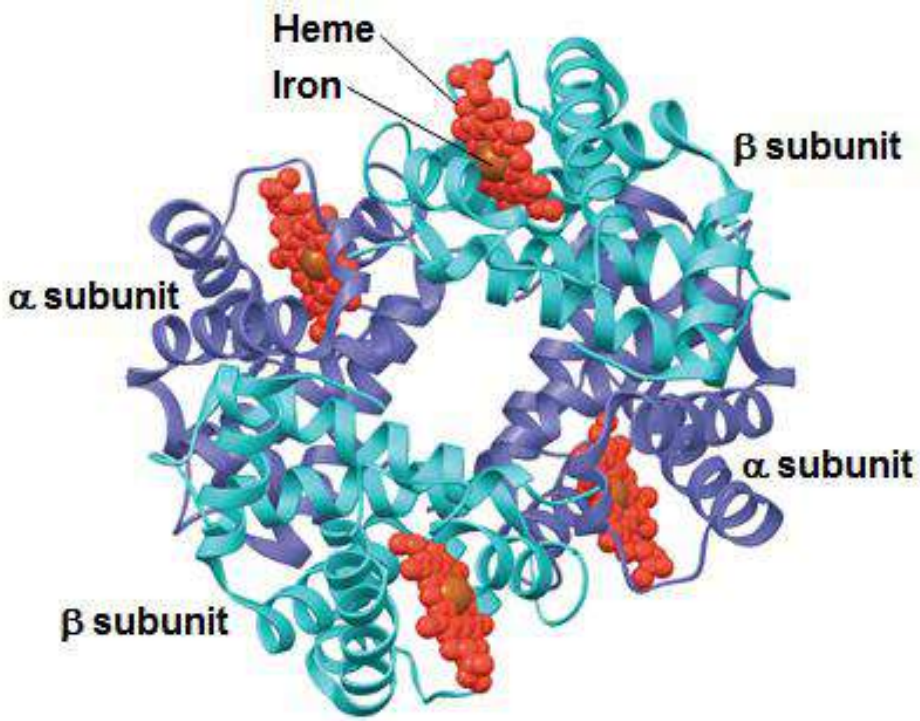
Quaternary Structure

- **Quaternary structure** results when two or more polypeptide chains form one macromolecule

- Collagen is a fibrous protein consisting of three polypeptides coiled like a rope

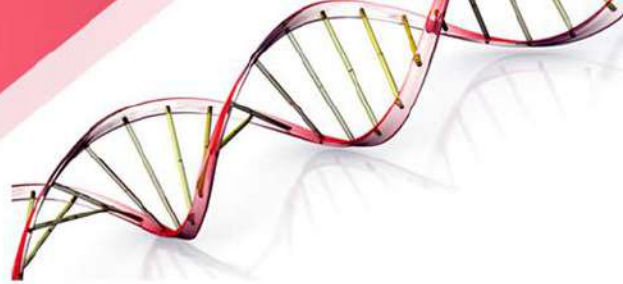
Quaternary Structure

- Hemoglobin is a globular protein consisting of four polypeptides: two alpha and two beta chains



Hemoglobin

Sickle-Cell Disease: Perubahan di Primary Structure

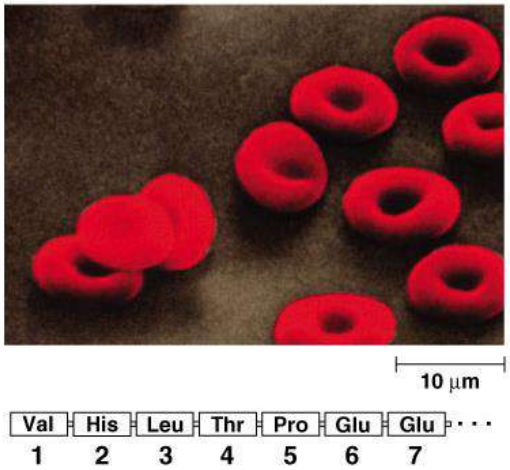


- A slight change in primary structure can affect a protein's structure and ability to function
- **Sickle-cell disease**, an inherited blood disorder, results from a single amino acid substitution in the protein hemoglobin

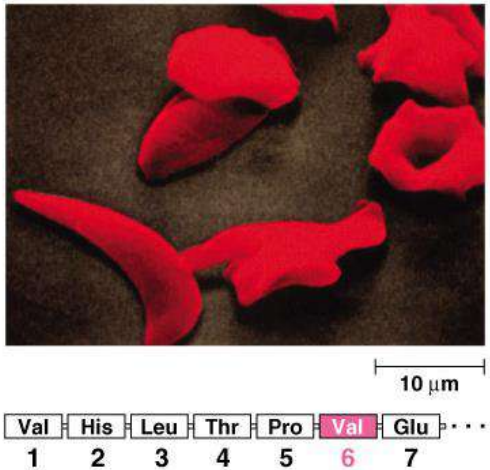
"Normal" Red Blood Cells

Sickle-Cell Disease: Perubahan di Primary Structure

- A slight change in primary structure can affect a protein's structure and ability to function
- **Sickle-cell disease**, an inherited blood disorder, results from a single amino acid substitution in the protein hemoglobin

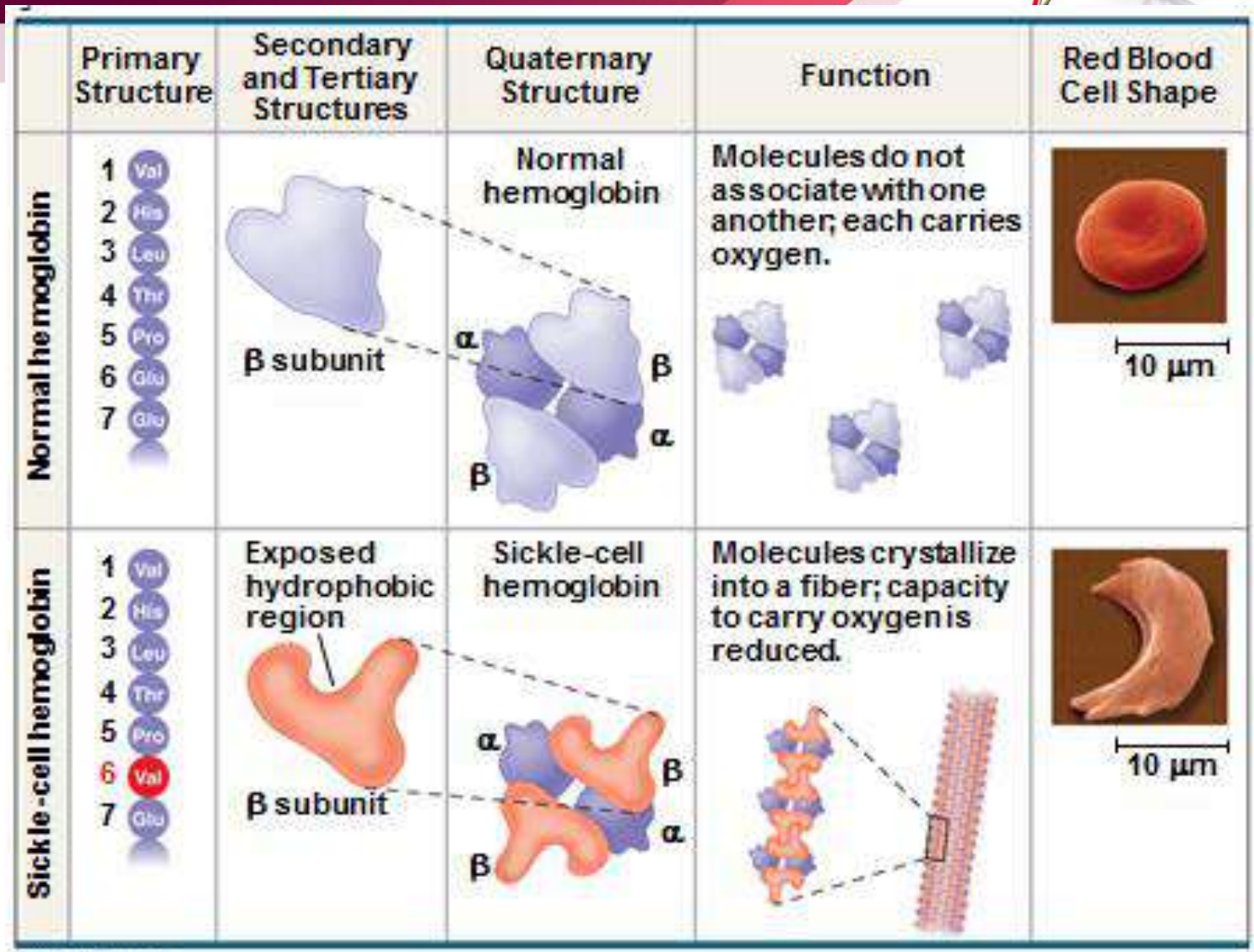


(a) Normal red blood cells and the primary structure of normal hemoglobin

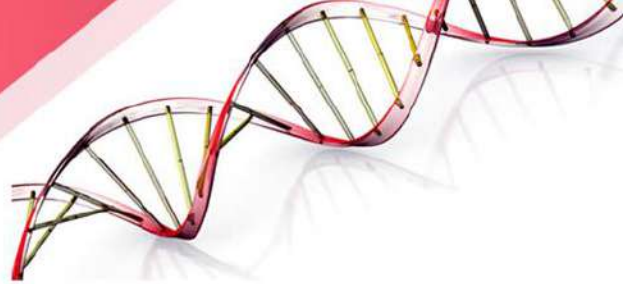


(b) Sickled red blood cells and the primary structure of sickle-cell hemoglobin

Sickle-Cell Disease:
A change in Primary Structure

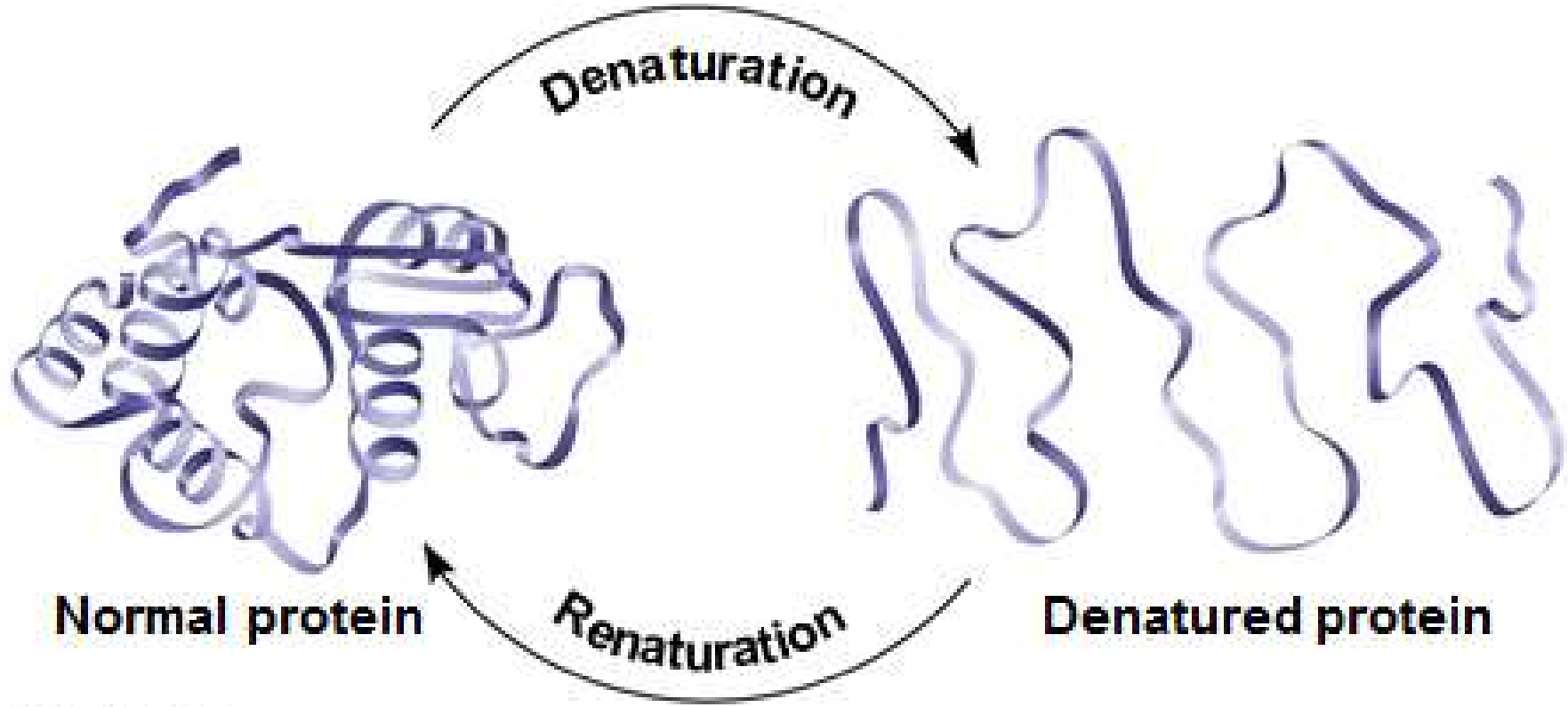


Apa yang menentukan struktur Protein?

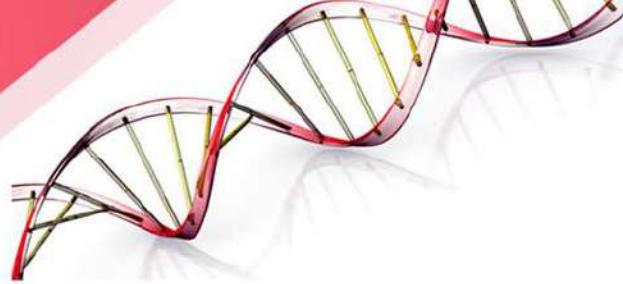


- Selain struktur primer, sifat fisik dan kimiawi protein dapat mempengaruhi struktur
- Perubahan lingkungan → pH, konsentrasi garam, suhu, dan faktor-faktor lain → perubahan struktur protein
- Perubahan struktur asli protein → **denaturasi**
- Protein yang terdenaturasi → inaktif secara biologis

Denaturasi: pemutusan ikatan atau kerusakan struktur

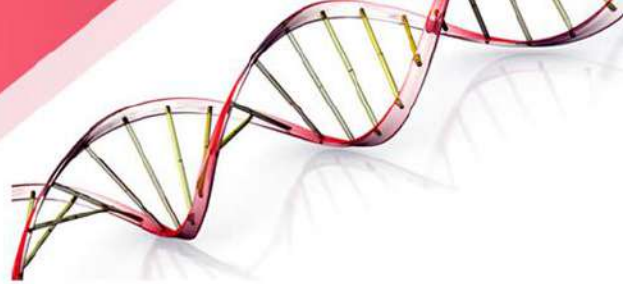


Asam Nukleat



- Nucleic acids store, transmit, and help express hereditary information
- The amino acid sequence of a polypeptide is programmed by a unit of inheritance called a **gene**
- Genes are made of DNA, a **nucleic acid** made of monomers called *nucleotides*

Two Types of Nucleic Acids



- There are two types of nucleic acids
  - **Deoxyribonucleic acid (DNA)**
  - **Ribonucleic acid (RNA)**
- DNA provides directions for its own replication
- DNA directs synthesis of messenger RNA (mRNA) and, through mRNA, controls protein synthesis
- *Protein synthesis occurs on ribosomes*

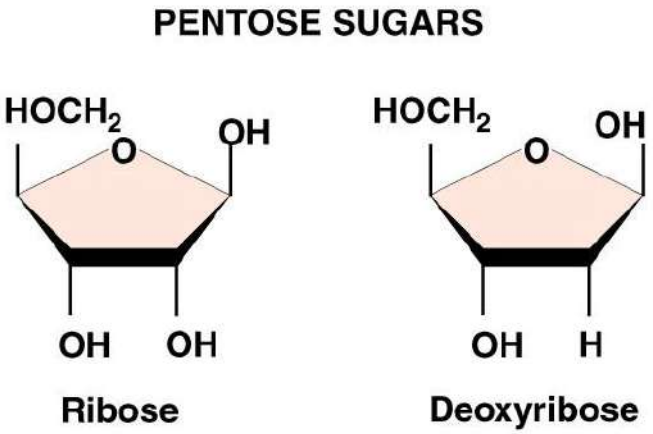


Figure 5.25-1

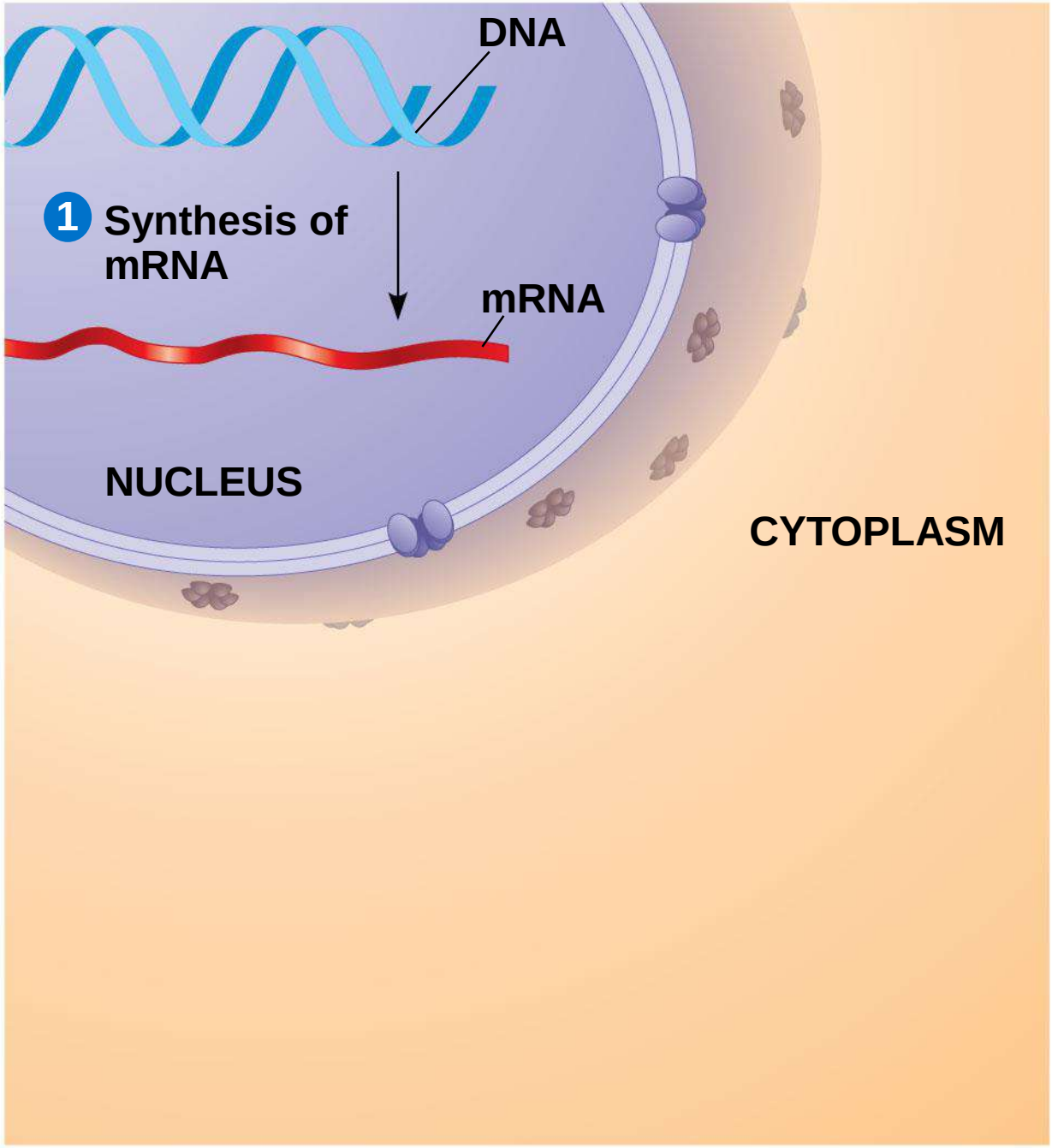


Figure 5.25-2

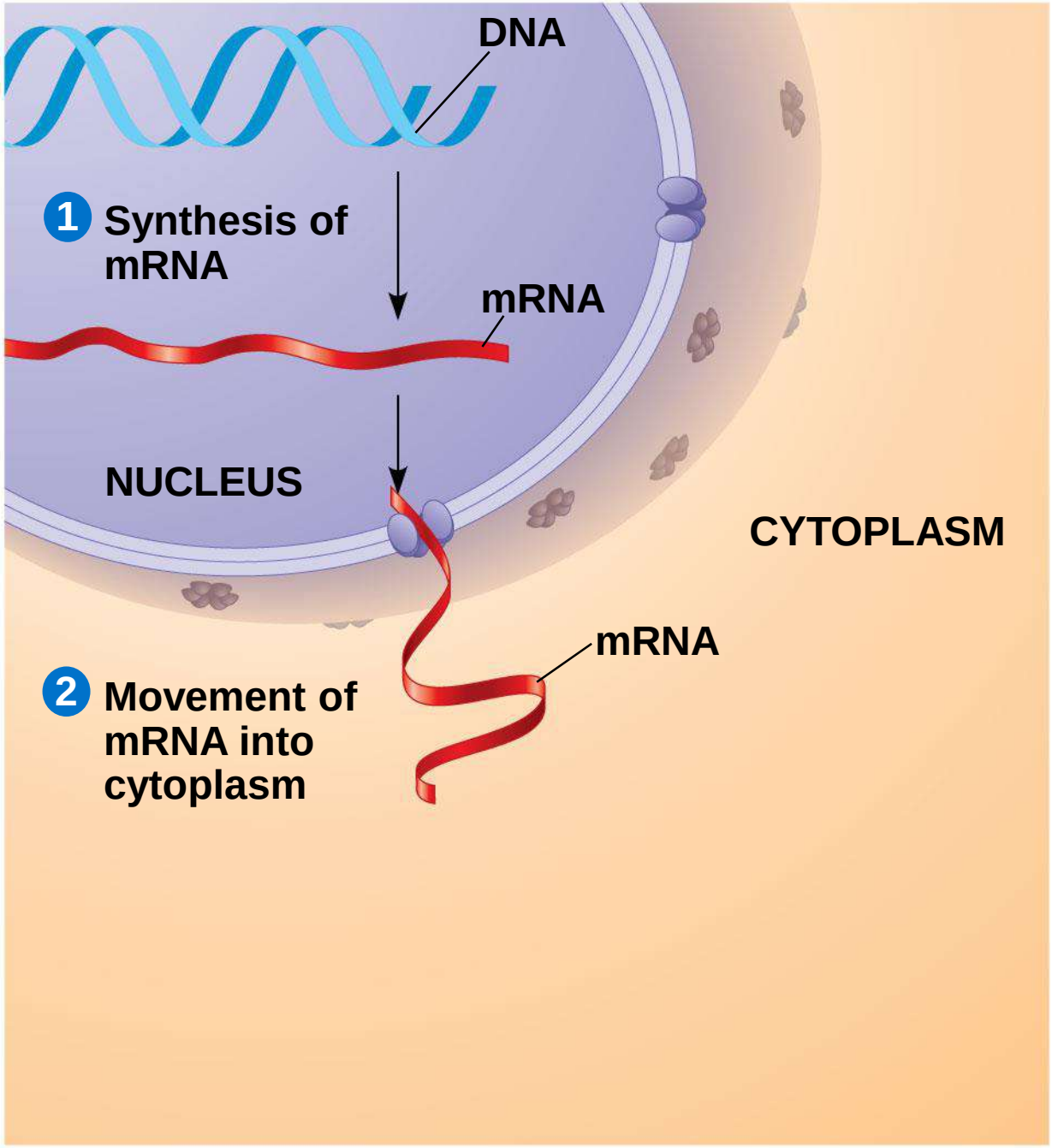
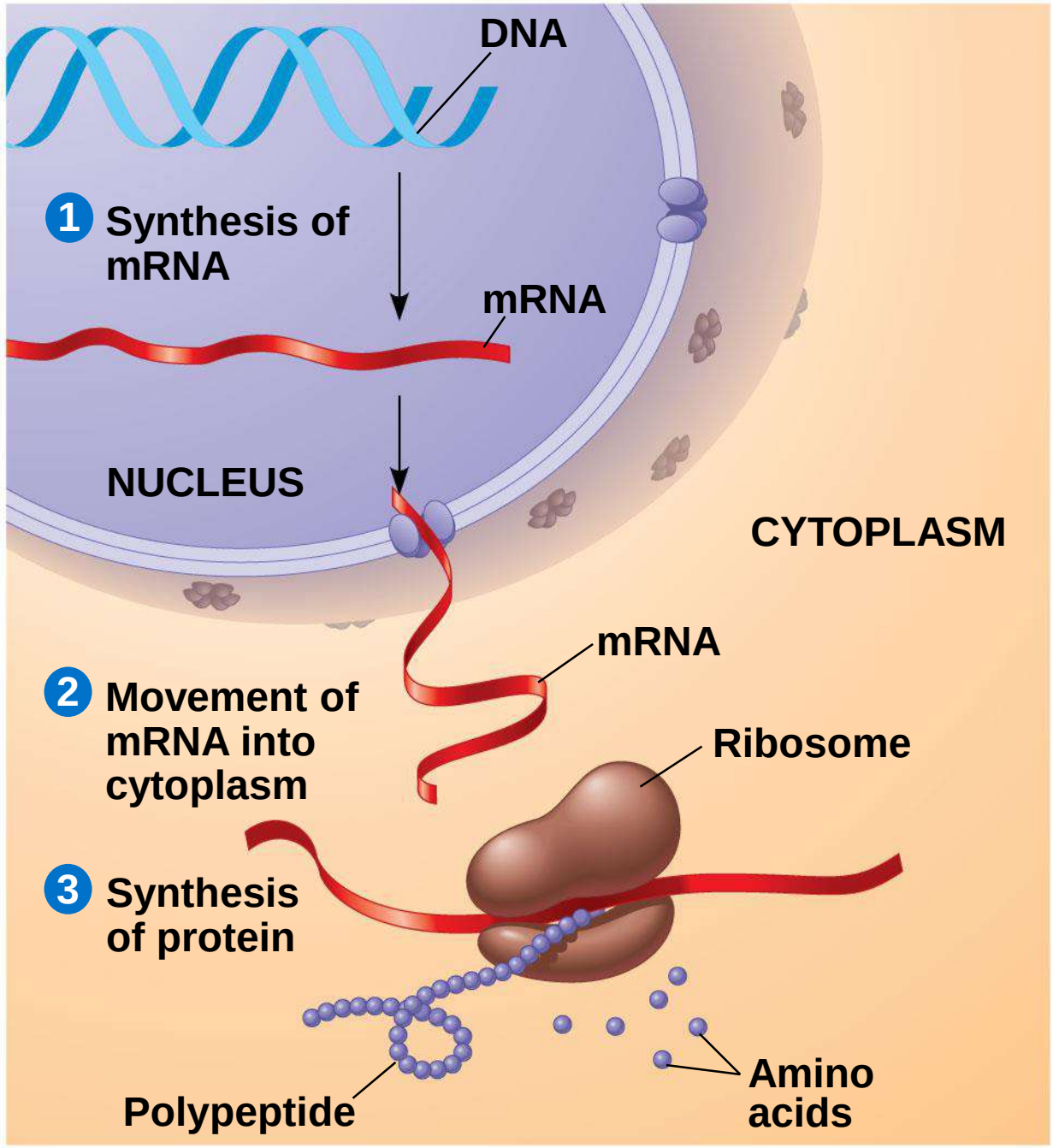
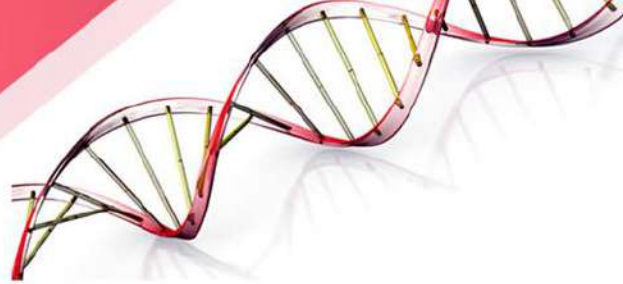


Figure 5.25-3



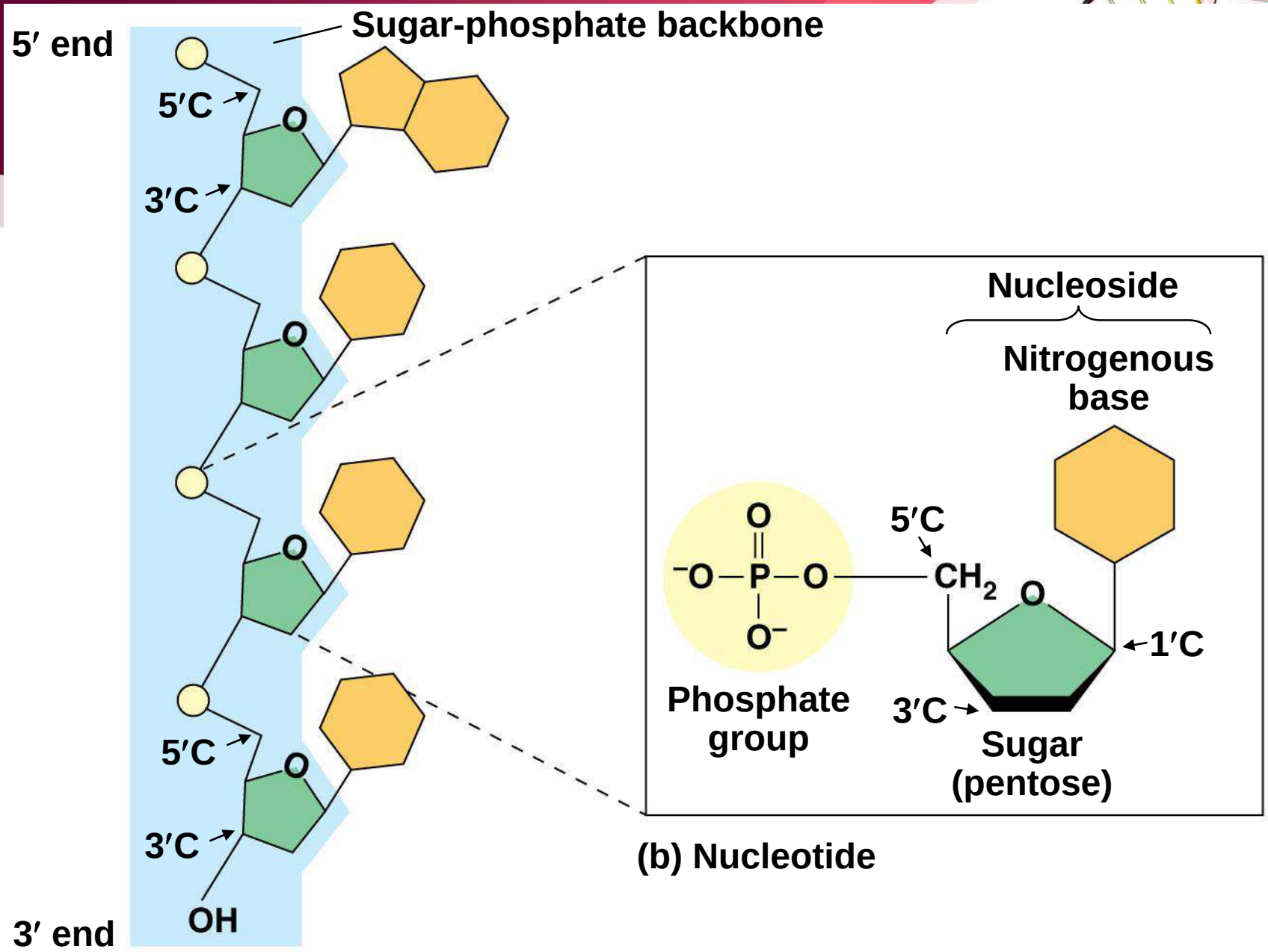
The Components of Nucleic Acids



- Each nucleic acid is made of monomers called **nucleotides**

- Each nucleotide consists of a *nitrogenous base, a pentose sugar, and one or more phosphate groups*

Figure 5.26ab



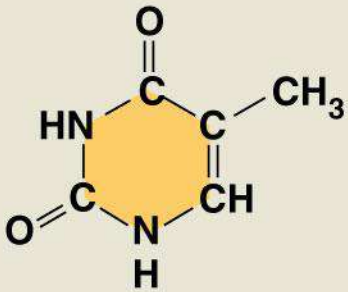
(a) Polynucleotide, or nucleic acid

Figure 5.26c

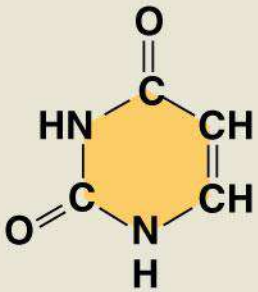
Nitrogenous bases

Pyrimidines

Cytosine (C)

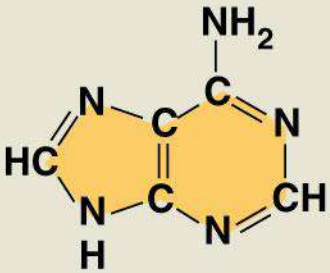


Thymine (T, in DNA)

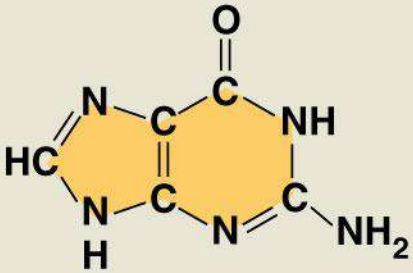


Uracil (U, in RNA)

Purines

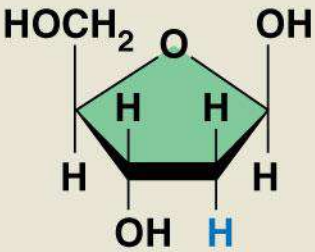


Adenine (A)

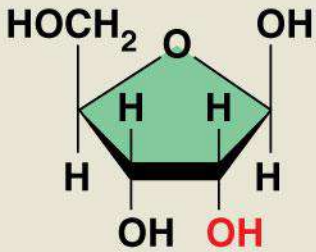


Guanine (G)

Sugars

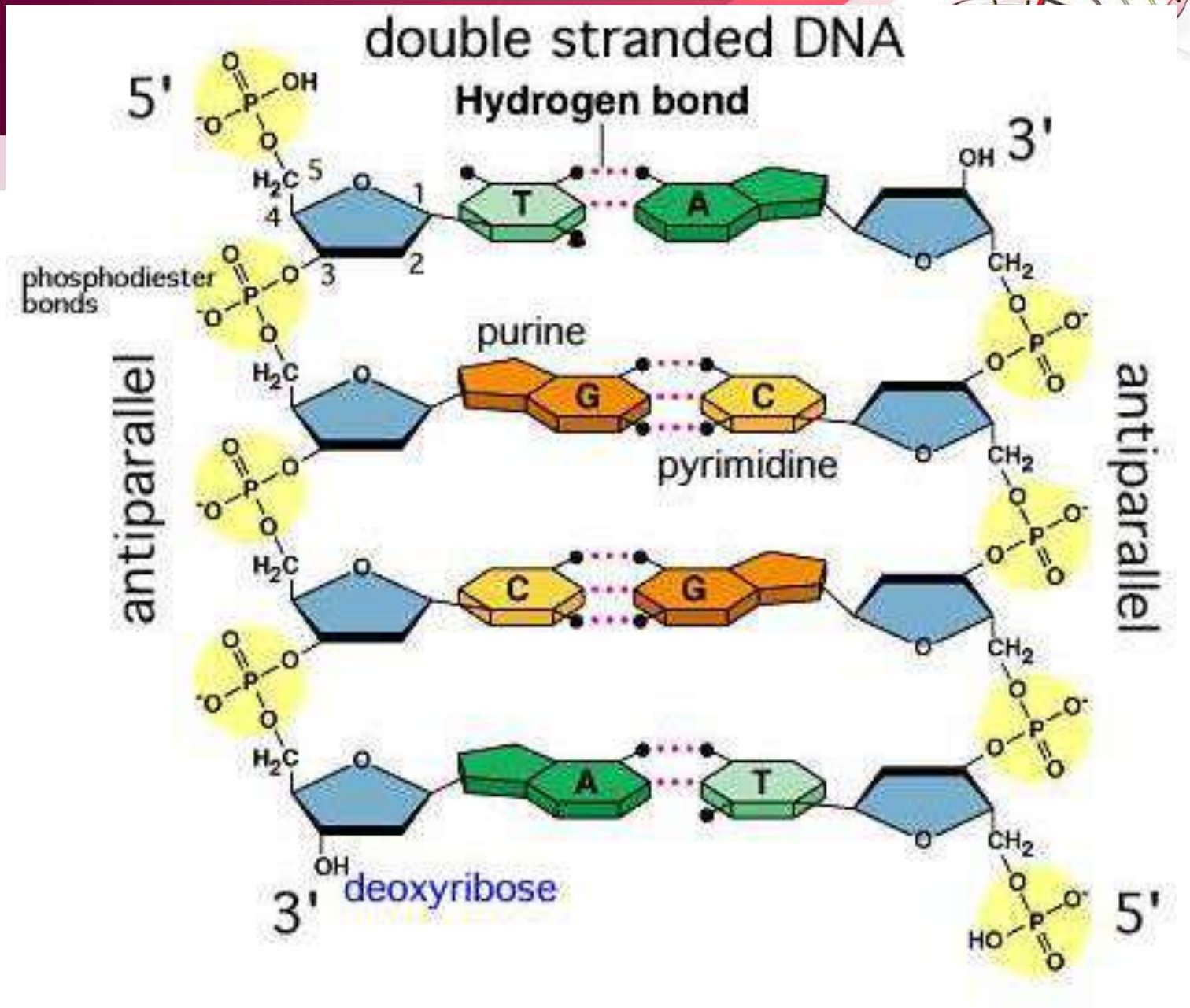


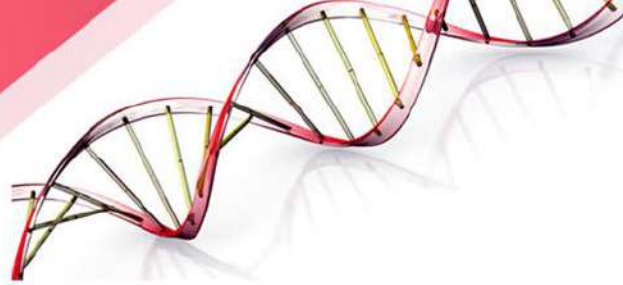
Deoxyribose (in DNA)



Ribose (in RNA)

(c) Nucleoside components

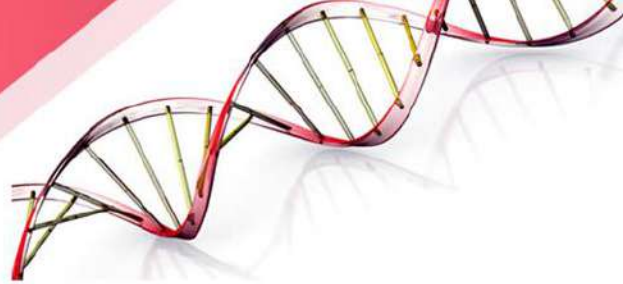

double stranded DNA
Hydrogen bond
5'
3'
phosphodiester bonds
purine
pyrimidine
antiparallel
antiparallel
T
A
G
C
C
G
A
T
deoxyribose
3'
5'



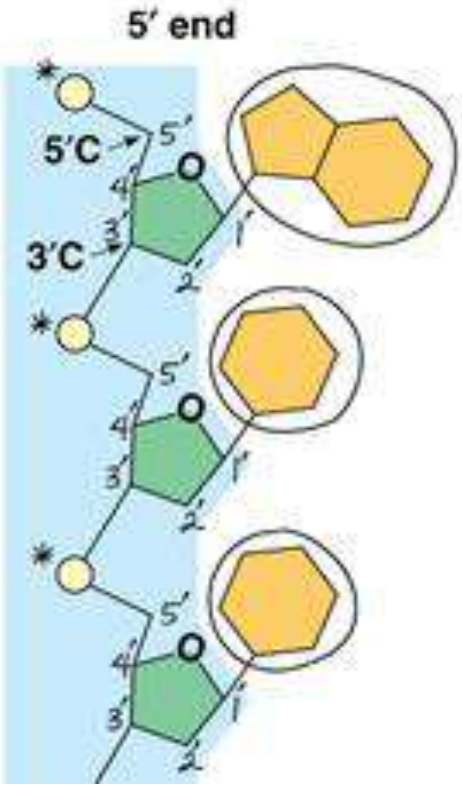
- There are two families of nitrogenous bases
  - **Pyrimidines** (cytosine, thymine, and uracil) have a single six-membered ring
  - **Purines** (adenine and guanine) have a six-membered ring fused to a five-membered ring

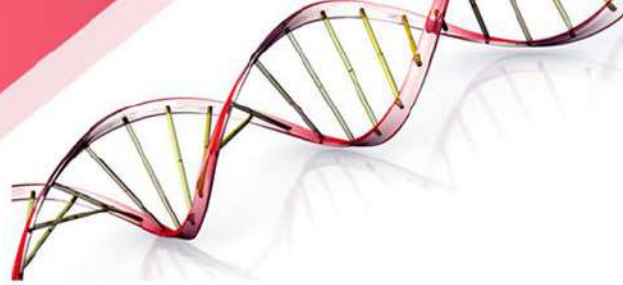
- In DNA, the sugar is **deoxyribose**; in RNA, the sugar is **ribose**

The Devil is in the Details



- Adjacent *nucleotides are joined by covalent bonds* that form between the —OH group on the 3′ carbon of one nucleotide and the phosphate on the 5′ carbon on the next
- These links create a backbone of sugar-phosphate units with nitrogenous bases as appendages
- The sequence of bases along a DNA or mRNA polymer is unique for each gene

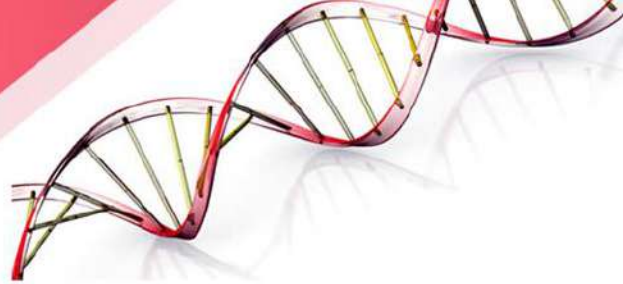




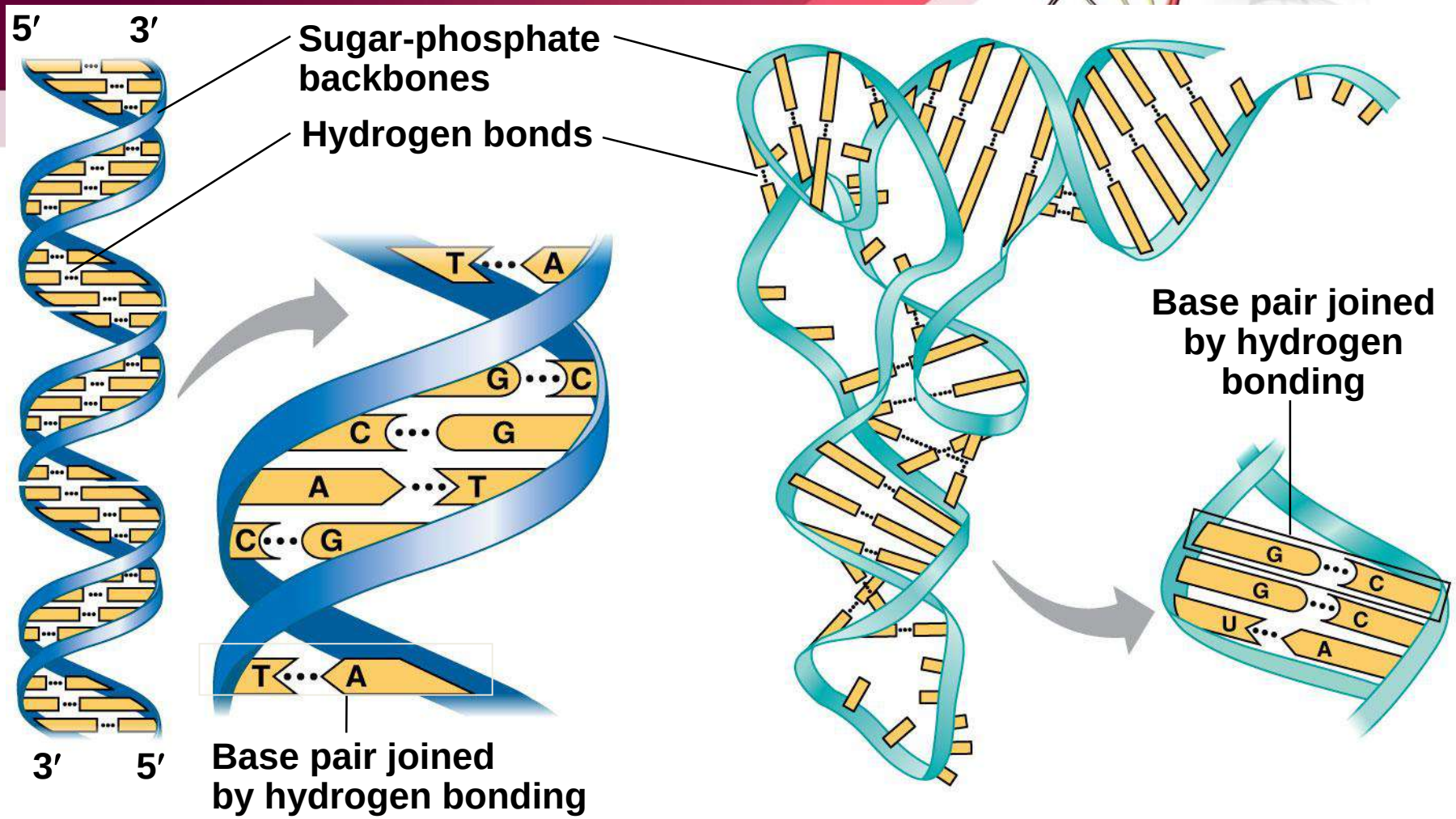
- RNA molecules usually exist as single polypeptide chains
- DNA molecules have two polynucleotides spiraling around an imaginary axis, forming a **double helix**
- In the DNA double helix, the two backbones run in opposite 5′→ 3′ directions from each other, an arrangement referred to as **antiparallel**
- One DNA molecule includes many genes

5′–T A G G C C T–3′

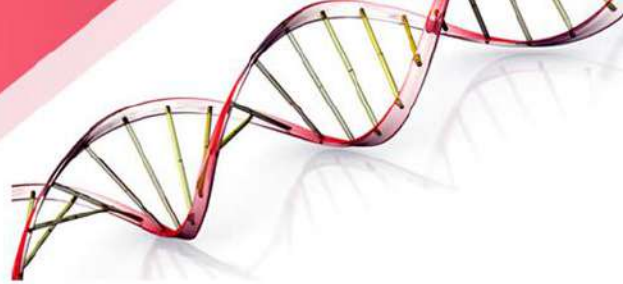
3′–A T C C G G A–5′



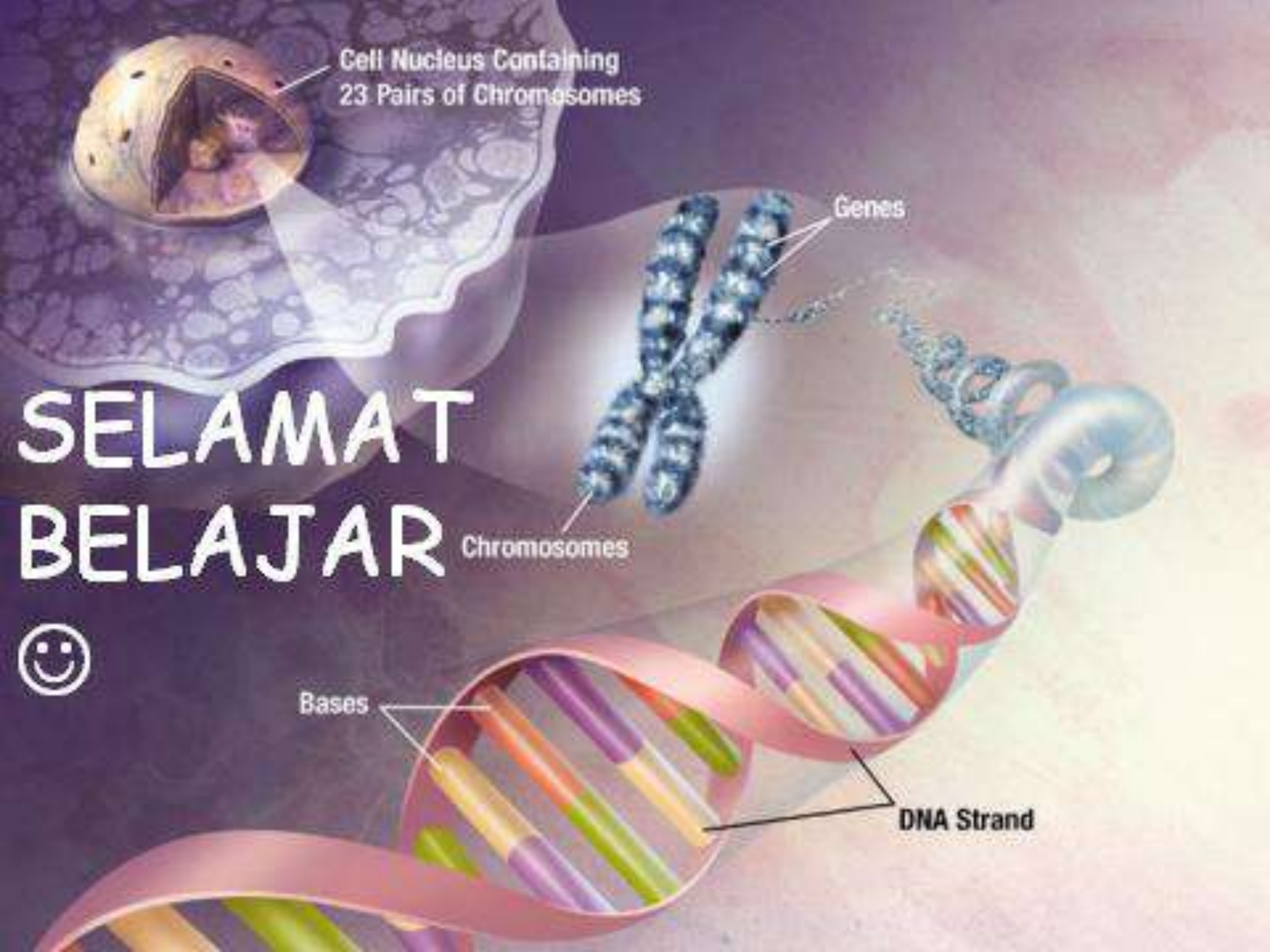
- The nitrogenous bases in DNA pair up and form hydrogen bonds: adenine (A) always with thymine (T), and guanine (G) always with cytosine (C)
- Called complementary base pairing
- Complementary pairing can also occur between two RNA molecules or between parts of the same molecule
- In RNA, thymine is replaced by uracil (U) so A and U pair



Link to Evolution



- The linear sequences of nucleotides in DNA molecules are passed from parents to offspring
- Two closely related species are more similar in DNA than are more distantly related species
- Molecular biology can be used to assess evolutionary kinship


Cell Nucleus Containing
23 Pairs of Chromosomes
Genes
SELAMAT
BELAJAR
Chromosomes
Bases
DNA Strand

MACROMOLECULE:
LIPID

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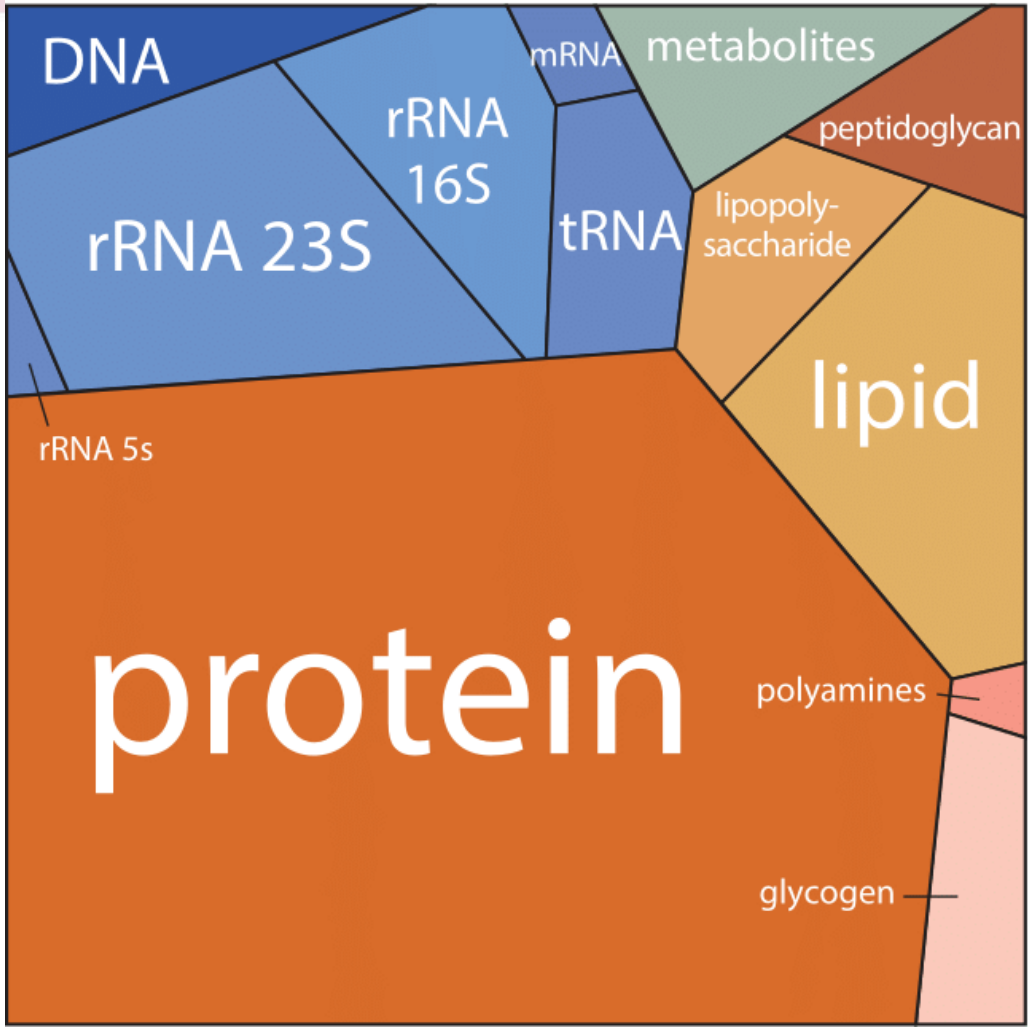
Macromolecules: Carbohydrates and Lipids

- Diagram of the composition of an E. coli cell



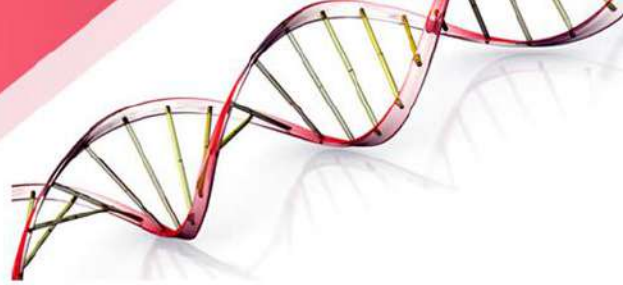
macromolecule	percentage of total dry weight	weight per cell (fg)	characteristic molecular weight (Da)	number of molecules per cell
protein	55	165	3×10^4	3,000,000
RNA	20	60		
23 S rRNA		32	1×10^6	20,000
16 S rRNA		16	5×10^5	20,000
5 S rRNA		1	4×10^4	20,000
transfer		9	2×10^4	200,000
messenger		2	1×10^6	1,400
DNA	3	9	3×10^9	2
lipid	9	27	800	20,000,000
lipopolysaccharide	3	9	8000	1,000,000
peptidoglycan	3	9	$(1000)_n$	1
glycogen	3	9	1×10^6	4,000
metabolites and cofactors pool	3	9		
inorganic ions	1	3		
total dry weight	100	300		
water (70% of cell)		700		
total cell weight		1000		

composition rules of thumb

- carbon atoms ~10^{10}
- 1 molecule per cell gives ~1 nM conc.
- ATP required to build and maintain cell over a cell cycle ~10^{10}
- glucose molecules needed per cell cycle ~3×10^9 (2/3 of carbons used for biomass and 1/3 used for ATP)

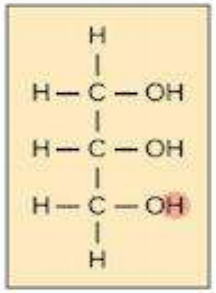
- **Lipid→** Lipid terdapat pada tumbuhan, hewan, manusia, dan mikroorganisme.
- Lipid merupakan zat lemak yang berperan dalam berbagai sel hidup.
- Tersusun atas unsur karbon (CH), hidrogen (H), dan oksigen (O), serta kadang kala ditambah fosfor (P) serta nitrogen (N).
- Disimpan sebagai sumber energi sekunder dan sebagian lain bertindak sebagai komponen penting dari membran sel.
- Lipid terdiri dari beberapa jenis, yang terpenting adalah lemak, fosfolipid, dan steroid.

- Lemak
- Penyusun lemak → sintesis dehidrasi antara molekul gliserol dan asam lemak.
- Gliserol adalah rangka karbon yang memiliki tiga gugus alkohol. Rumus empirisnya adalah $C_3H_4(OH)_3$.
- Asam lemak merupakan rantai karbon yang panjang yang memiliki gugus karboksil.
- Rantai karbon yang memiliki banyak ikatan hidrogen→ asam lemak jenuh.
- Tidak jenuh jika atom-atom karbonnya memiliki ikatan rangkap lebih dari satu.



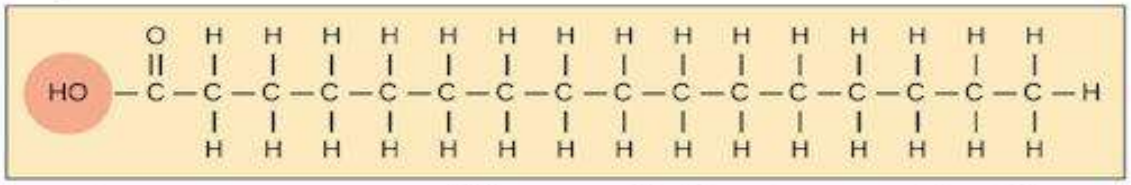
- To make a fat molecule, the hydroxyl groups on the glycerol backbone react with the carboxyl groups of fatty acids in a dehydration synthesis reaction.
- This yields a fat molecule with three fatty acid tails bound to the glycerol backbone via ester linkages (linkages containing an oxygen atom next to a carbonyl, or C=O, group).
- Triglycerides may contain three identical fatty acid tails, or three different fatty acid tails (with different lengths or patterns of double bonds).

Glycerol



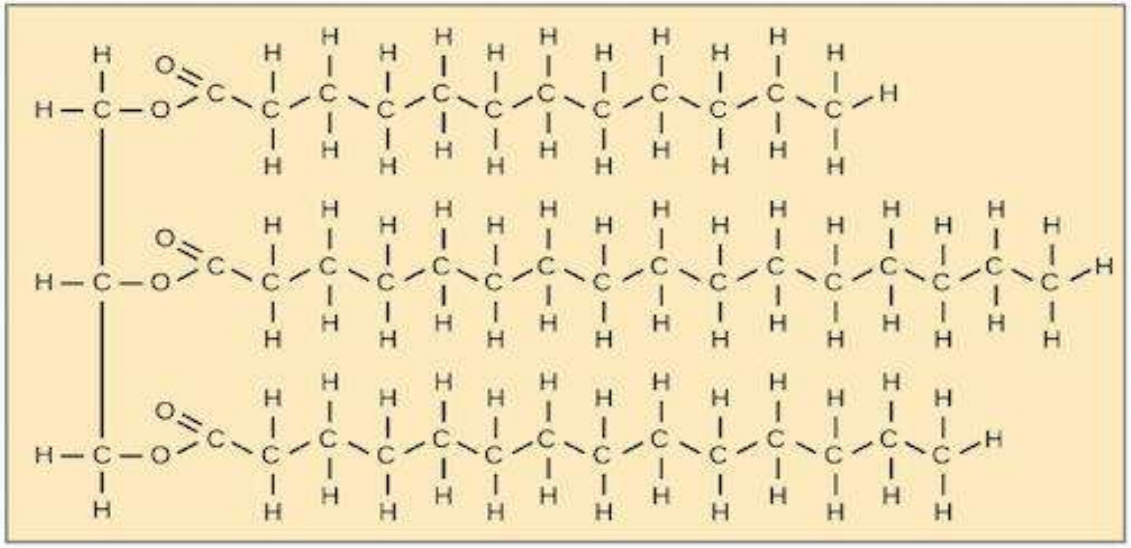
+

Fatty Acid

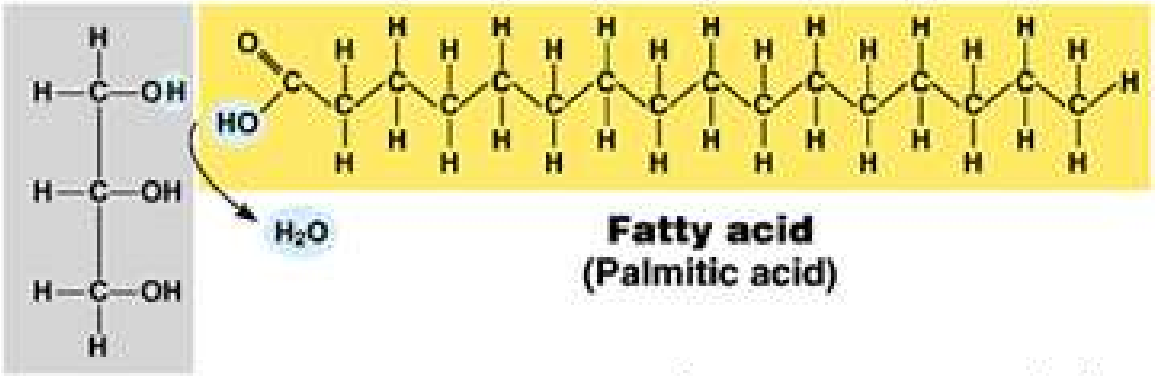


(three of these, may have different structures)

Triacylglycerol



$+ 3H_2O$



(a) Dehydration synthesis

Gambar 4.1 Sintesis Dehidrasi

(sumber: Campbell)

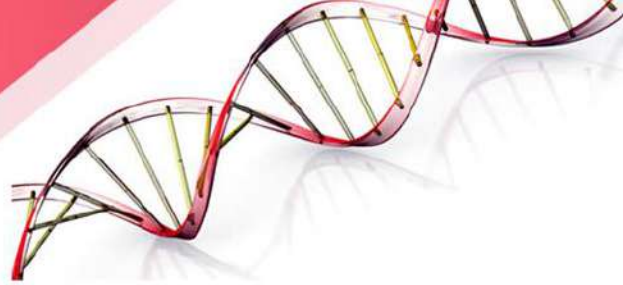
(a) Asam Stearat

(b) Asam Linoleat (Omega-6)

(c) Asam Alfa-Linoleat (Omega-3)

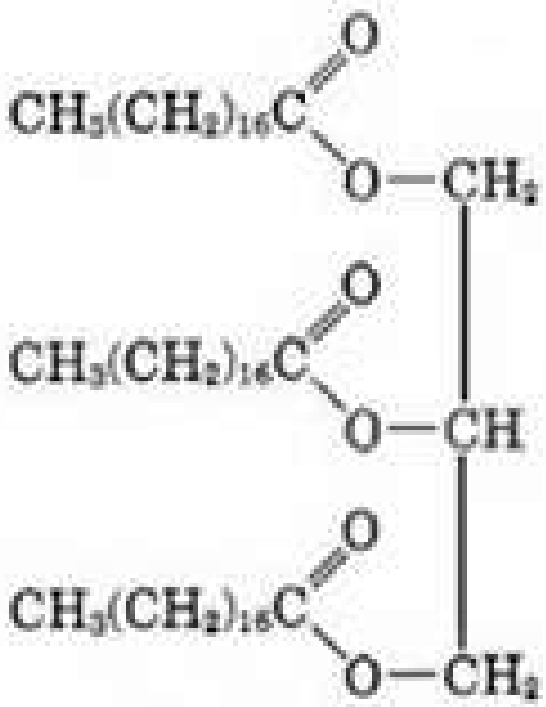
Struktur Asam Lemak Jenuh dan Tak jenuh

(a) Asam stearat merupakan asam lemak jenuh. *(b)* Asam linoleat merupakan asam emak tak jenuh. *(c)* Asam Alfa-Linoleat merupakan asam lemak essensial untuk manusia

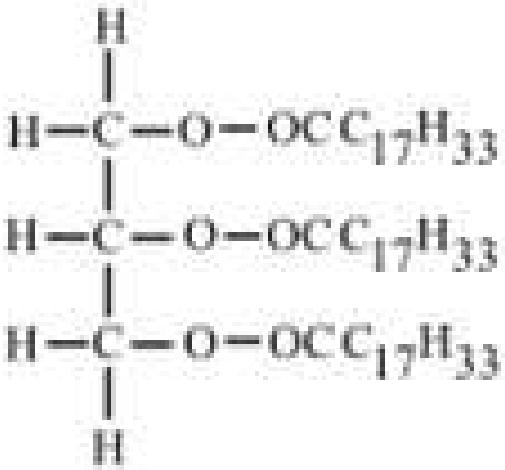


- Lemak identik dengan minyak hewani dan minyak nabati yang terutama terdiri dari gliserida.
- Lemak merupakan ester yang terbentuk melalui reaksi tiga molekul asam lemak dan sebuah molekul gliserol.
- Lemak bersifat tidak mudah menguap, tidak larut dalam air, terasa berminyak atau licin ketika disentuh, dan berbentuk padat pada suhu kamar.

Beberapa jenis lemak



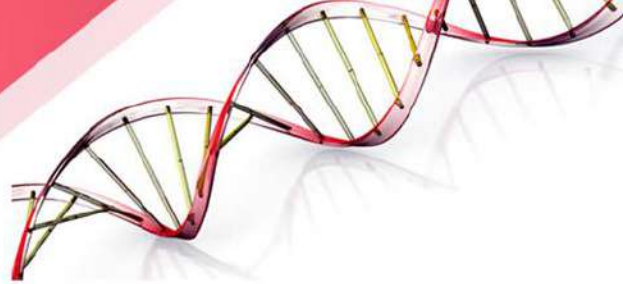
Glycerol tristearate



Glyceryl Trioleate

Struktur beberapa jenis lemak

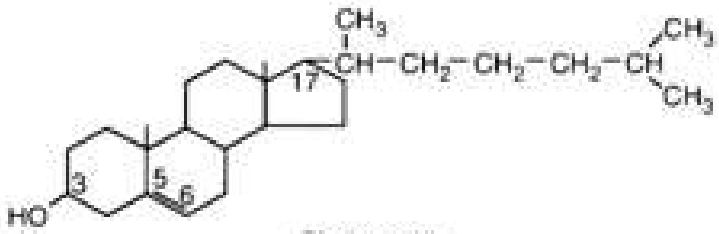
Fungsi



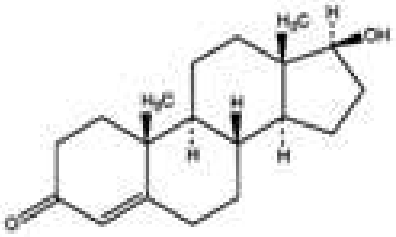
- Lebih dari 90 persen lemak diperoleh dari sekitar 20 jenis tumbuhan dan hewan. Lemak berfungsi sebagai cadangan makanan atau sumber energi di dalam tubuh.

Steroid

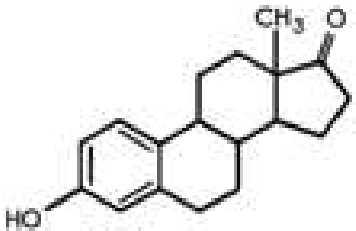
- Steroid merupakan senyawa turunan lipid yang tidak terhidrolisis.
- Steroid berfungsi sebagai hormon, seperti hormon seks, hormon adrenal kortikal, asam empedu, sterol.
- Contoh-contoh steroid antara lain adalah kolesterol, esterogen, dan testosteron.



Cholesterol



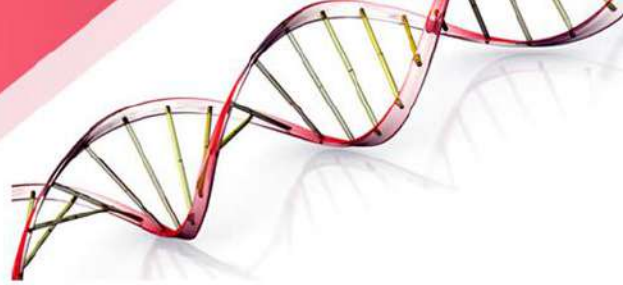
Testosterone



Estrogen

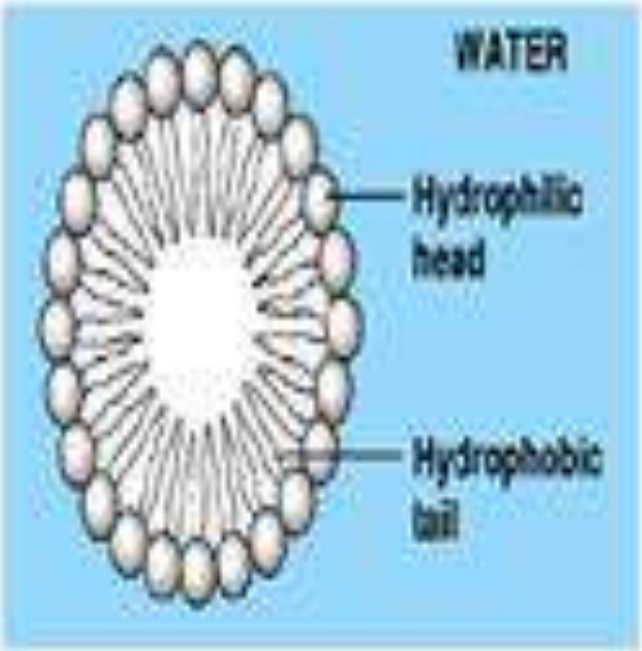
Sruktur beberapa steroid

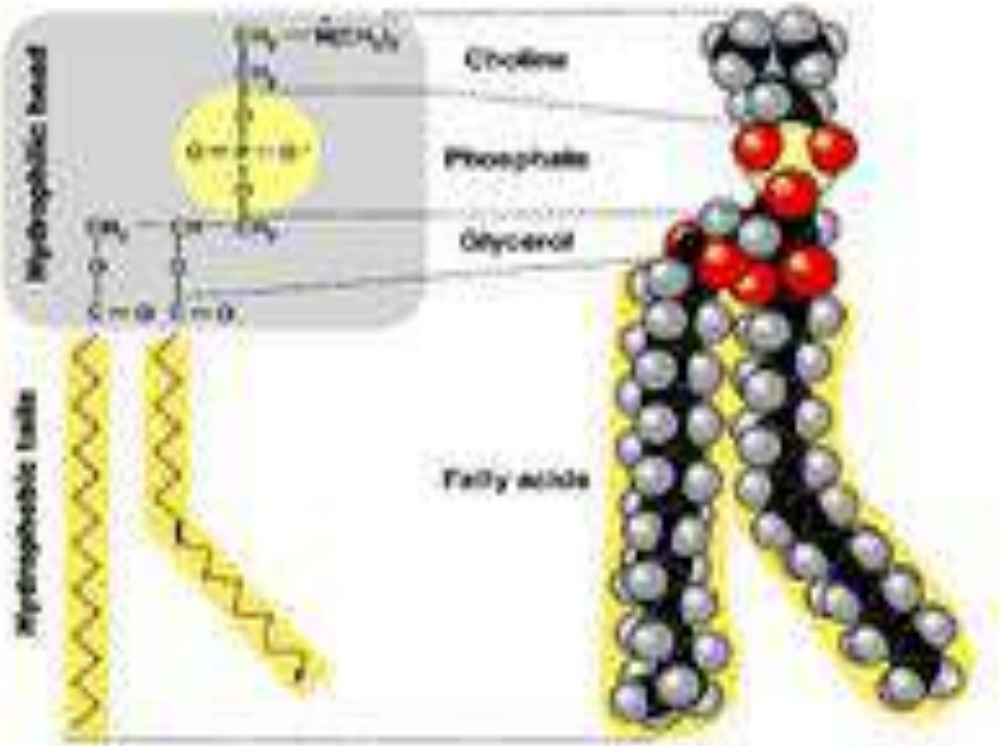
Fosfolipid



- Fosfolipid merupakan jenis lemak majemuk.
- Fosfolipid merupakan lipid yang berjumlah banyak (sebagai lesitin atau fosfatidietanolamin) yang di dalamnya asam fosfat serta asam lemak diesterifikasi menjadi gliserol dan terdapat dalam semua sel hidup serta dalam plasma membran

- Fats have received a lot of bad publicity, and it's true that eating large amounts of fried foods and other "fatty" foods can lead to weight gain and cause health problems. However, fats are essential to the body and have a number of important functions.
- For instance, many vitamins are fat-soluble, meaning that they must be associated with fat molecules in order to be effectively absorbed by the body.
- Fats also provide an efficient way to store energy over long time periods, since they contain over twice as much energy per gram as carbohydrates, and they additionally provide insulation for the body.
- Like all the other large biological molecules, fats in the right amounts are necessary to keep your body (and the bodies of other organisms) functioning correctly.

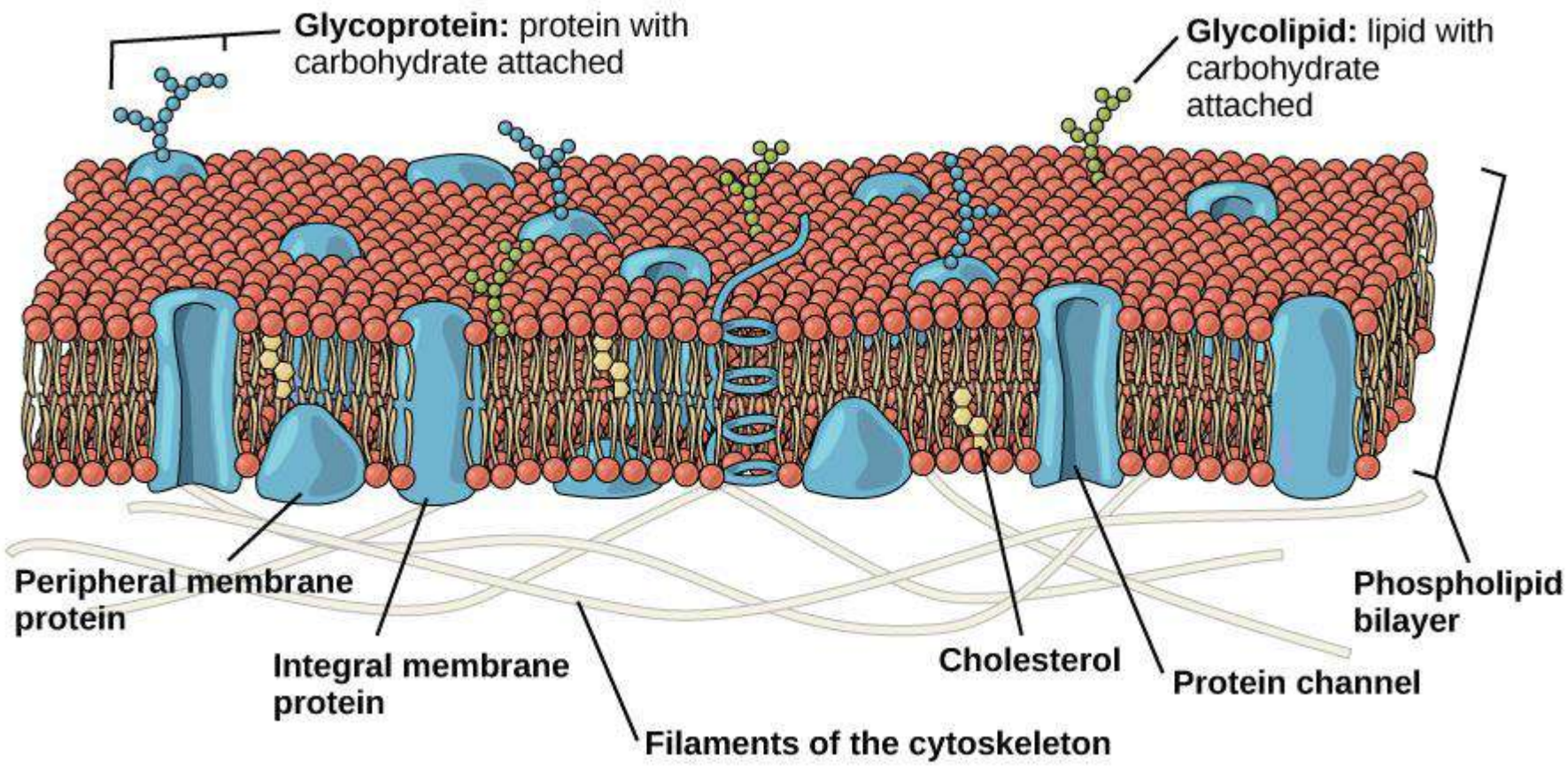


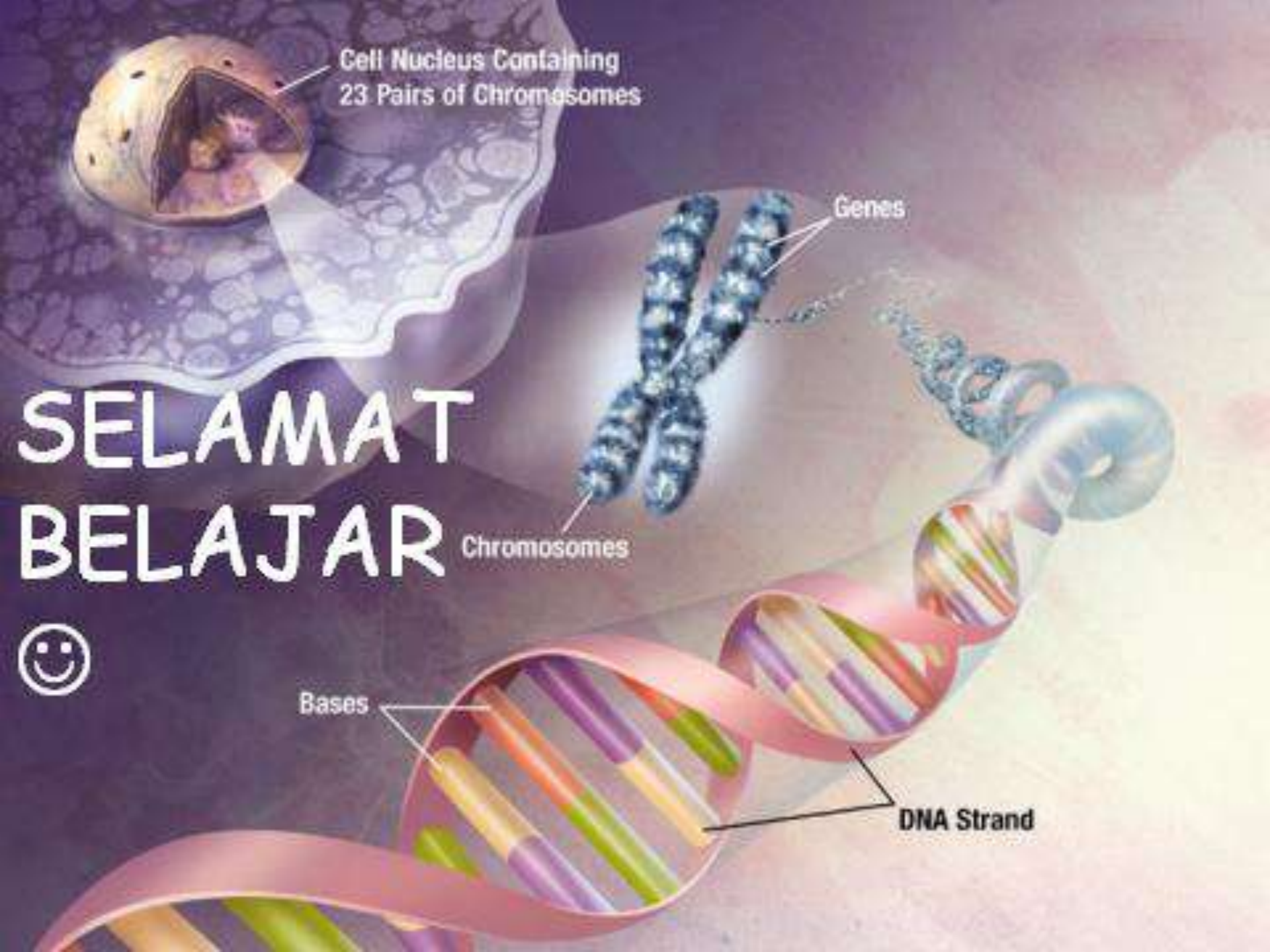


(a) Structural formula (b) Space-filling model

- Beberapa fungsi fosfolipid antara lain adalah:
- Lesitin membawa lemak dalam aliran darah dari satu jaringan ke jaringan lainya;
- Fosfatidiletanolamin berperan dalam proses pembekuan darah;
- Fosfolipid merupakan komponen utama dinding sel → **phospholipid** bilayer

Cell wall




Cell Nucleus Containing
23 Pairs of Chromosomes
Genes
SELAMAT
BELAJAR
Chromosomes
Bases
DNA Strand

SINTESIS ASAM NUKLEAT

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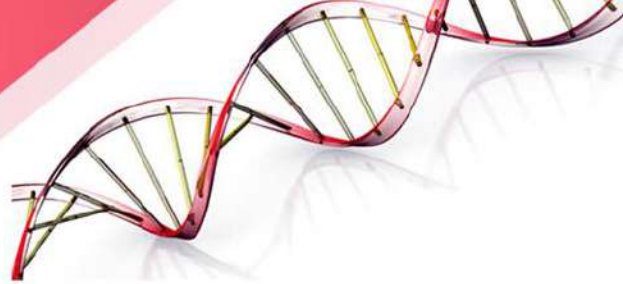
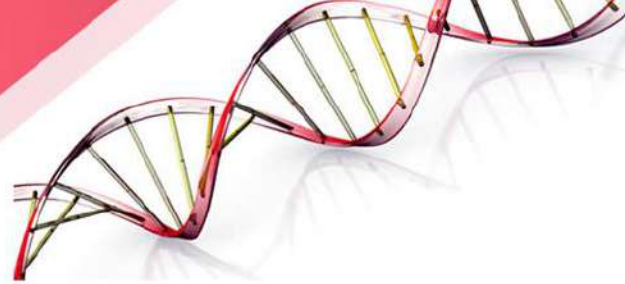
UNIVERSITAS AHMAD DAHLAN

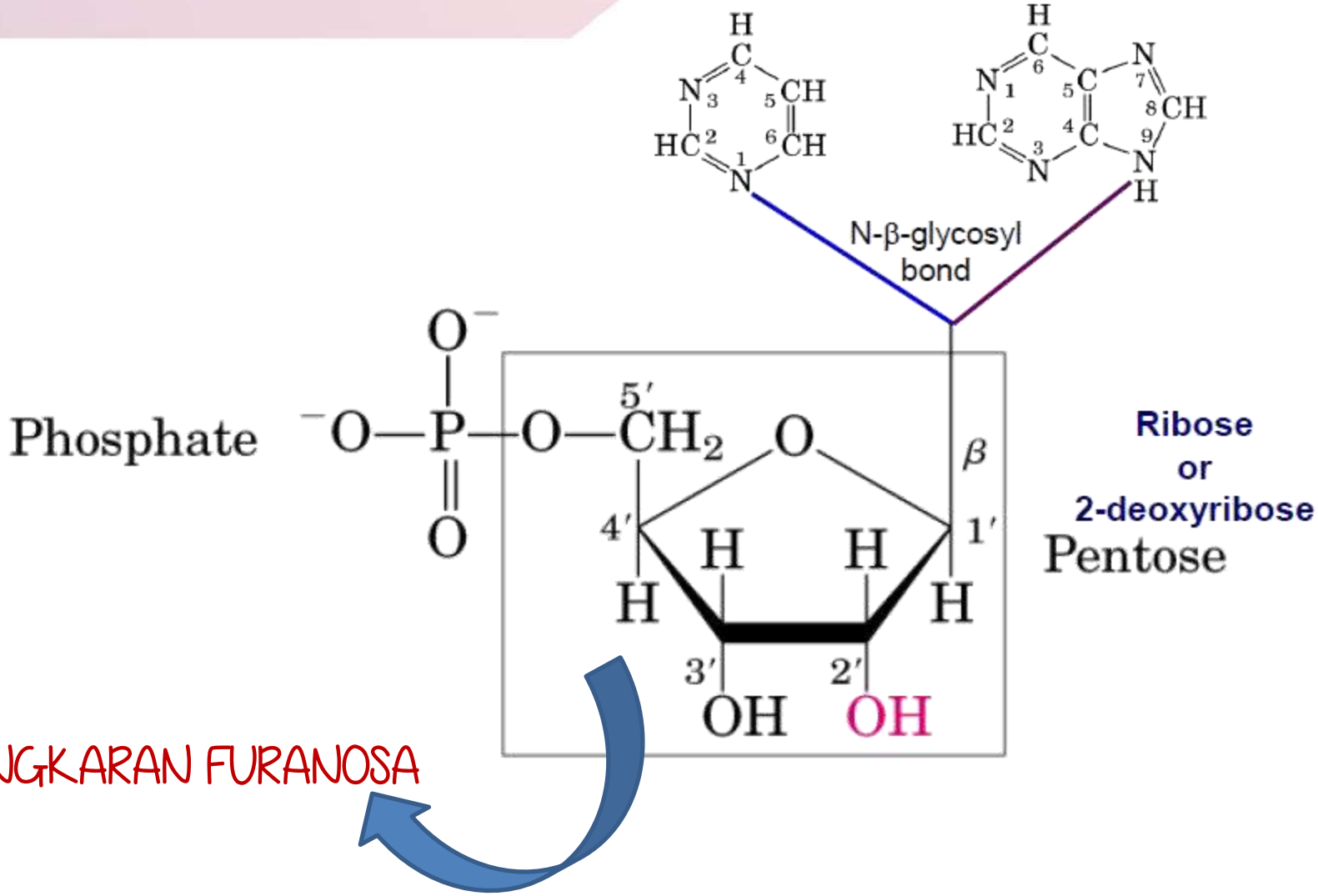
PENGERTIAN



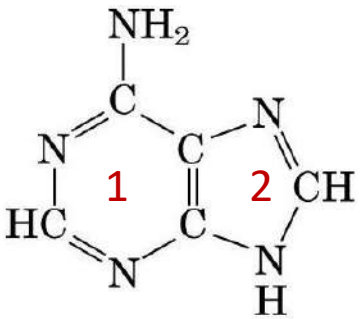
- Asam nukleat → senyawa kimia polimer nukleotida yang terdapat di dalam inti sel (Nukleus),
- Asam nukleat → biopolymer berbobot molekul tinggi → unit monomernya mononukleotida.
- Asam nukleat → terdapat pada semua sel hidup dan bertugas untuk menyimpan dan mentransfer genetic, kemudian menerjemahkan informasi ini secara tepat untuk mensintesis protein yang khas bagi masing- masing sel.
- berperan dalam penyimpanan & pemindahan informasi genetik yang berhubungan dengan pewarisan sifat turunan.

STRUKTUR & NOMENKLATUR

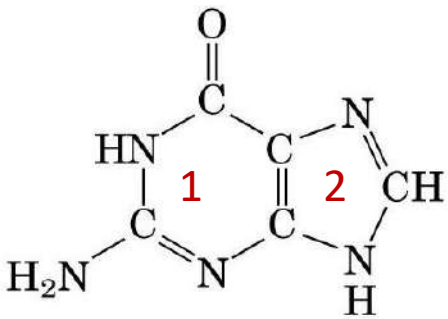




STRUKTUR & NOMENKLATUR

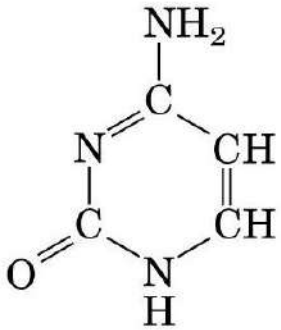


Adenine

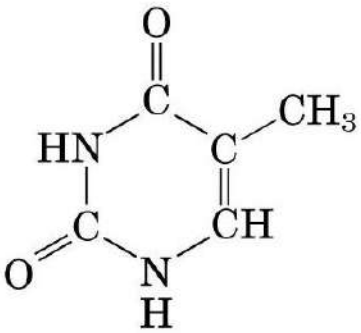


Guanine

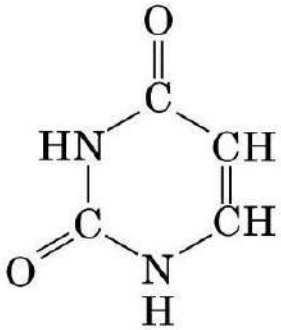
Purines



Cytosine



Thymine
(DNA)



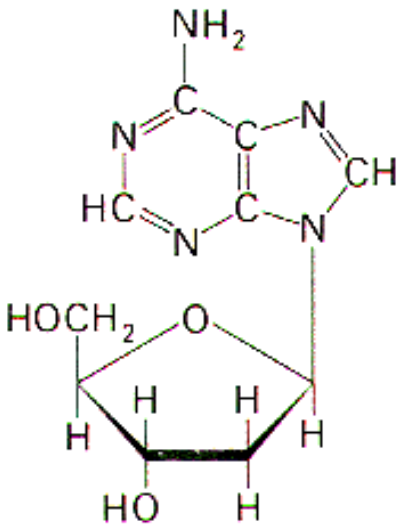
Uracil
(RNA)

Pyrimidines

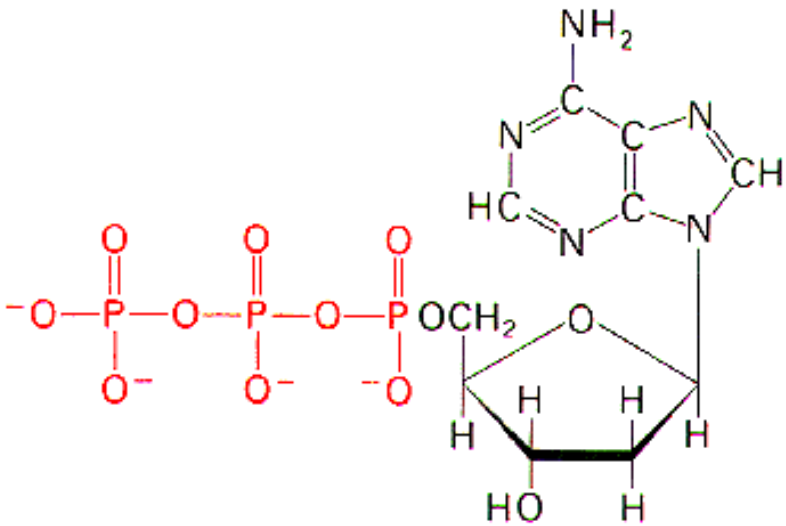
1. Lingkar pirimidina

2. Lingkar imidazole

- NUKLEOSIDA vs NUKELOTIDA?



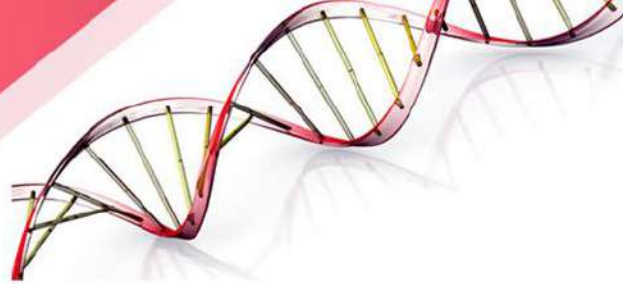
Deoxyadenosine
(A nucleoside)



Deoxyadenosine 5′-triphosphate
(dATP)
(A nucleotide)

Nucleoside = Nitrogenous base – ribose
Nucleotide = Nitrogenous base – ribose – phosphate

SIFAT-SIFAT ASAM NUKLEAT



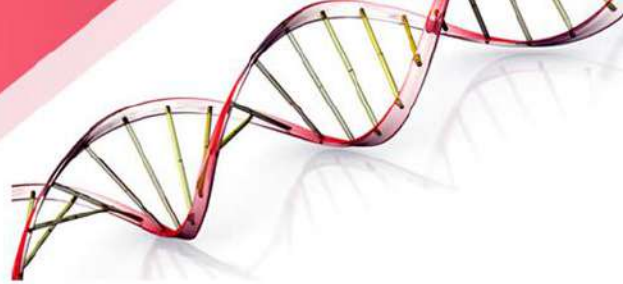
- Stabilitas asam nukleat

➢ Ketika melihat struktur tangga berpilin molekul DNA atau struktur sekunder RNA, sepintas akan terlihat bahwa struktur tersebut menjadi stabil karena adanya ikatan hidrogen.

➢ PADAHAL penentu stabilitas struktur asam nukleat terletak pada interaksi penempatan *(stacking interactions)* antara pasangan-pasangan basa.

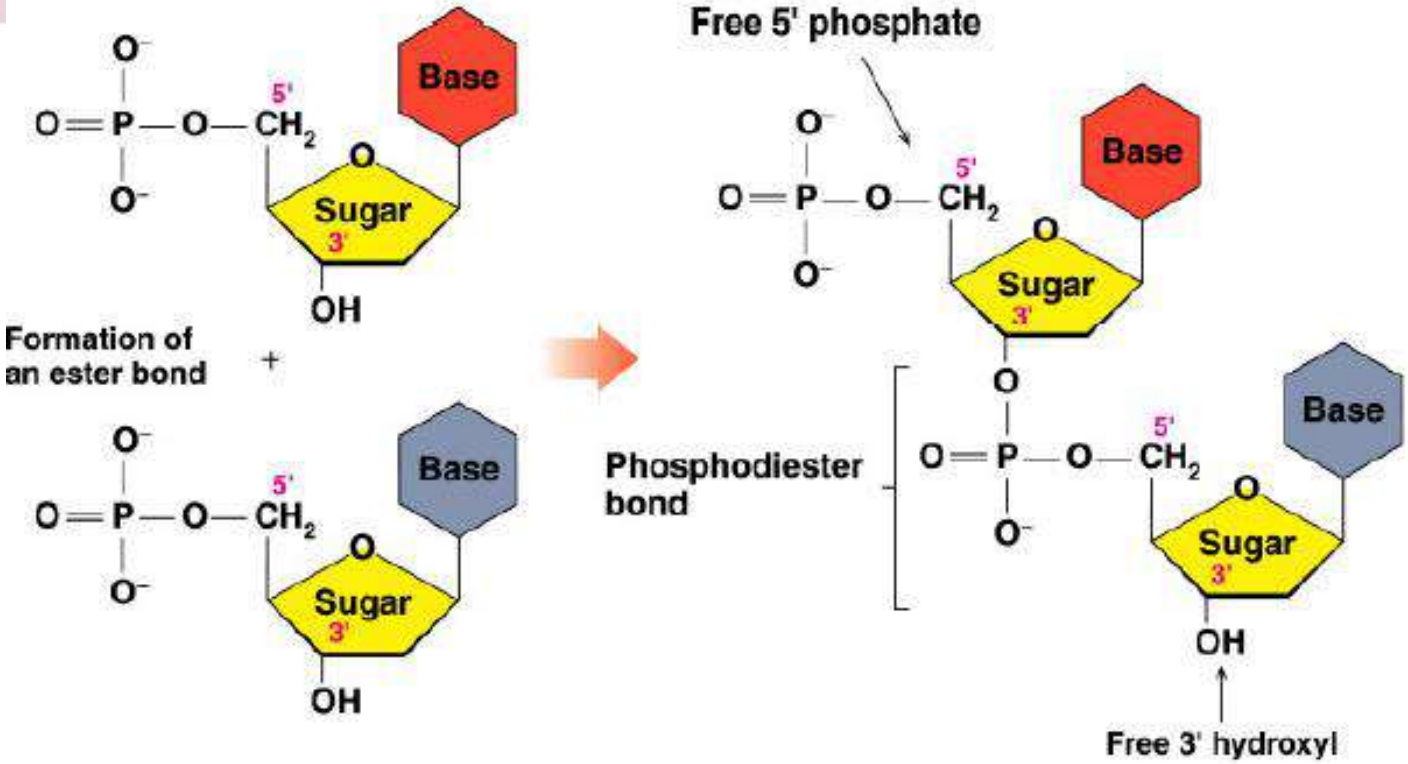
➢ Permukaan basa yang bersifat hidrofobik menyebabkan molekul-molekul air dikeluarkan dari sela-sela perpasangan basa sehingga perpasangan tersebut menjadi kuat.

SIFAT-SIFAT ASAM NUKLEAT



- Pengaruh asam
- Di dalam asam pekat dan suhu tinggi, misalnya $HClO_4$ dengan suhu lebih dari 100ºC, asam nukleat akan mengalami hidrolisis sempurna menjadi komponen-komponennya. Namun, di dalam asam mineral yang lebih encer, hanya ikatan glikosidik antara gula dan basa purin saja yang putus sehingga asam nukleat dikatakan bersifat apurinik.

Primary Structure of Nucleic Acids

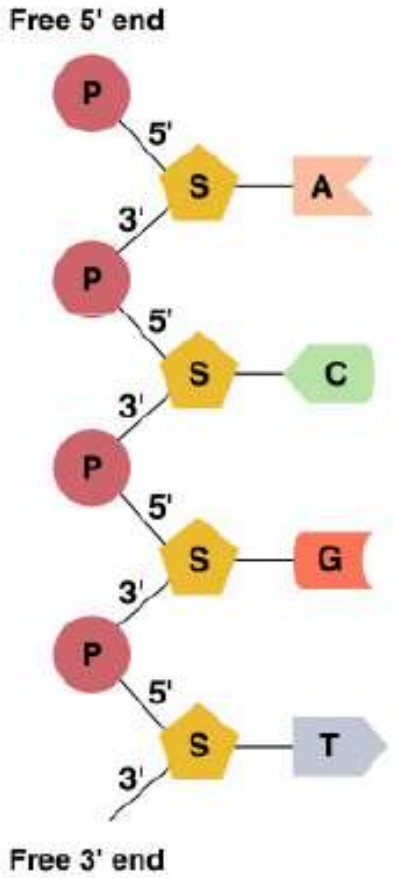


Structure of Nucleic Acids

A nucleic acid polymer

- Has a free 5'-phosphate group at one end and a free 3'-OH group at the other end.
- Is read from the free 5'-end using the letters of the bases.
- This section is read as:

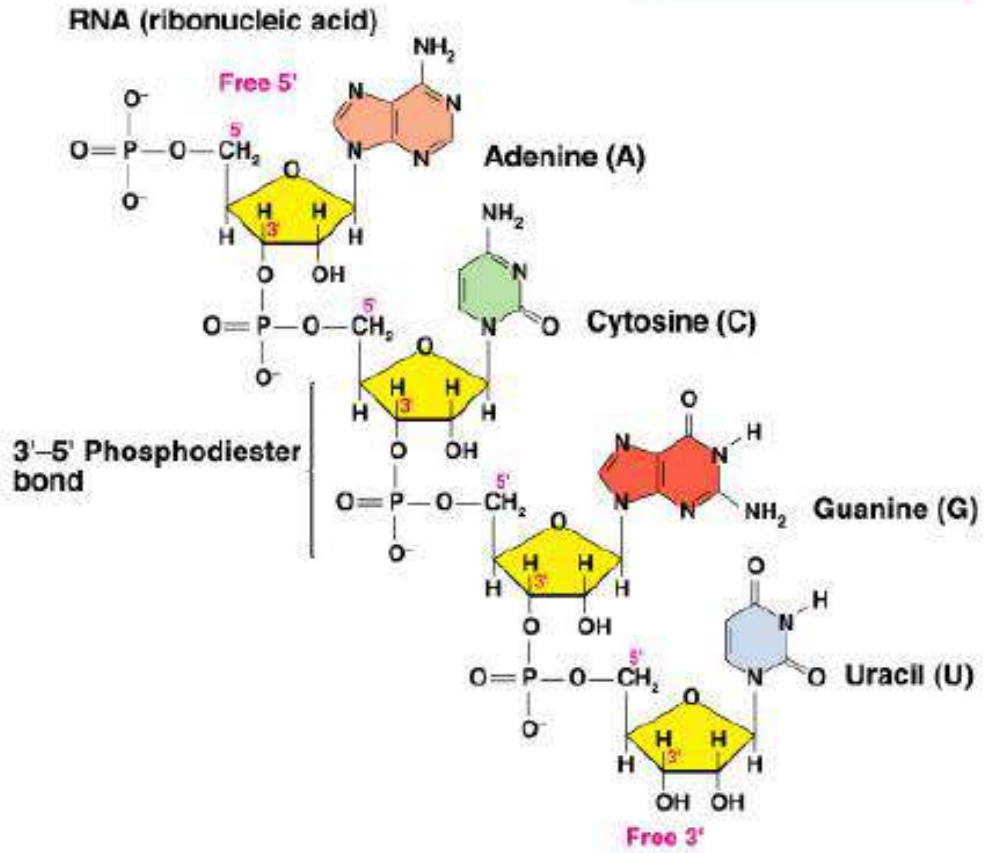
  5'—A—C—G—T—3'.



Example of RNA

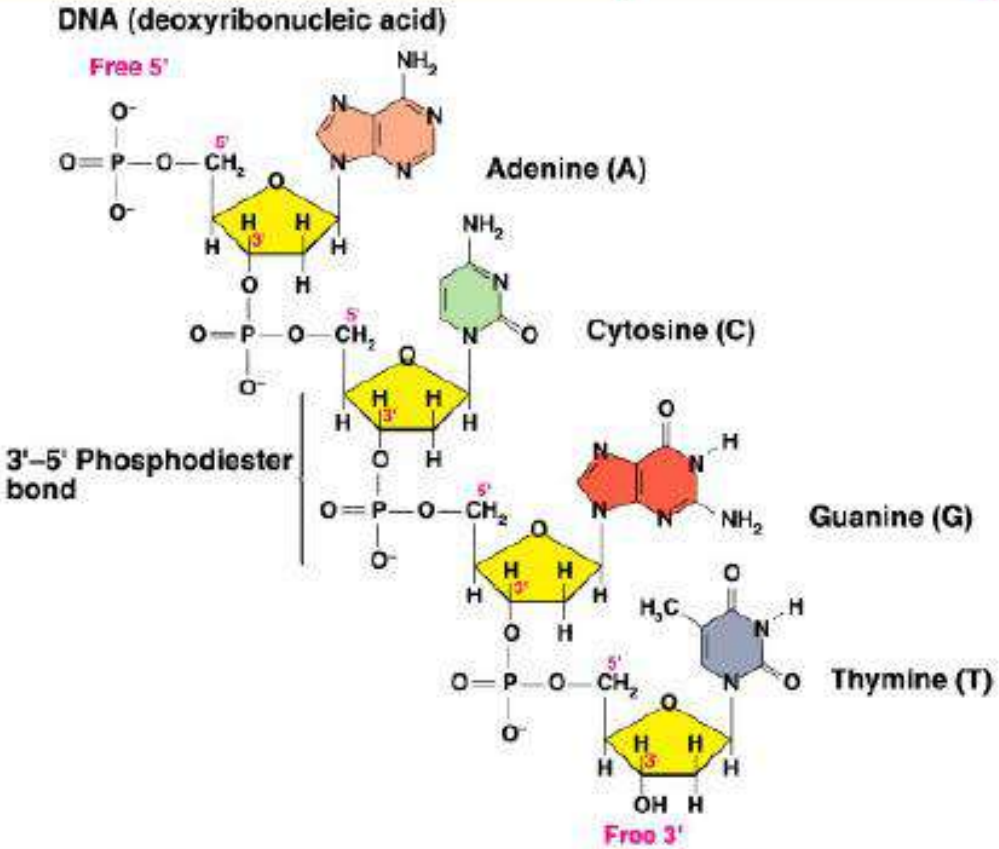
The primary structure of RNA

- Is a single strand of nucleotides.
- Consists of the bases A, C, G, and U linked by 3'-5' ester bonds between ribose and phosphate.

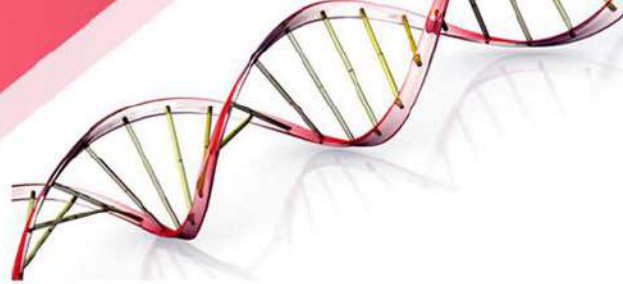


Example of DNA

In the primary structure of DNA, A, C, G, and T are linked by 3'-5' ester bonds between deoxyribose and phosphate.



SIFAT-SIFAT ASAM NUKLEAT

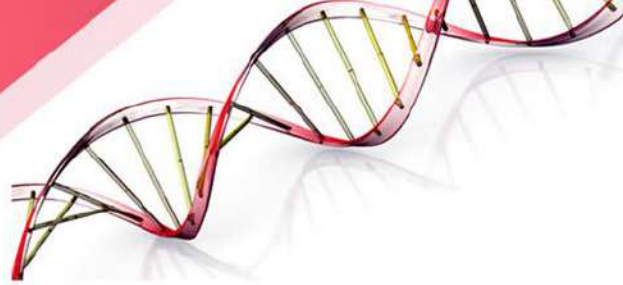


- Pengaruh alkali

➢ Peningkatan pH akan menyebabkan perubahan struktur guanin dari bentuk keto menjadi bentuk enolat karena molekul tersebut kehilangan sebuah proton → menyebabkan terputusnya sejumlah ikatan hidrogen sehingga pada akhirnya rantai ganda DNA mengalami denaturasi.

➢ Hal yang sama terjadi pula pada RNA. Bahkan pada pH netral sekalipun, RNA jauh lebih rentan terhadap hidrolisis bila dibadingkan dengan DNA karena adanya gugus OH pada atom C nomor 2 di dalam gula ribosanya.

SIFAT-SIFAT ASAM NUKLEAT



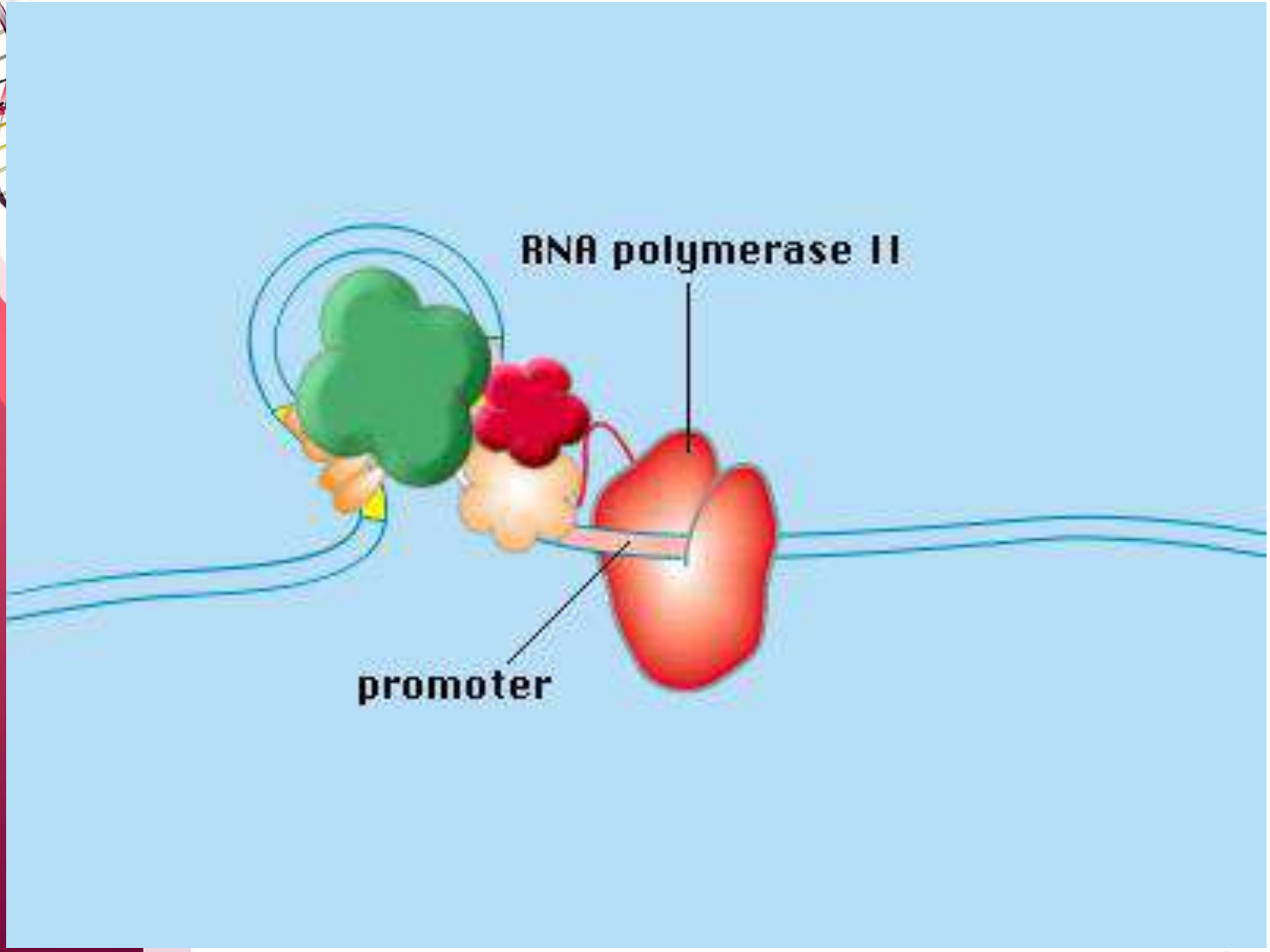
- Denaturasi kimia

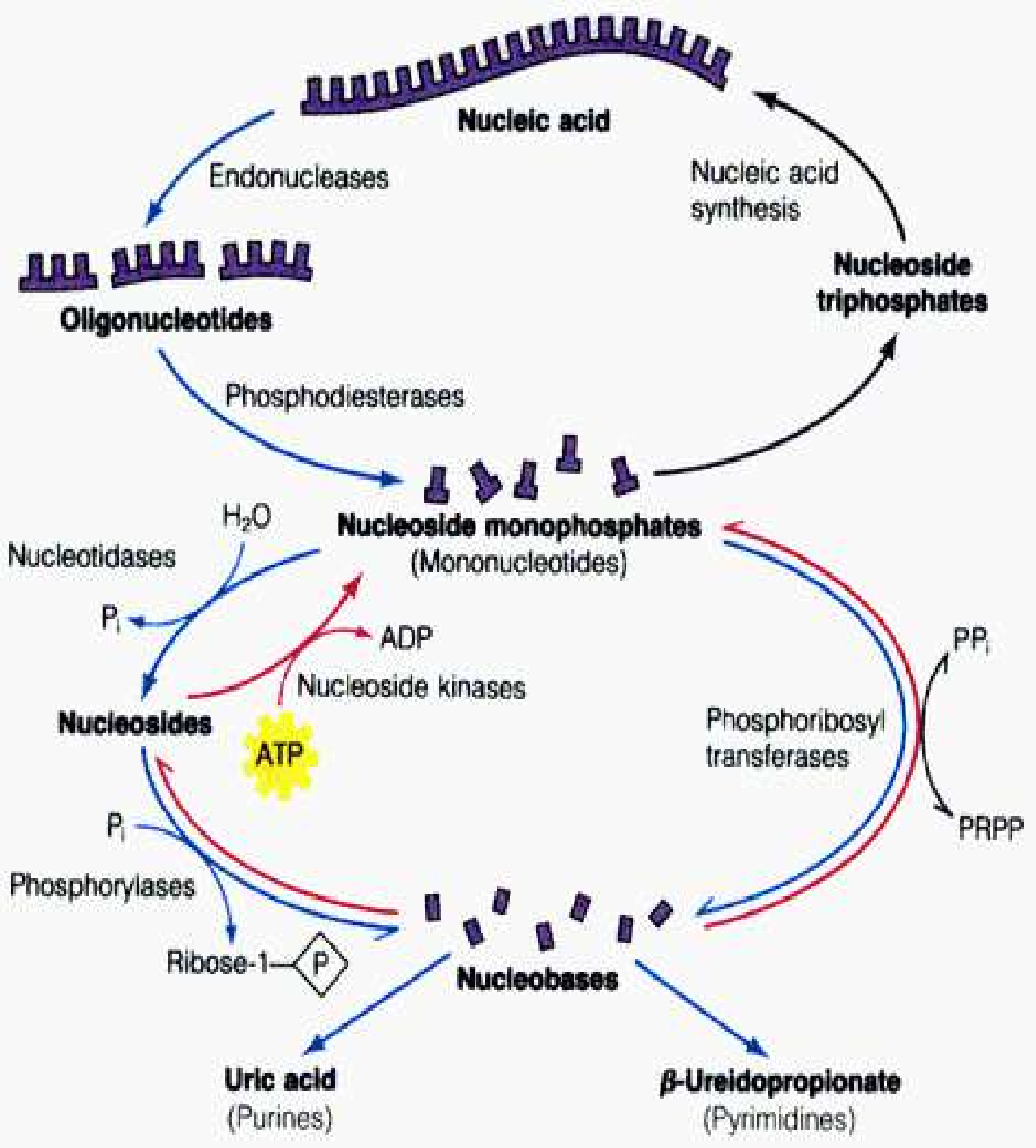
➢ Sejumlah bahan kimia diketahui dapat menyebabkan denaturasi asam nukleat pada pH netral.

➢ Contoh yang paling dikenal adalah urea ($CO(NH_2)_2$) dan formamid ($COHNH_2$). Pada konsentrasi yang relatif tinggi, senyawa-senyawa tersebut dapat merusak ikatan hidrogen. Artinya, stabilitas struktur sekunder asam nukleat menjadi berkurang dan rantai ganda mengalami denaturasi.

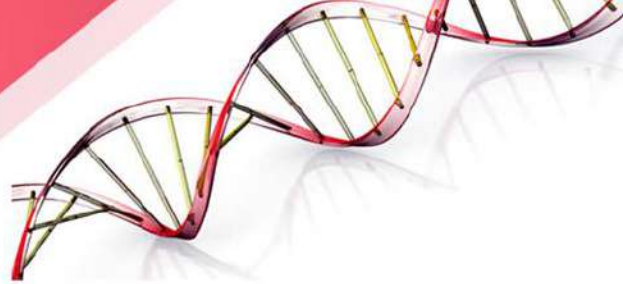
SINTESIS ASAM NUKLEAT

- SINTESIS PURIN via jalur de Novo dan SALVAGE
- SINTESIS PRIMIDIN via jalur de Novo dan SALVAGE
- TAKE A LOOK AT THIS FIRST




Nucleic acid
Endonucleases
Nucleic acid synthesis
Oligonucleotides
Nucleoside triphosphates
Phosphodiesterases
H_2O
Nucleotidases
Nucleoside monophosphates
(Mononucleotides)
P_i
ADP
Nucleoside kinases
PP_i
Nucleosides
ATP
Phosphoribosyl transferases
PRPP
P_i
Phosphorylases
Ribose-1—P
Nucleobases
Uric acid
(Purines)
β-Ureidopropionate
(Pyrimidines)

JALUR DE NOVO



- Sintesis nukleotida dimulai dengan prekursor metaboliknya:
  - asam amino
  - ribosa-5-fosfat,
  - CO_2,
  - unit satu karbon.

JALUR DE NOVO

Nukleus fosfat penyusun purin dan pirimidin berasal dari 5-Phospho- D-ribosyl-1-pyrophosphate (PRPP).

PRPP berasal dari Ribosa 5 fosfat + ATP.

Ribosa 5 fosfat berasal dari *HMP shunt*.

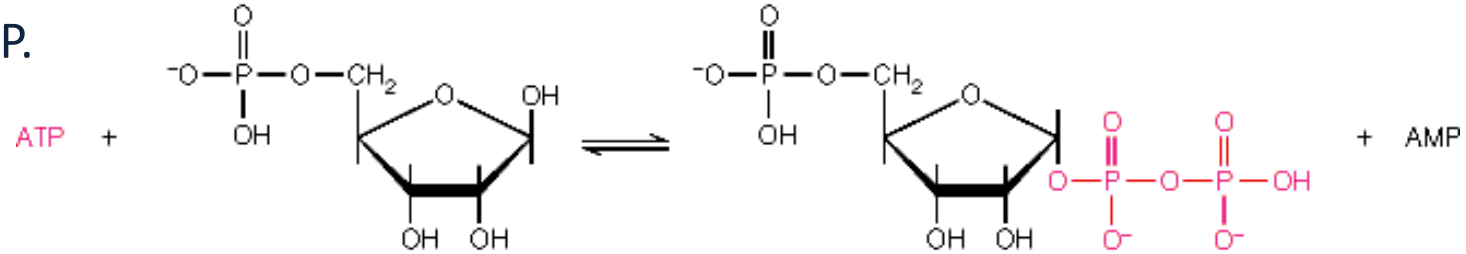
PRPP → fosfo ribosil 1 amin

Enzim amidofosforibosil transferase dengan bantuan glutamin sebagai pendonor NH_3 → melewati 10 rangkaian reaksi akan membentuk IMP.

IMP → adenilosuksinat dan xantilat.

Adenilosuksinat → AMP

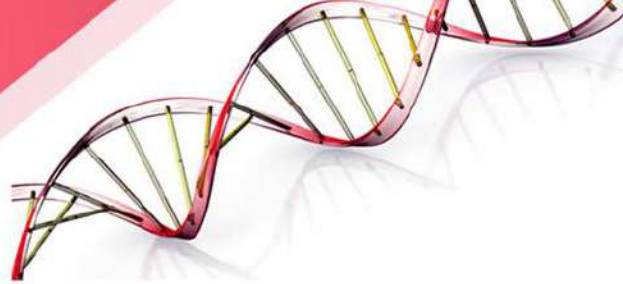
xantilat → GMP.



Ribose-5-phosphate

5-Phospho-α-D-ribosyl-1-pyrophosphate (PRPP)

JALUR SALVAGE

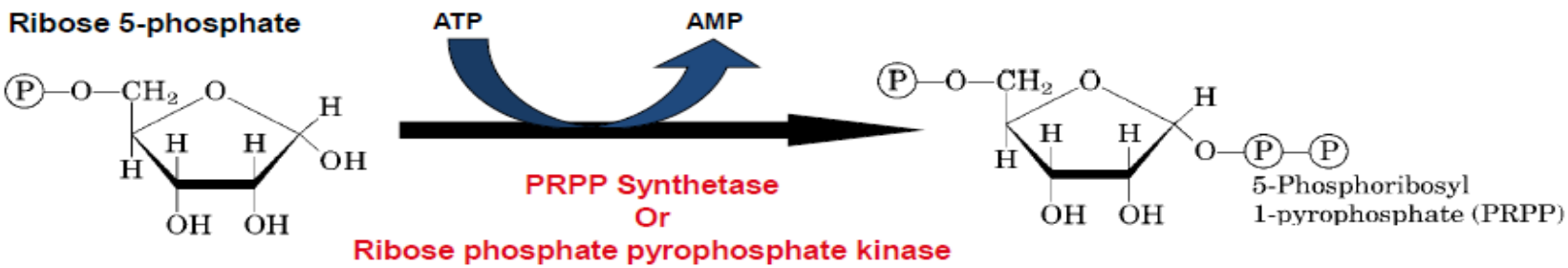


- Jalur Salvage: sintesis nukleotida dengan daur ulang dari basa bebas atau nukleosida yg dilepaskan dari pemecahan asam nukleat.
- Disini PRPP akan diubah menjadi purin-ribonukleotida.
- Contohnya

  Adenin + PRPP → adenilat + Ppi.

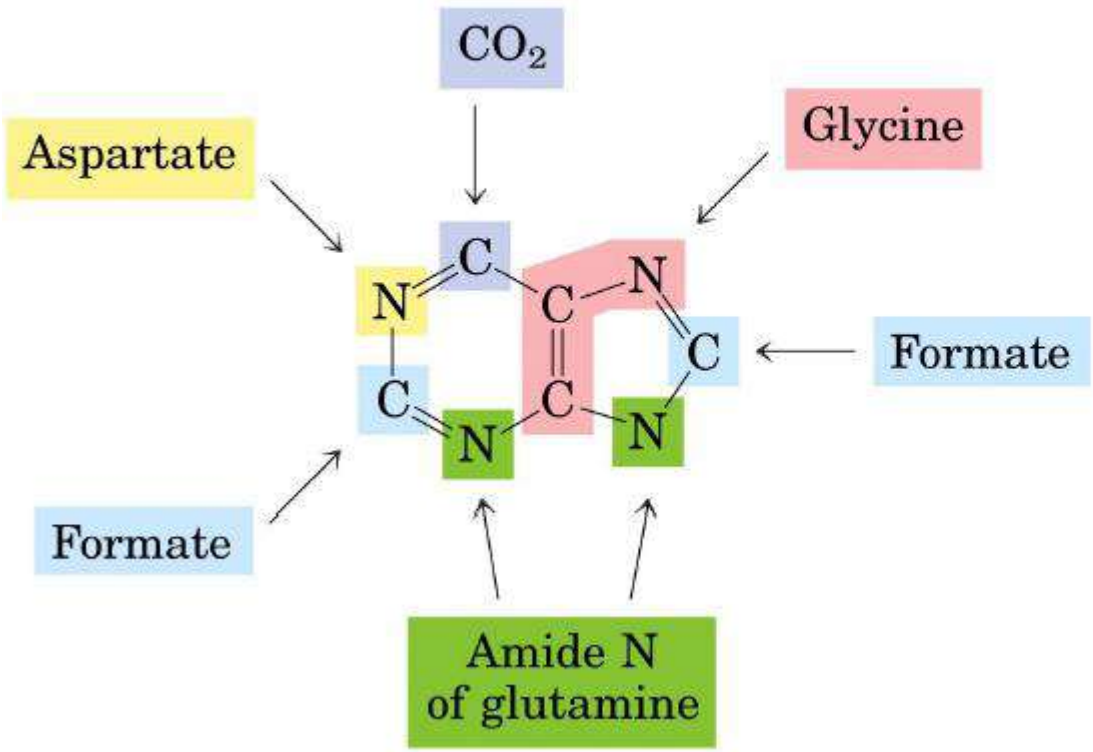
Purine Biosynthesis
in the beginning there was PRPP

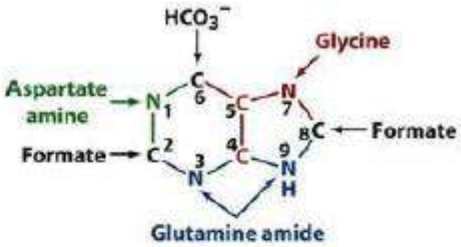
The use of ribose links the pentose phosphate pathway to nucleotide metabolism.



The purine ring is built upon ribose using PRPP

PREKURSOR

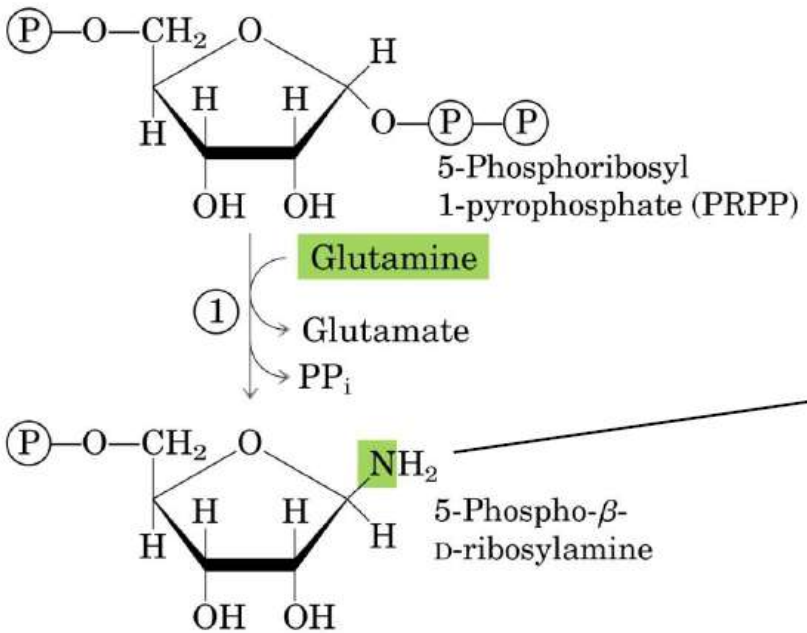


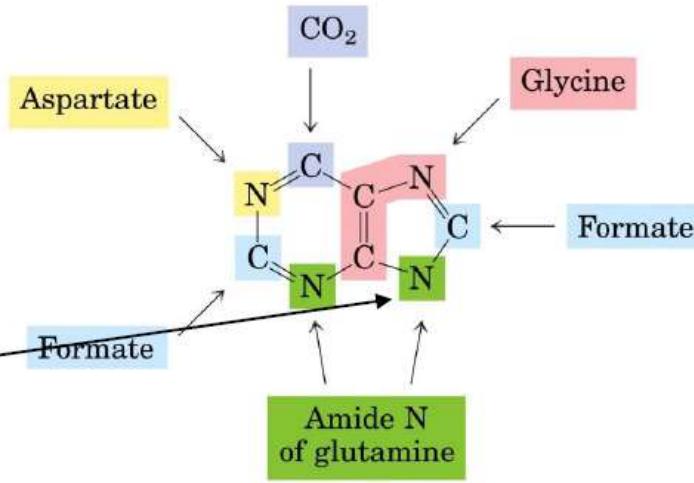


Isotopic labeling experiments defined the precursors.

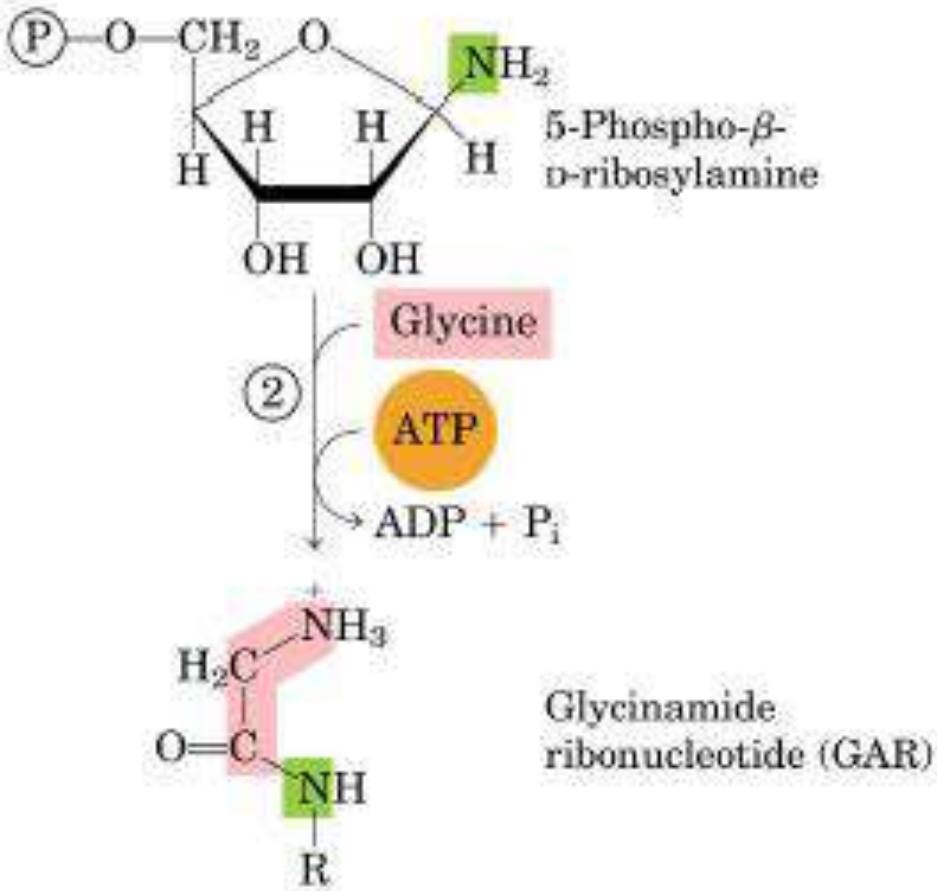
FIRST COMMITED STEP

① Glutamine-PRPP amidotransferase

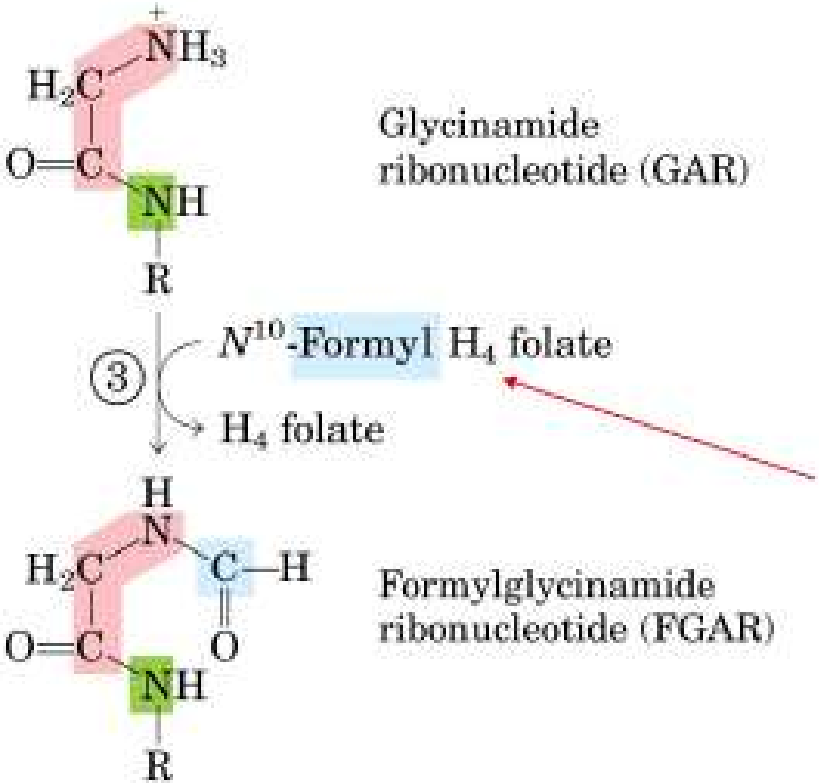


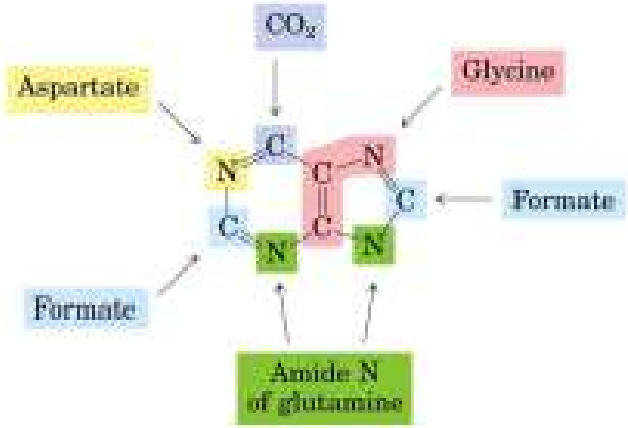


ADDITION OF GLYCINE

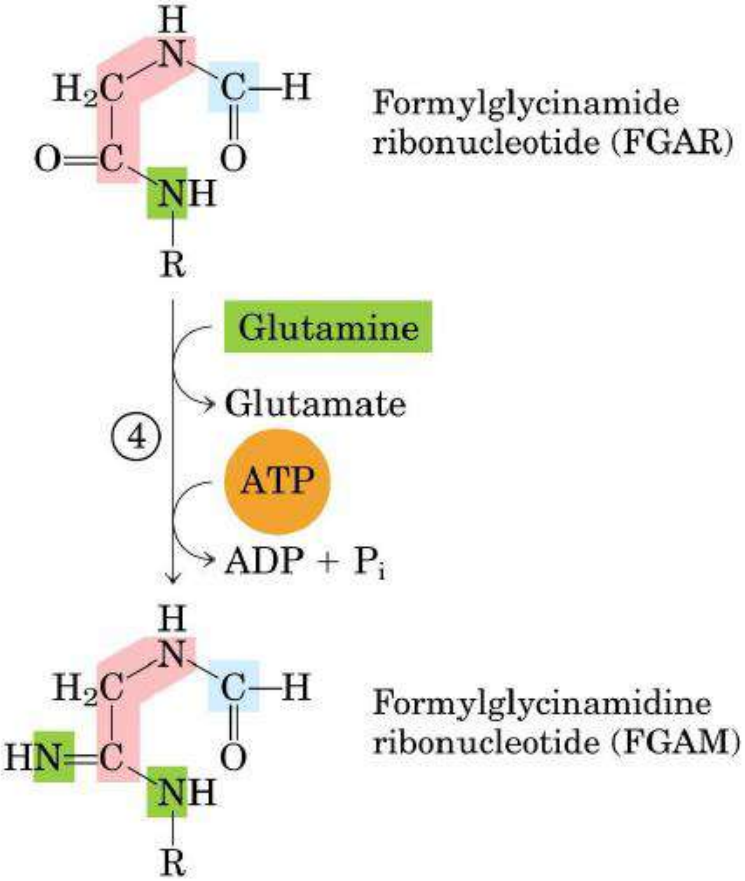


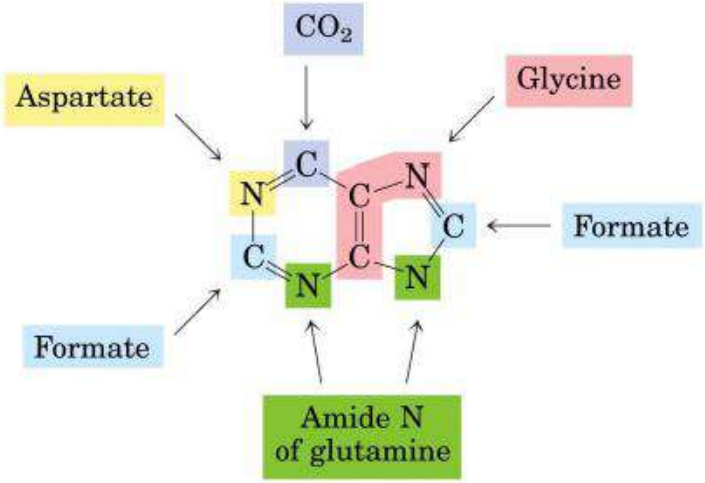
FORMYLATION



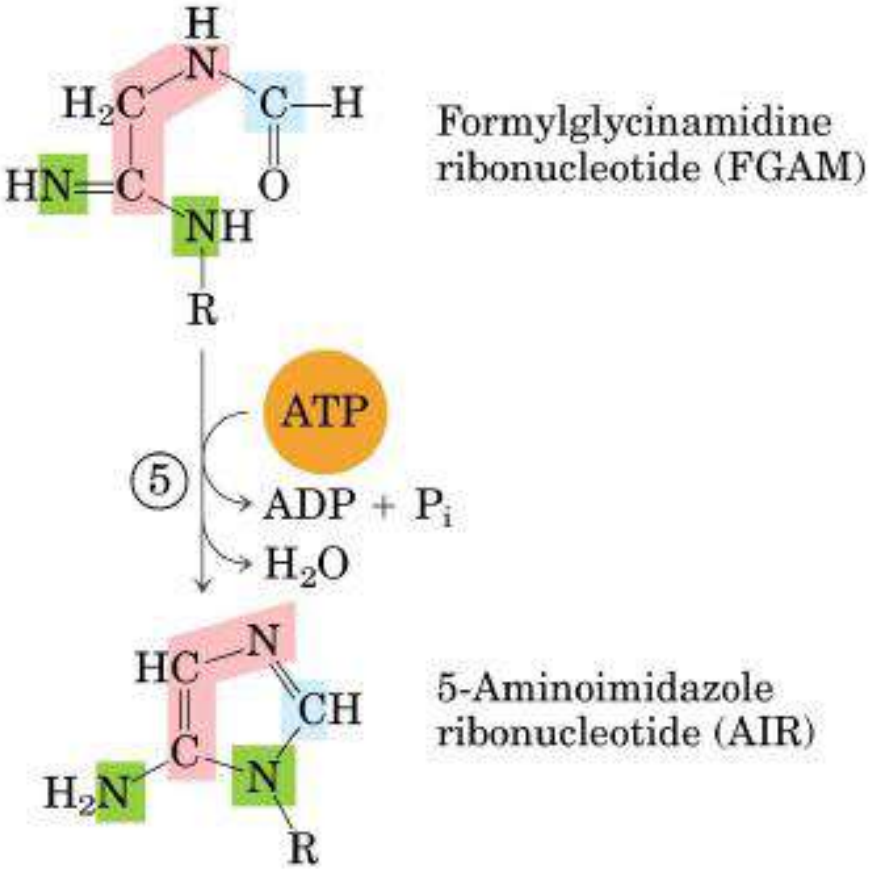


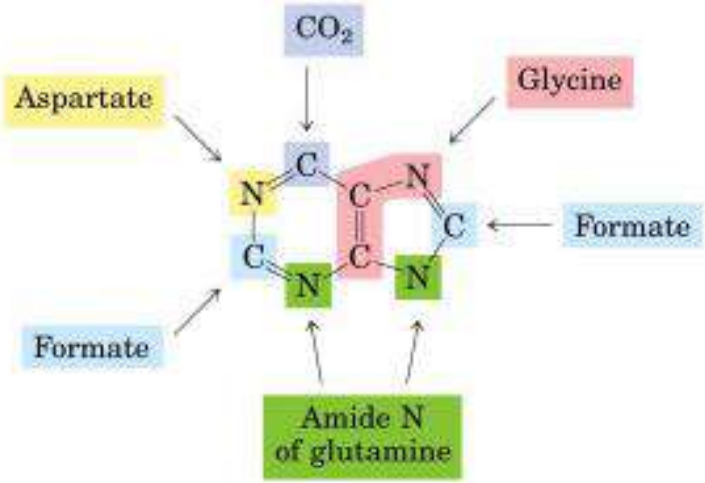
AMIDOTRANSFERASE



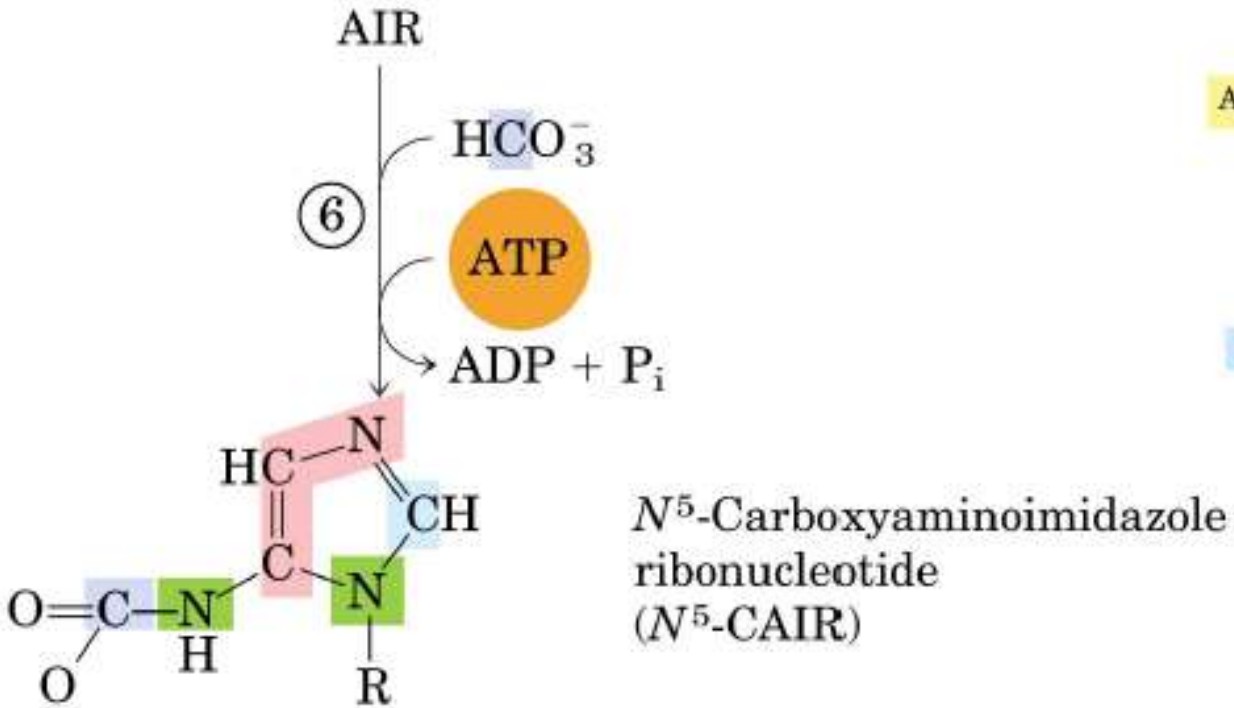


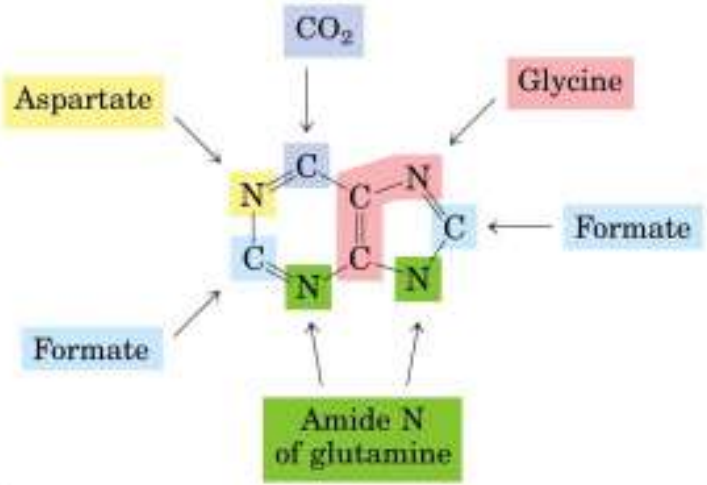
IMIDAZOLE RING CLOSURE





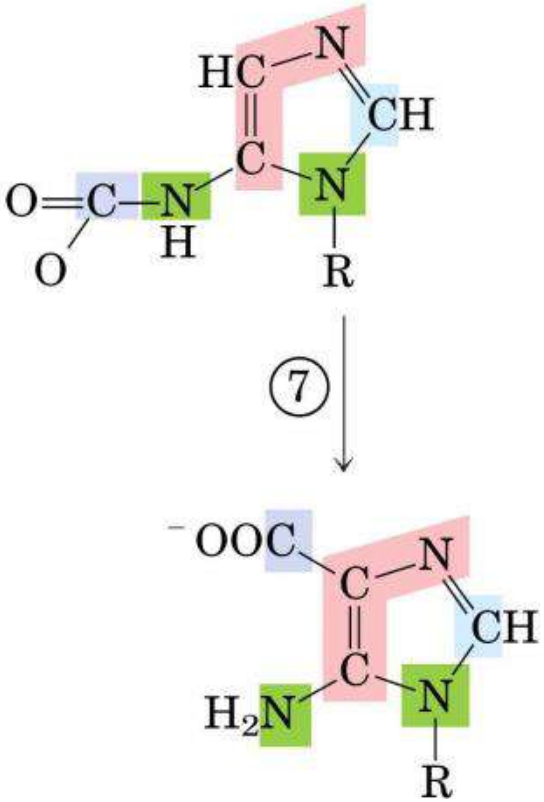
CARBOXYLATION





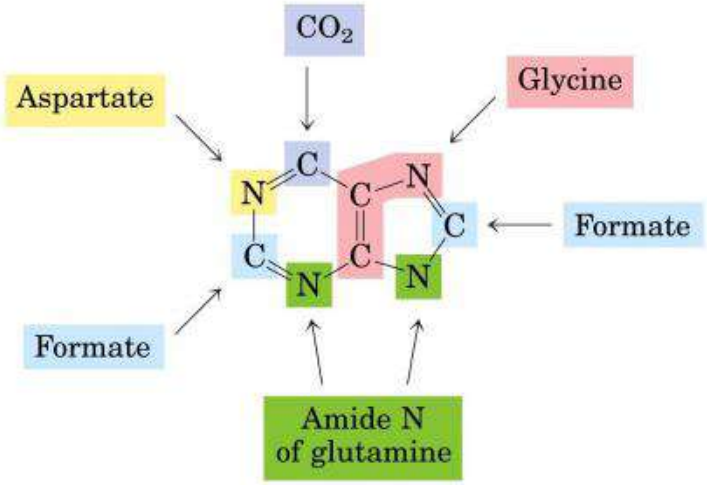
Carbon is the wrong position based on isotope labeling exp.

CARBON MOVING



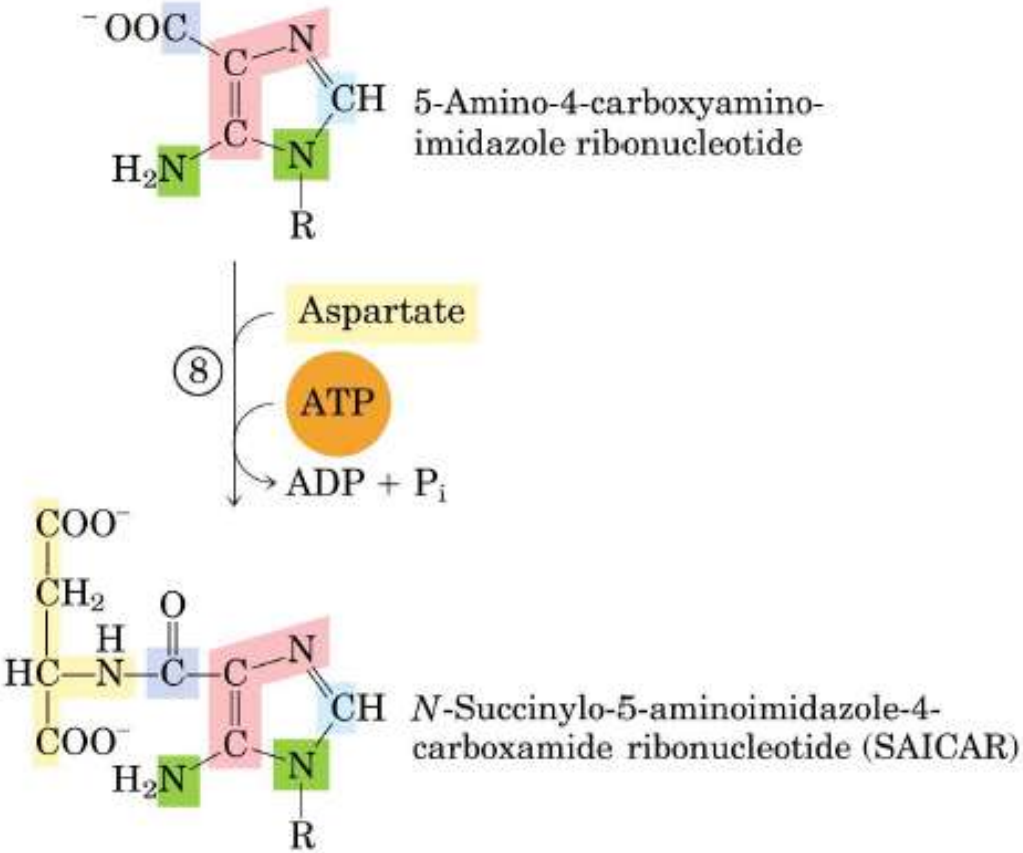
N^5-Carboxyaminoimidazole ribonucleotide (N^5-CAIR)

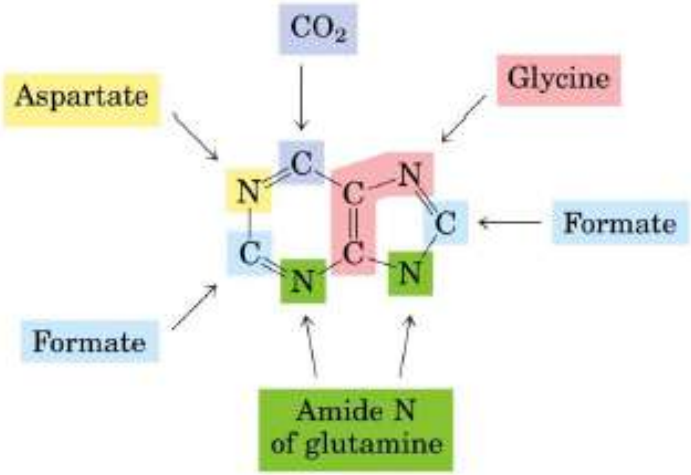
5-Amino-4-carboxyamino-imidazole ribonucleotide



Now the carbon is placed correctly.

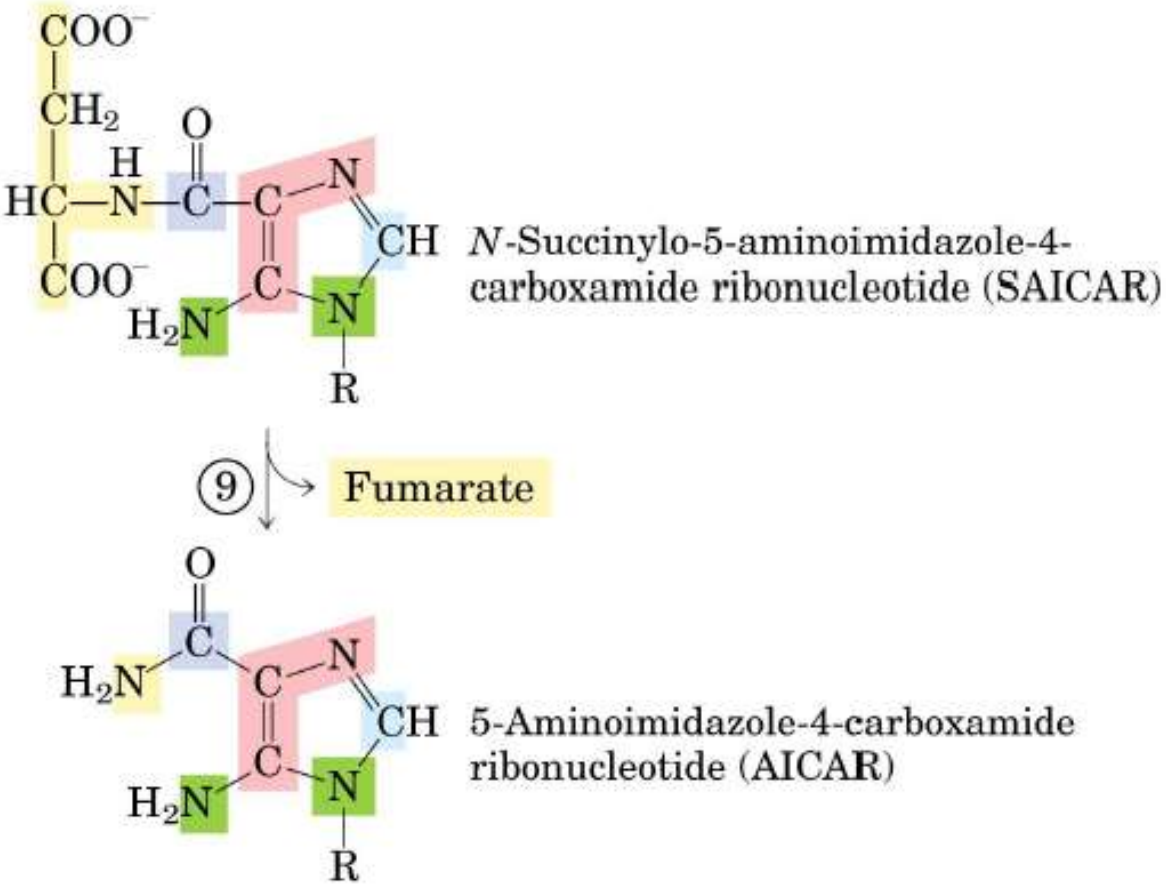
ASPARTATE ADDITION

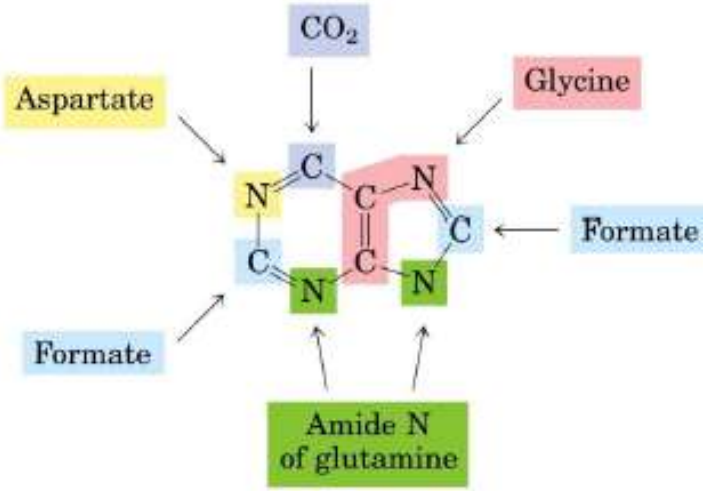




But all we need is the nitrogen.

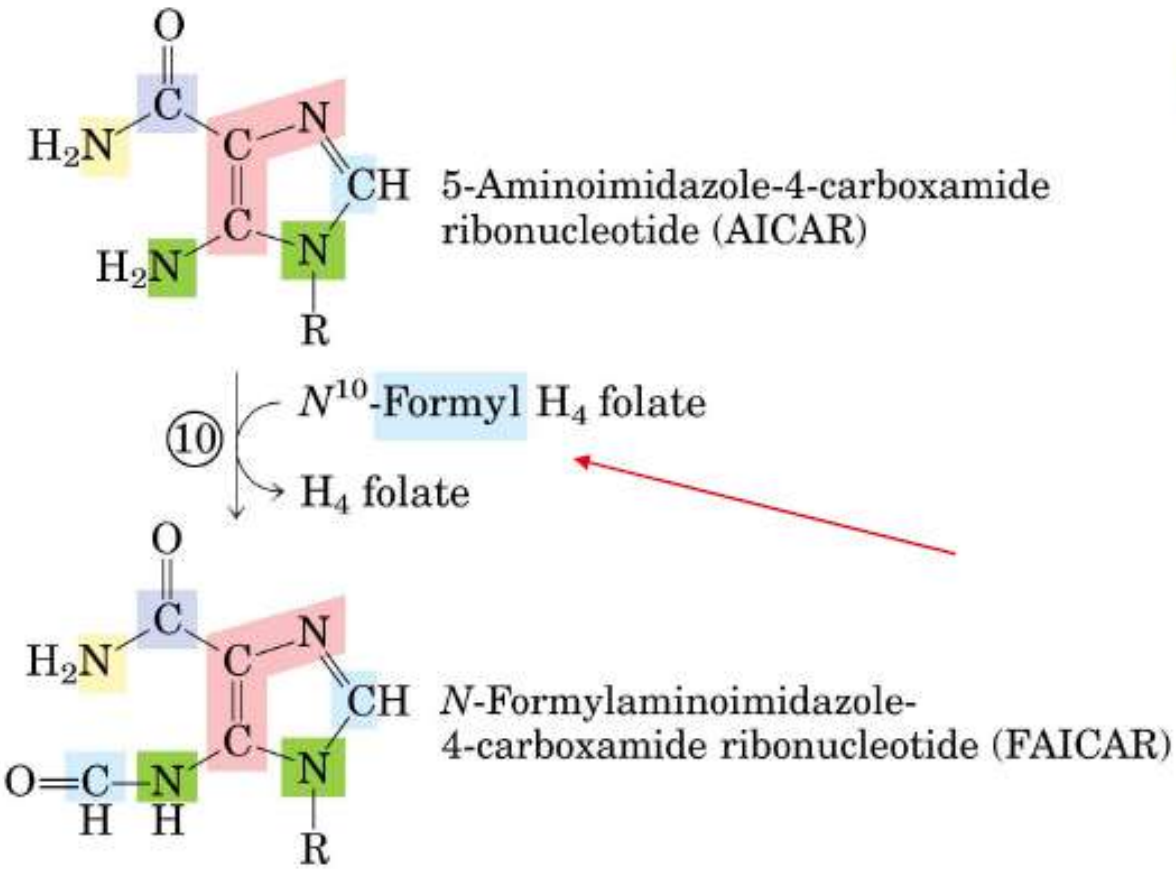
FUMARATE SUBSTRACTION

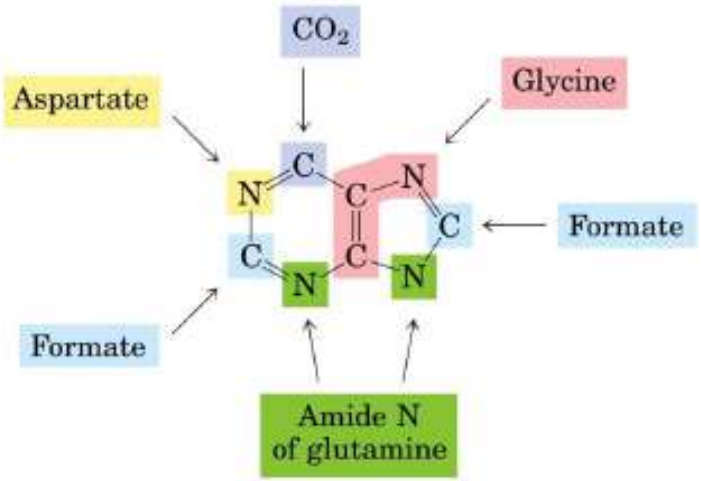




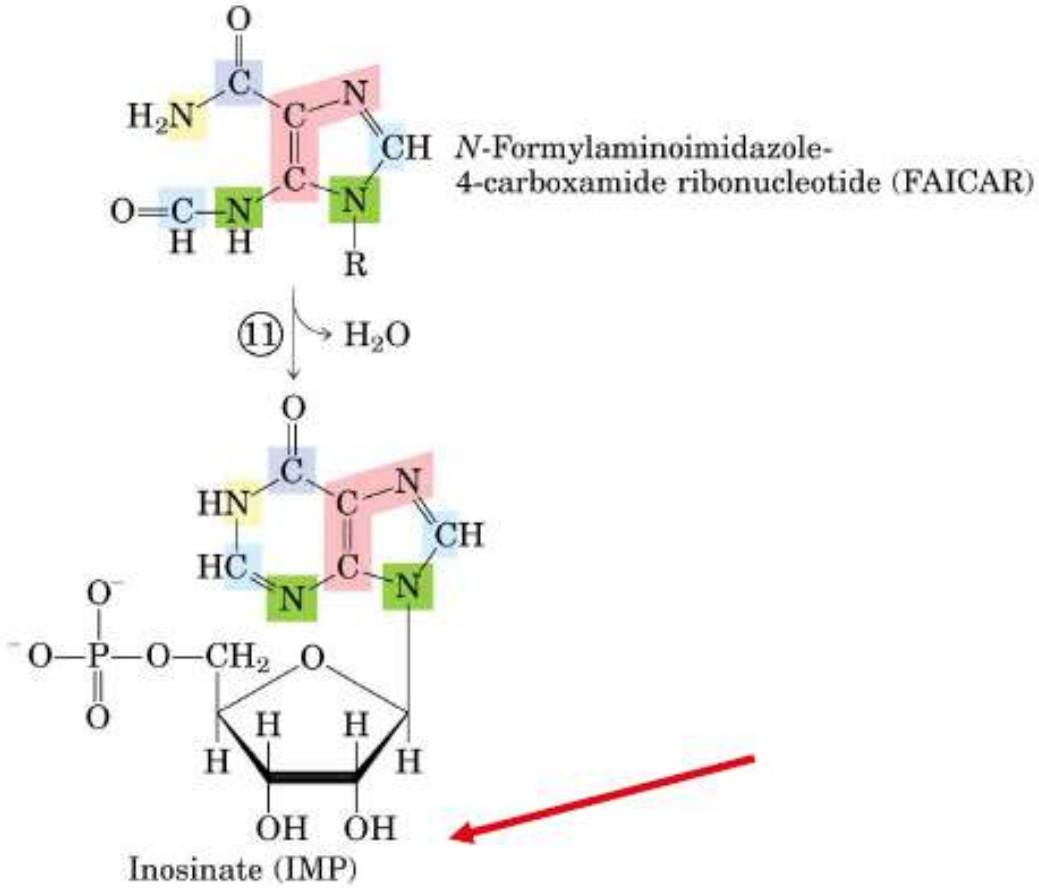
One more carbon.

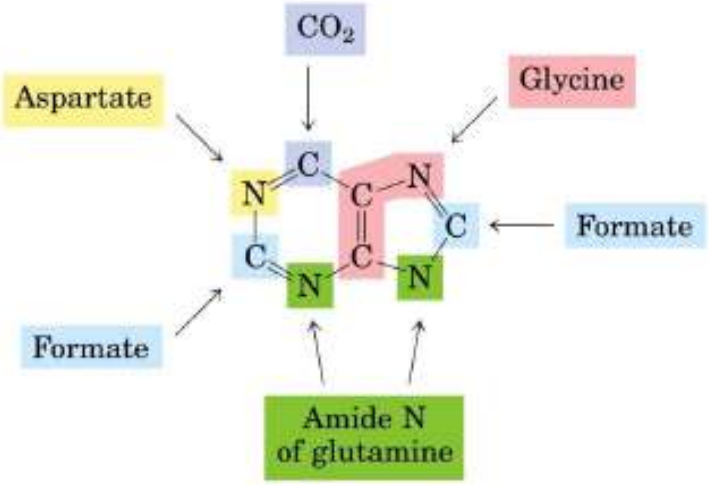
2ND FORMYLATION





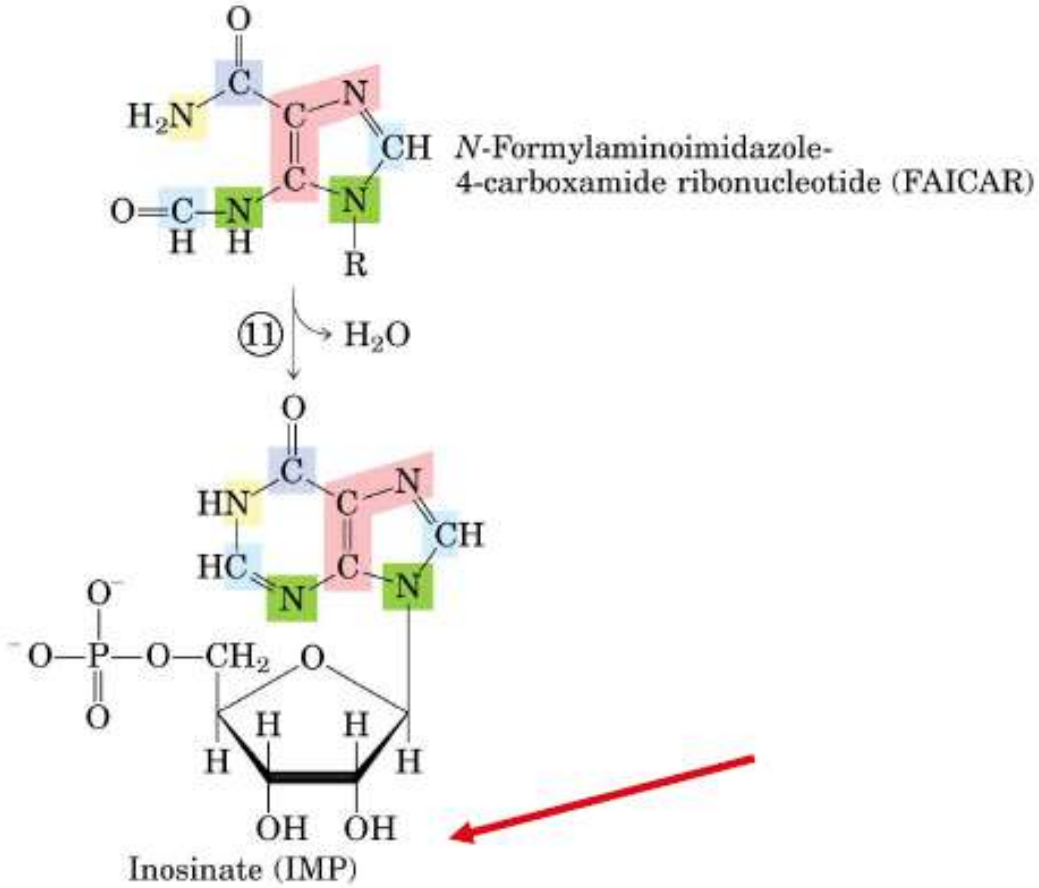
Finally… INOSINATE

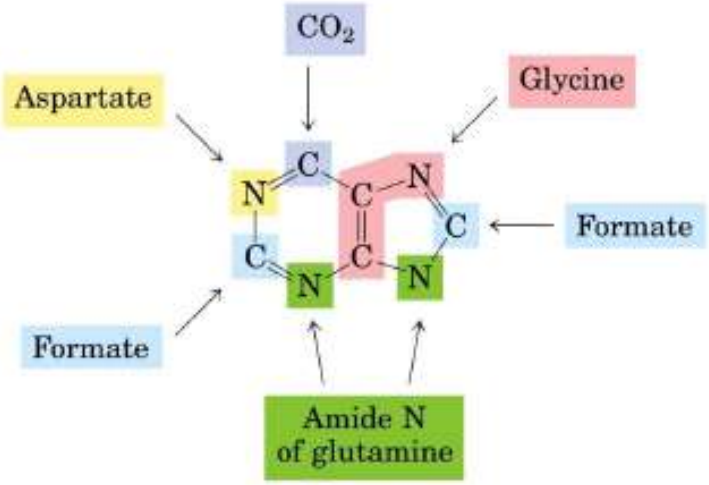




But who needs inosinate?

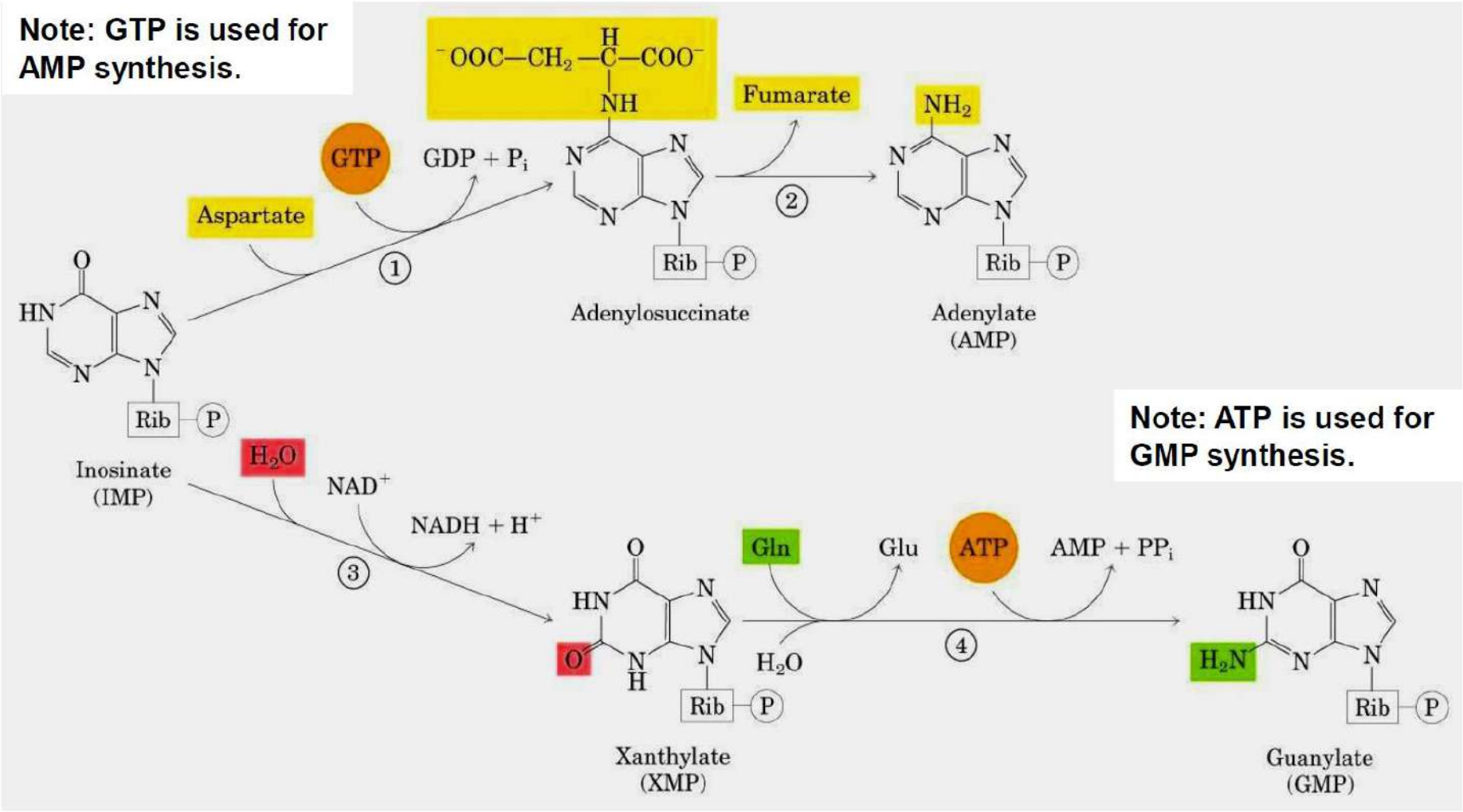
Finally… INOSINATE



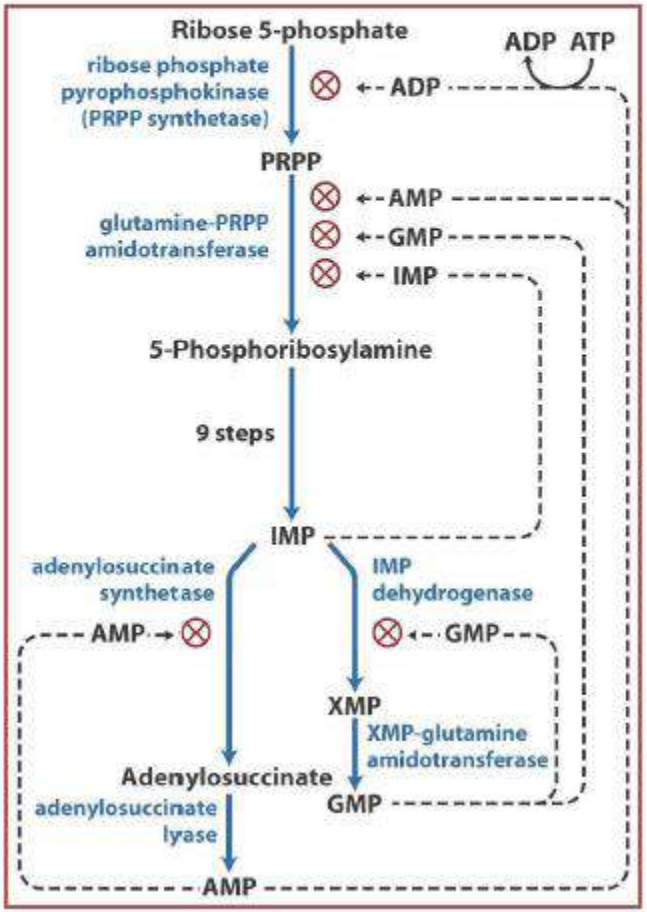


But who needs inosinate?

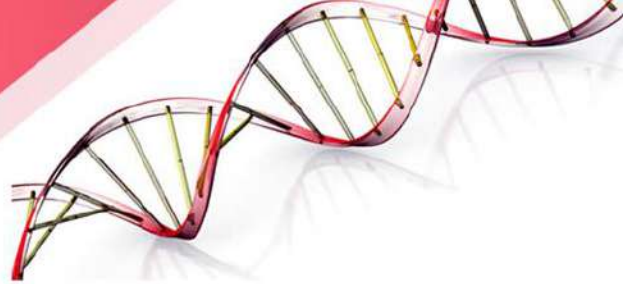
Conversion of IMP to AMP and GMP



Feedback Inhibitors of purine nucleotide biosynthesis



Regulatory control of purine biosynthesis



- GTP is involved in AMP synthesis and ATP is involved in GMP synthesis (reciprocal control of production)
- PRPP is a biosynthetically "central" molecule (why?)
  - ADP/GDP levels – negative feedback on Ribose Phosphate Pyrophosphokinase
  - Amidophosphoribosyl transferase is activated by PRPP levels
  - APRT activity has negative feedback at two sites
    - ATP, ADP, AMP bound at one site
    - GTP,GDP AND GMP bound at the other site
- Rate of AMP production increases with increasing concentrations of GTP; rate of GMP production increases with increasing concentrations of ATP

Purine Catabolism uric acid

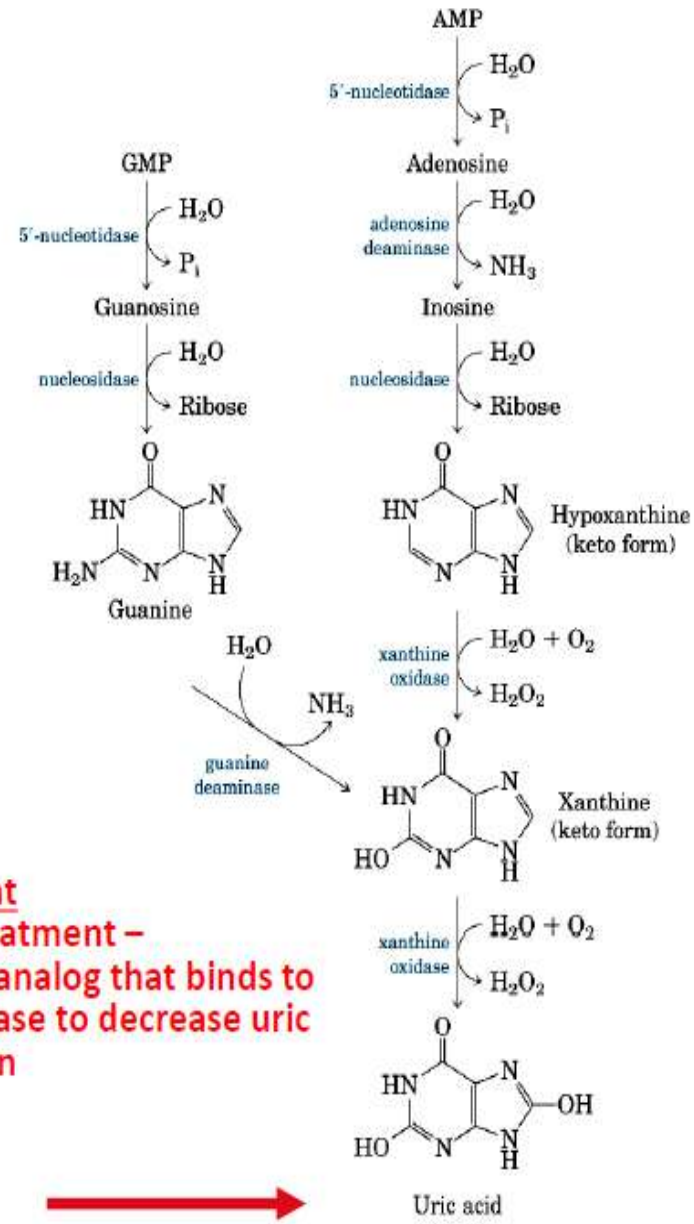
1. Remove phosphate.
   - -5'-nucleotidase

   1a. Deaminate adenosine
   - -Adenosine deaminase

2. Remove the ribose.
   - -nucleosidase

3. Generate xanthine.
   - -oxidize hypoxanthine
   - -deaminate guanine

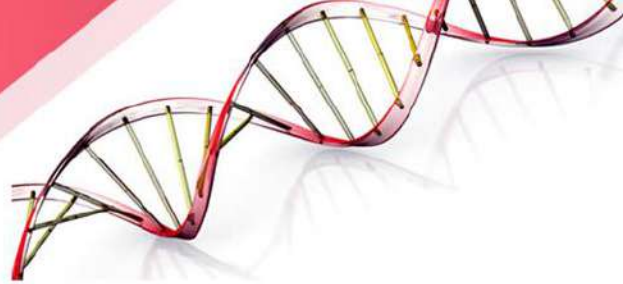
4. Make uric acid.
   - -xanthine oxidase



Gout treatment

Allopurinol treatment – hypoxanthine analog that binds to Xanthine Oxidase to decrease uric acid production

PURINE SALVAGE



- Adenine phosphoribosyl transferase (APRT)

  Adenine + PRPP → AMP + PP_i

- Hypoxanthine-Guanine phosphoribosyl transferase (HGPRT)

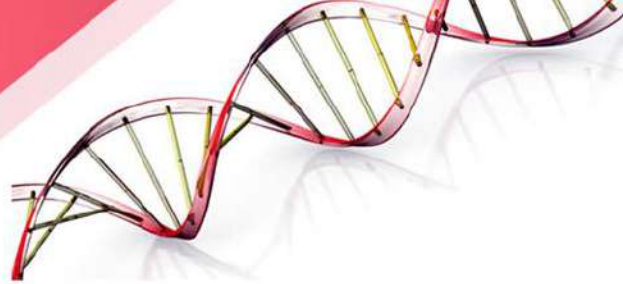
  Hypoxanthine + PRPP → IMP + PP_i

  Guanine + PRPP → GMP + PP_i

(NOTE: THESE ARE ALL <u>REVERSIBLE</u> REACTIONS)

AMP,IMP,GMP do not need to be resynthesized *de novo !*

PURINE CATABOLISM & SALVAGE



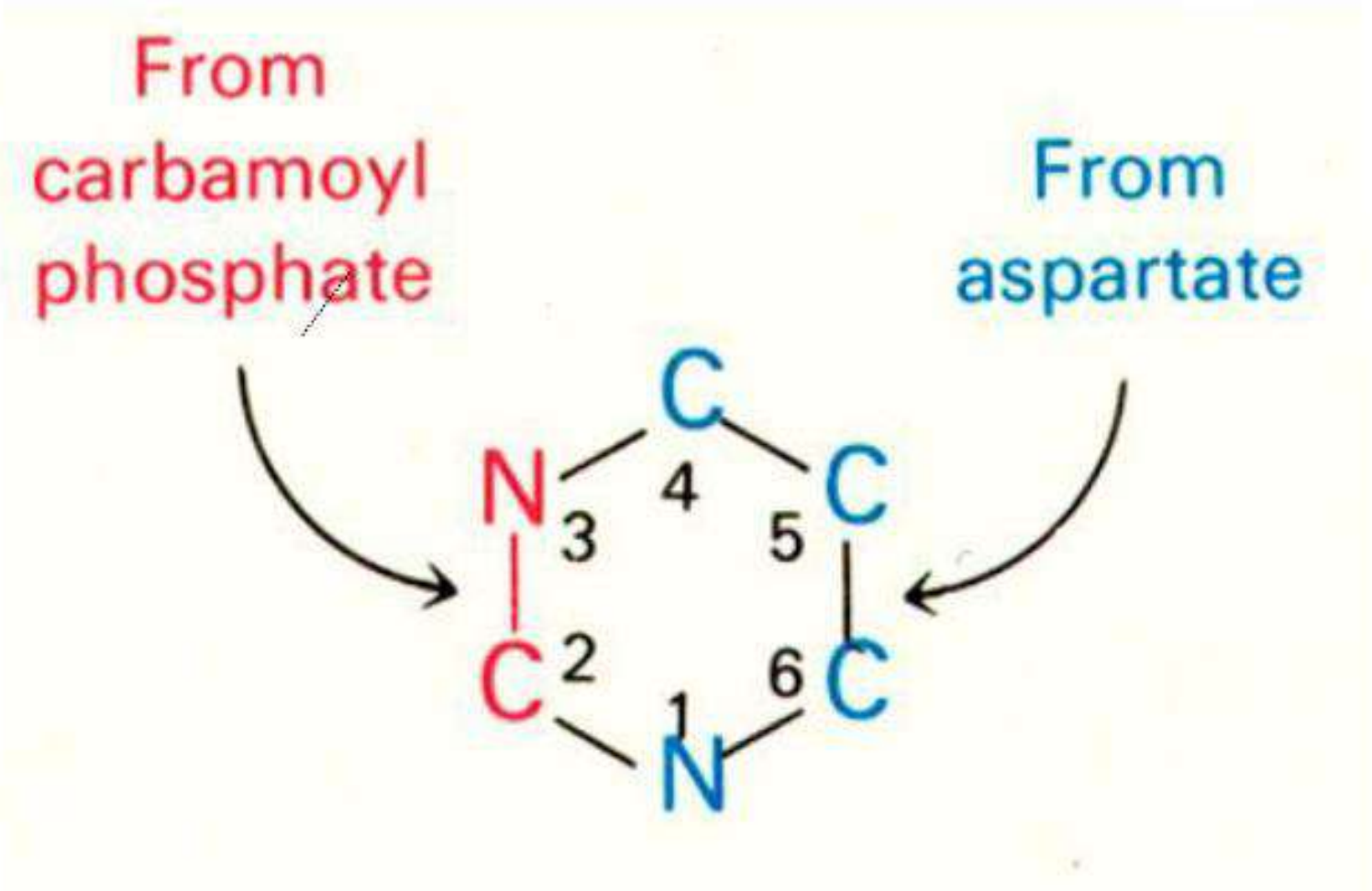
- All purine degradation leads to uric acid (but it might not stop there)
- Ingested nucleic acids are degraded to nucleotides by pancreatic nucleases, and intestinal phosphodiesterases in the intestine
- Group-specific nucleotidases and non-specific phosphatases degrade nucleotides into nucleosides
  - Direct absorption of nucleosides
  - Further degradation

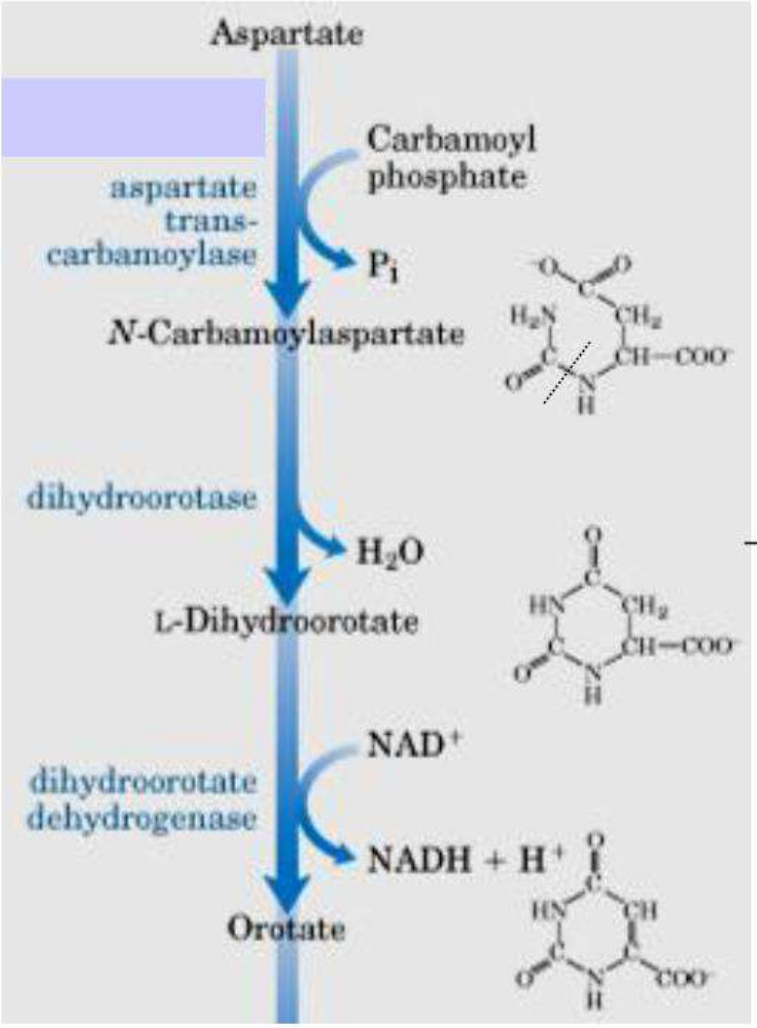
    Nucleoside + H_2O → base + ribose (nucleosidase)

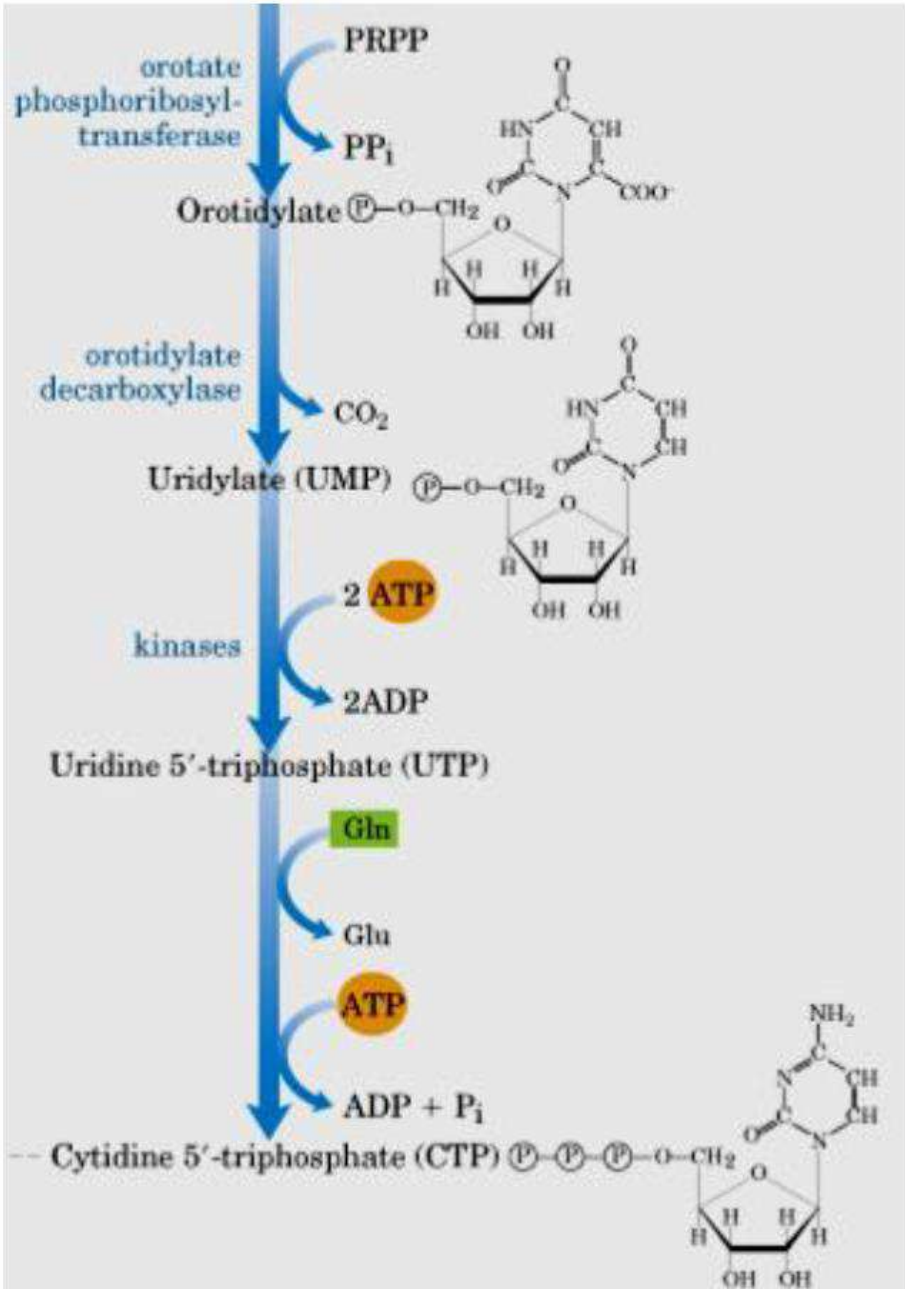
    Nucleoside + P_i → base + r-1-phosphate (n. phosphorylase)

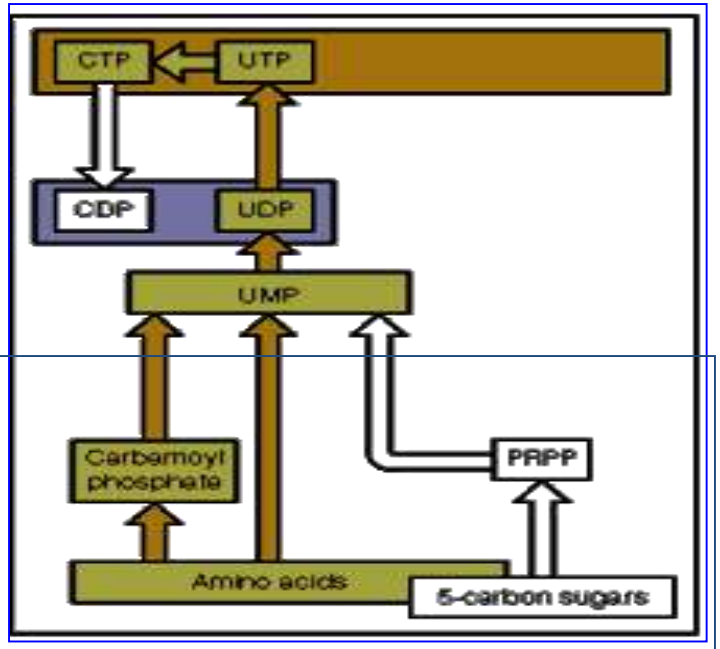
NOTE: MOST INGESTED NUCLEIC ACIDS ARE DEGRADED AND EXCRETED.

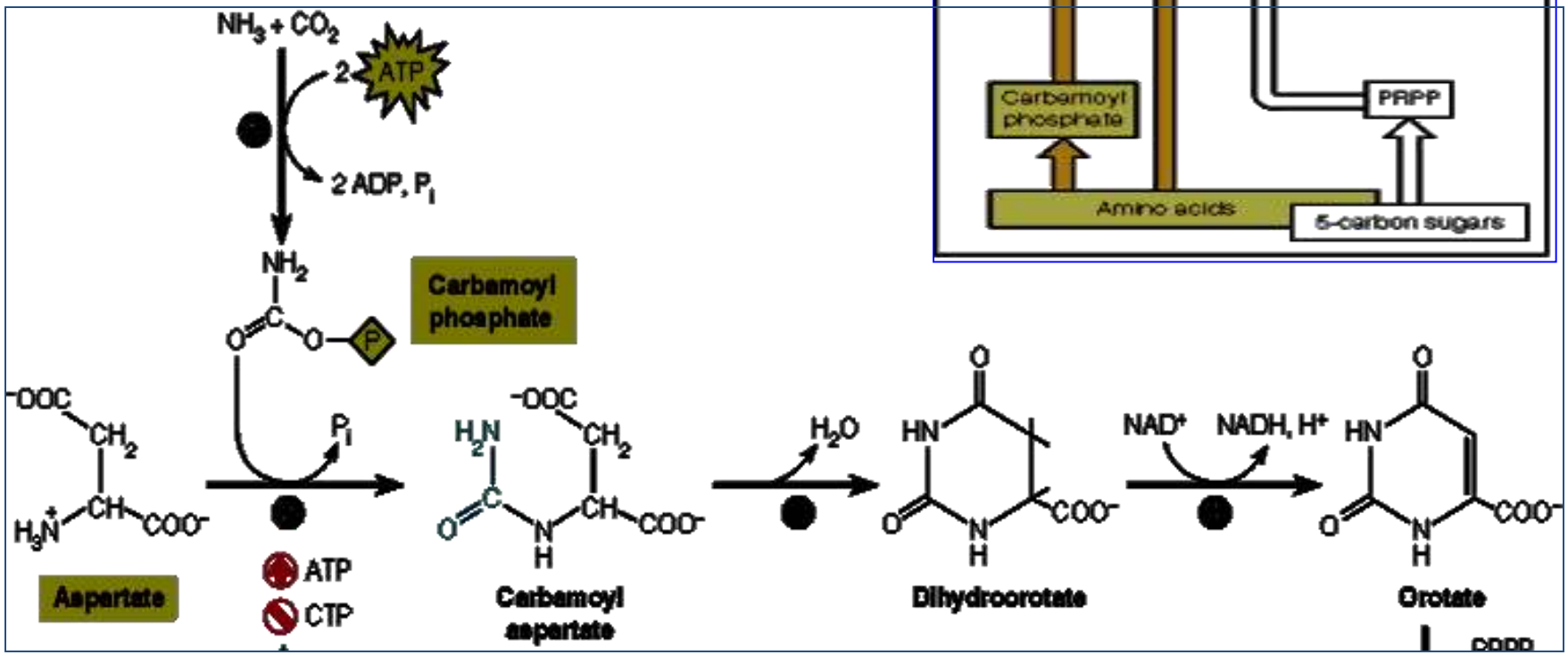
PYRIMIDINE BIOSYNTHESIS

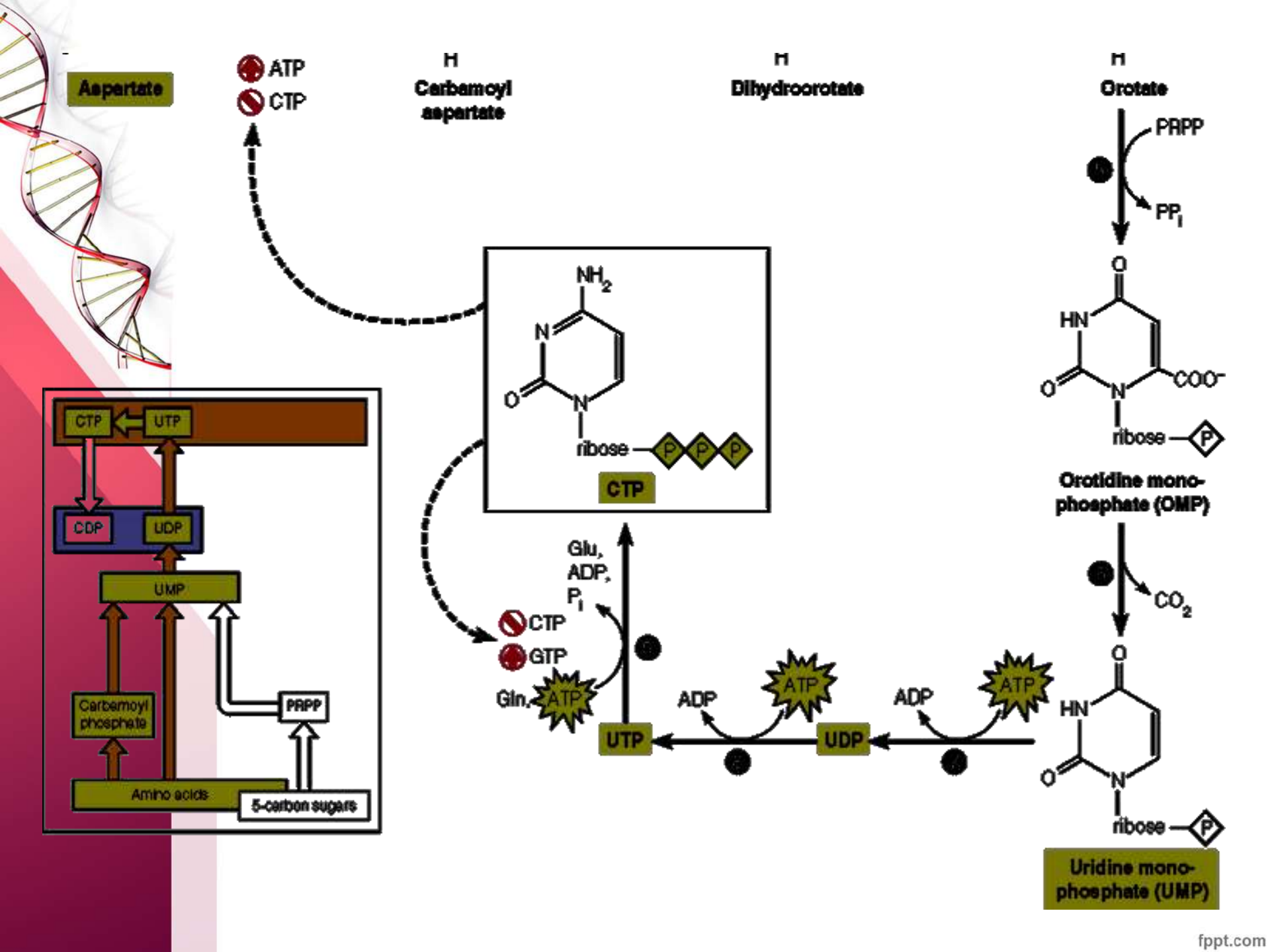



Aspartate
Carbamoyl phosphate
aspartate trans-carbamoylase
P_i
N-Carbamoylaspartate
dihydroorotase
H_2O
L-Dihydroorotate
NAD^+
dihydroorotate dehydrogenase
$NADH + H^+$
Orotate

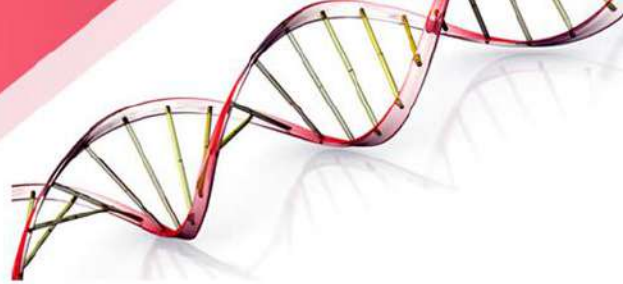

PRPP
orotate phosphoribosyl-transferase
PP_i
Orotidylate
orotidylate decarboxylase
CO_2
Uridylate (UMP)
2 ATP
kinases
2ADP
Uridine 5′-triphosphate (UTP)
Gln
Glu
ATP
$ADP + P_i$
Cytidine 5′-triphosphate (CTP)


CTP
UTP
CDP
UDP
UMP
Carbamoyl phosphate
PRPP
Amino acids
5-carbon sugars


NH3 + CO2
2 ATP
2 ADP, Pi
NH2
Carbamoyl phosphate
Pi
Aspartate
ATP
CTP
Carbamoyl aspartate
H2O
Dihydroorotate
NAD+
NADH, H+
Orotate

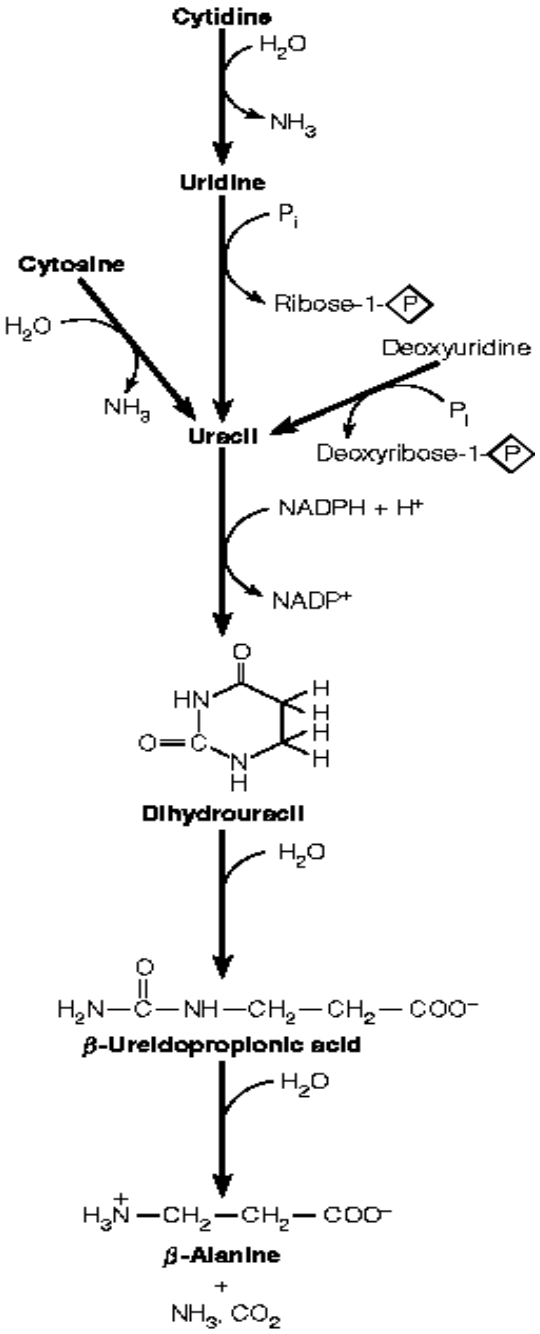

Aspartate
ATP
CTP
Carbamoyl aspartate
Dihydroorotate
Orotate
PRPP
PPi
Orotidine mono-phosphate (OMP)
CO2
Uridine mono-phosphate (UMP)
ribose
CTP
UTP
UDP
ADP
ATP
Glu, ADP, Pi
CTP
GTP
Gln,
CTP
UTP
CDP
UDP
UMP
Carbamoyl phosphate
PRPP
Amino acids
5-carbon sugars

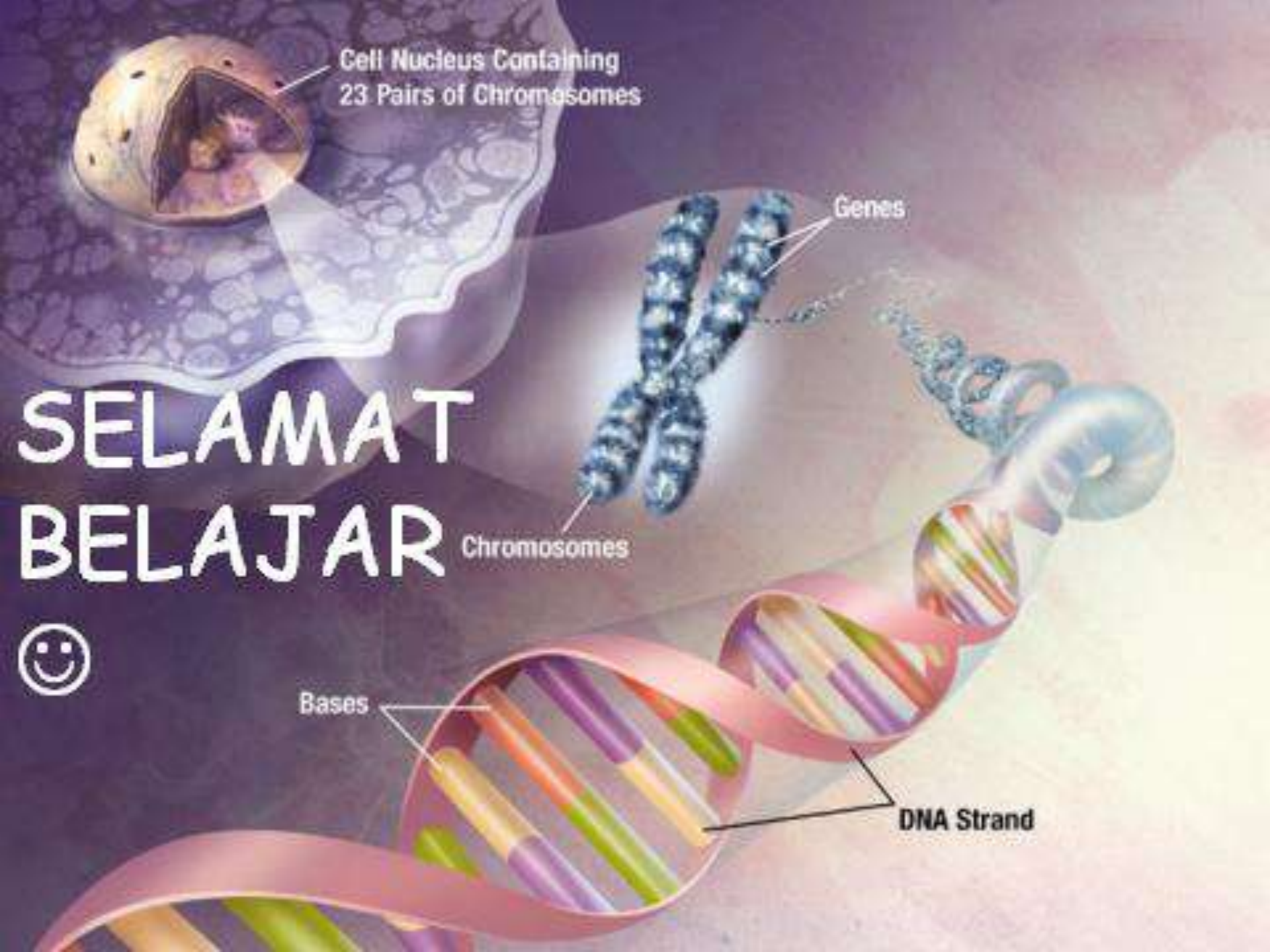
Hal-hal penting dalam sintesis de novo pirimidine:



- cincin pirimidine disintesis terpisah dr gula ribosa nya
- Daur pirimidine de novo tidak bercabang →produk akhir dr daur adalah UMP yang mrpkn bahan dari CMP
- Reaksi pertama → pembtkan karbamoyl aspartate dr asp dan carbomoyl-P→ titik regulasi yg penting dlm daur tsb
- Aspartat transcarbomoylase (ATCase) → diaktivasi oleh ATP dan dihambat oleh CTP sbg produk akhir

Degradasi pirimidin

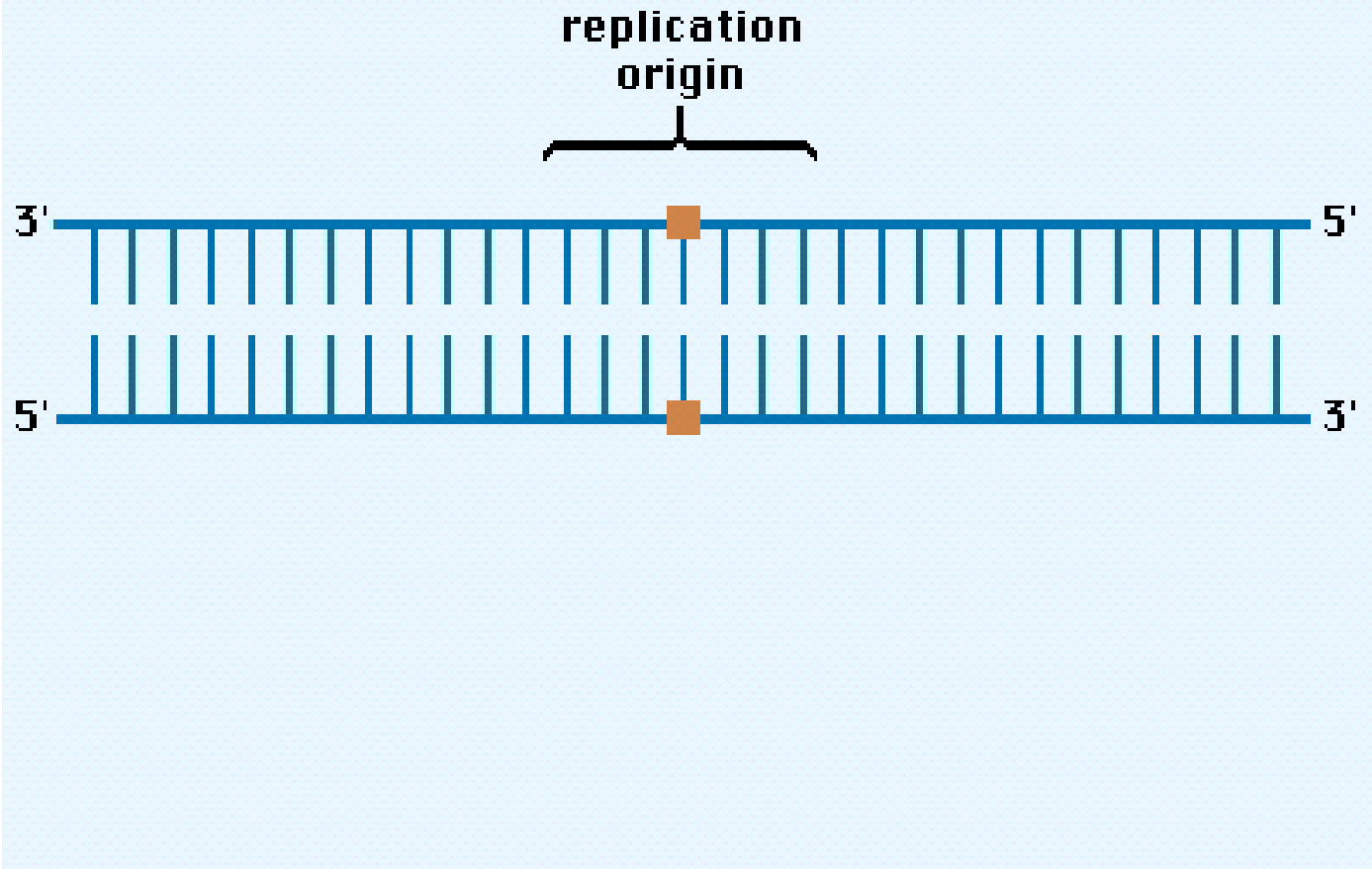



Cell Nucleus Containing
23 Pairs of Chromosomes
Genes
SELAMAT
BELAJAR
Chromosomes
Bases
DNA Strand

REPLIKASI DNA & SINTESIS PROTEIN

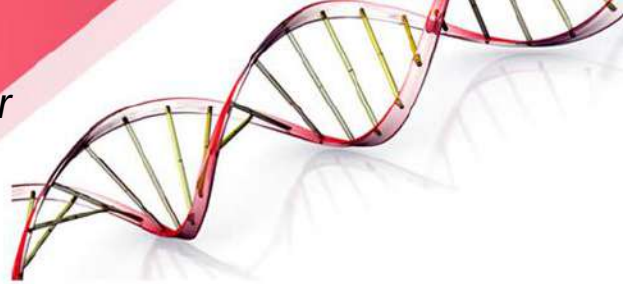
Wahidah Mahanani R., S.T.P., M.Sc.
TEKNOLOGI PANGAN
UNIVERSITAS AHMAD DAHLAN

CHECK THIS OUT FIRST!



"It has not escaped our attention that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material." Watson & Crick

Replication of DNA.



The Central Dogma (F. Crick):

DNA $\xleftarrow{\text{replication}}$ DNA (genome) $\xrightarrow{\text{transcription}}$ mRNA (transcriptome) $\xrightarrow{\text{translation}}$ Protein (proteome)

Expression and transfer of genetic information:

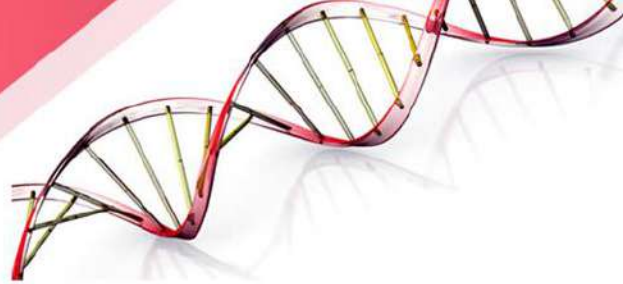
Replication: process by which DNA is copied with very high fidelity.

Transcription: process by which the DNA genetic code is read and transferred to messenger RNA (mRNA). This is an intermediate step in protein expression

Translation: The process by which the genetic code is converted to a protein, the end product of gene expression.

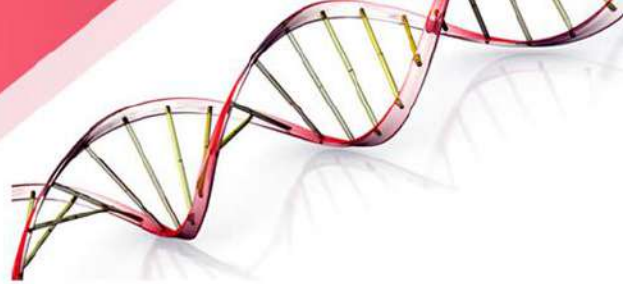
The DNA sequence codes for the mRNA sequence, which codes for the protein sequence

Replication



Replisomes: assemblies of “enzyme factories”.

Component	Function
Helicase	Unwinds the DNA double helix
Primase	Synthesizes primers
Clamp protein	Threads leading strand
DNA polymerase	Joins assembled nucleotides
Ligase	Joins Okazaki fragments in lagging strand



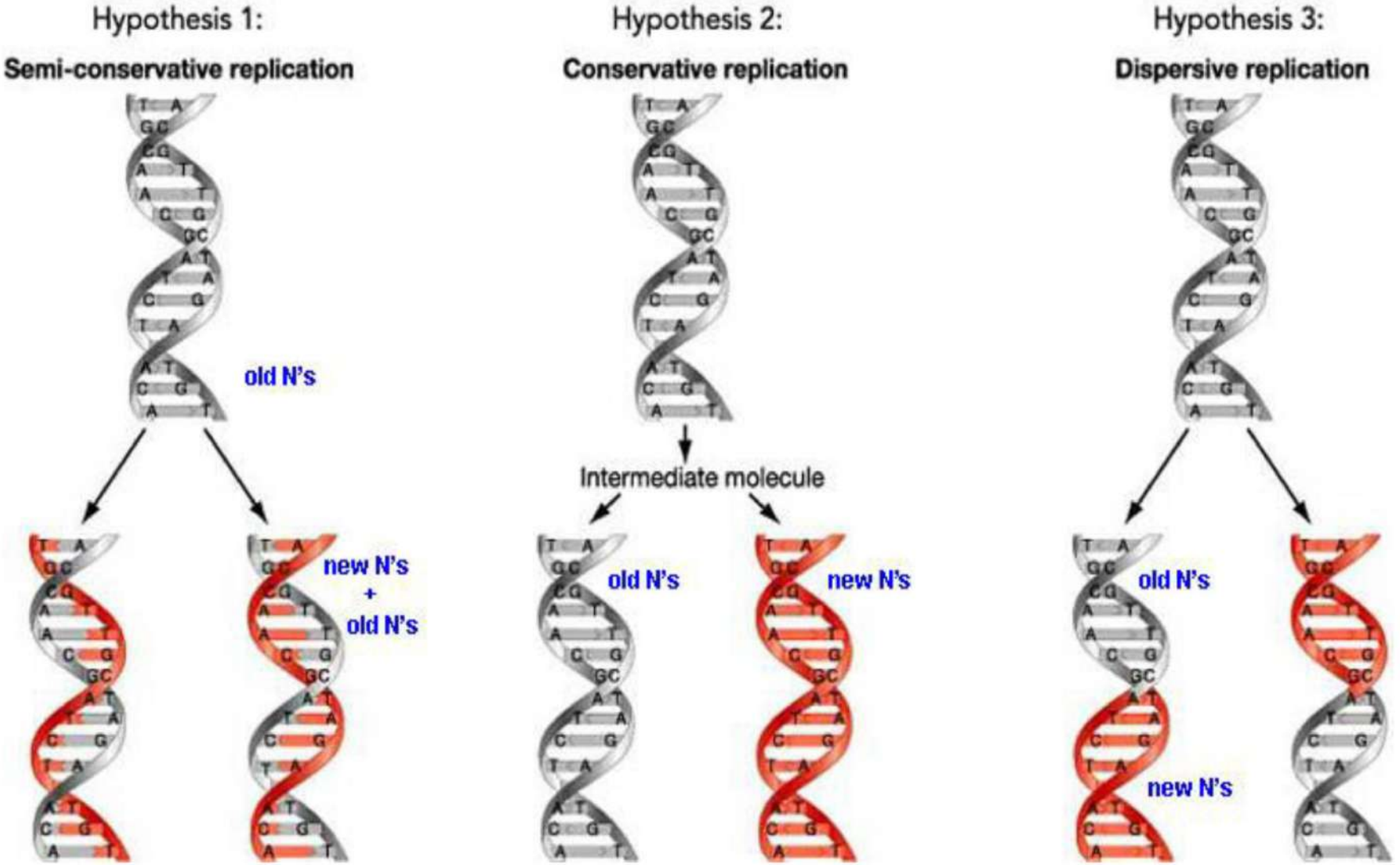
DNA is replicated by the coordinated efforts of a number of proteins and enzymes.

For replication, DNA must be unknotted, uncoiled and the double helix unwound.

Topoisomerase: Enzyme that unknots and uncoils DNA
Helicase: Protein that unwinds the DNA double helix.
DNA polymerase: Enzyme that replicates DNA using each strand as a template for the newly synthesized strand.
DNA ligase: enzyme that catalyzes the formation of the phosphodiester bond between pieces of DNA.

DNA replication is *semi-conservative*: Each new strand of DNA contains one parental (old, template) strand and one daughter (newly synthesized) strand

DNA Replication



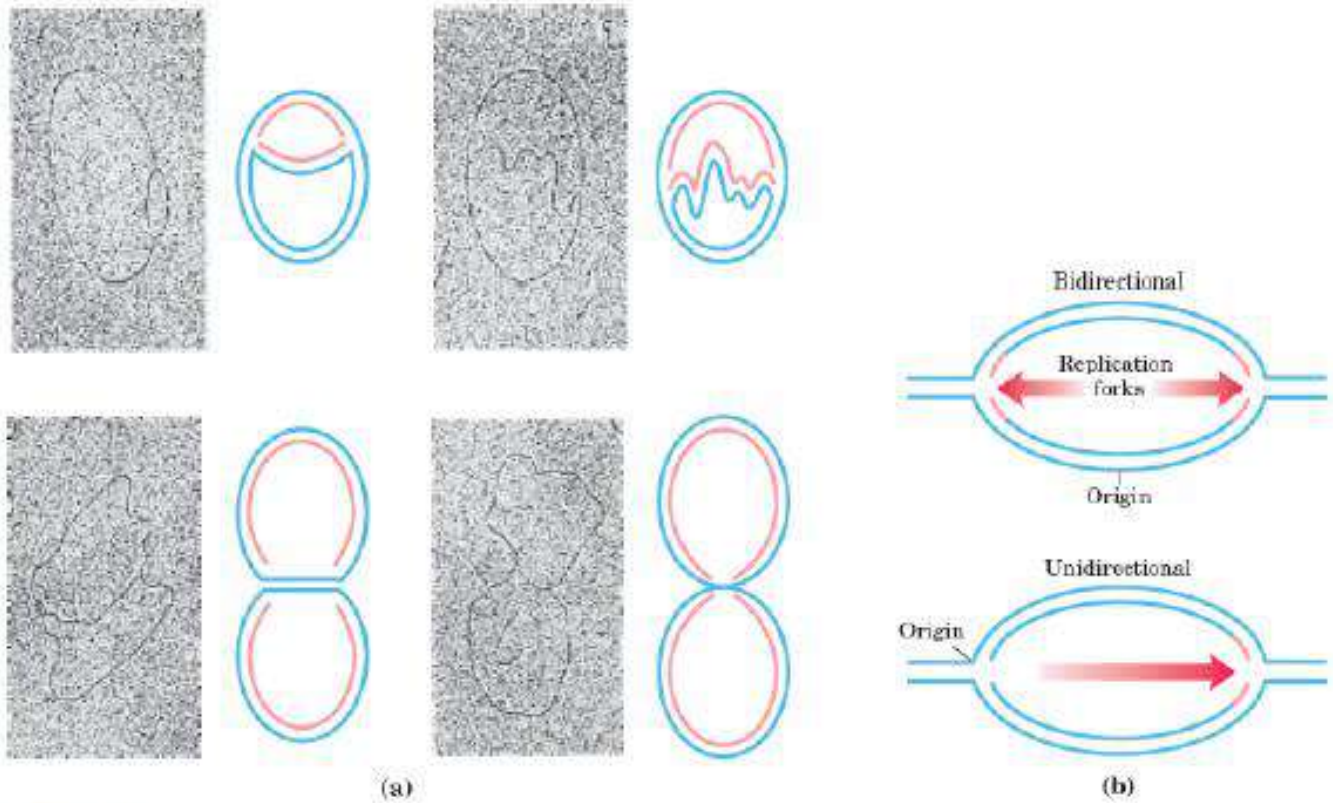
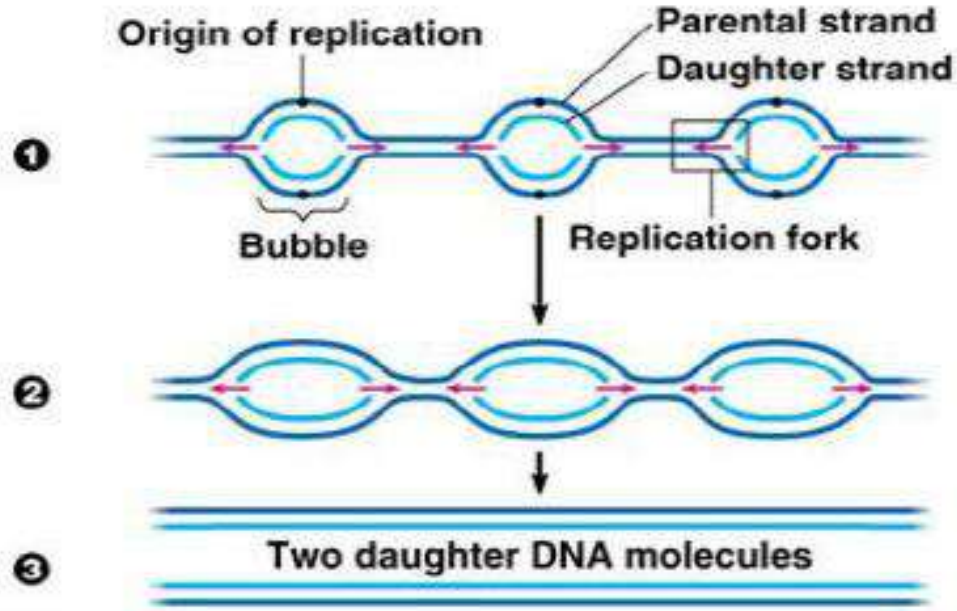
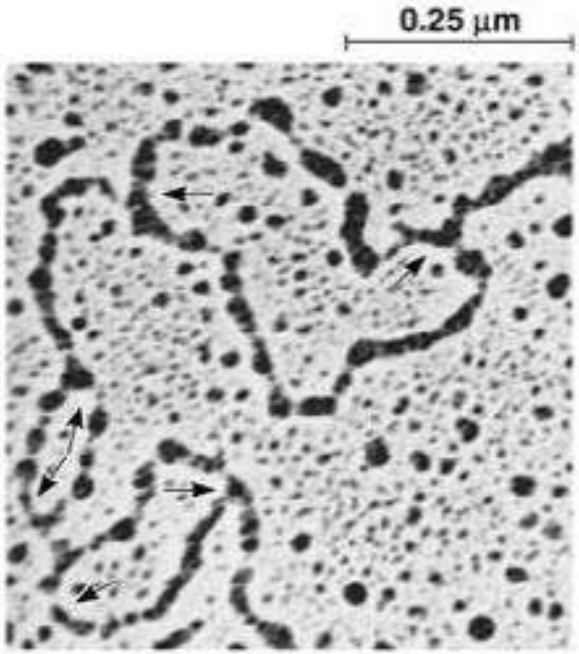


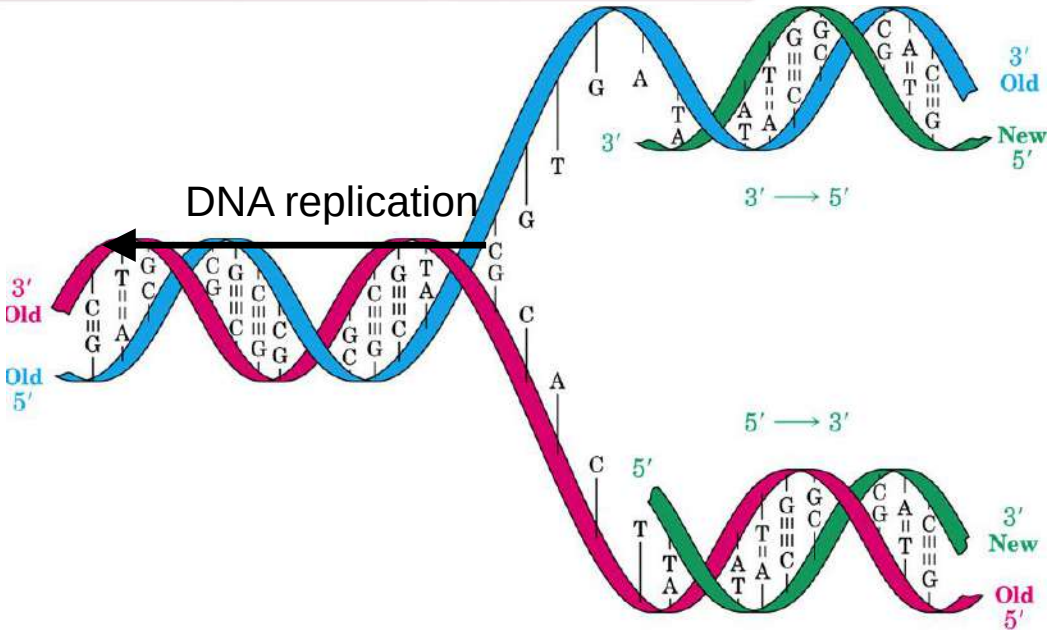
FIGURE 25-3 Visualization of bidirectional DNA replication. Replication of a circular chromosome produces a structure resembling the Greek letter theta (θ). (a) Labeling with tritium (^{3}H) shows that both strands are replicated at the same time (new strands shown in red). The electron micrographs illustrate the replication of a circular *E. coli* plasmid as visualized by autoradiography. (b) Addition of ^{3}H for a short period just before the reaction is stopped allows a distinction to be made between unidirectional and bidirectional replication, by determining whether label (red) is found at one or both replication forks in autoradiograms. This technique has revealed bidirectional replication in *E. coli*, *Bacillus subtilis*, and other bacteria.



(a) In eukaryotes, DNA replication begins at many sites along the giant DNA molecule of each chromosome.



(b) In this micrograph, three replication bubbles are visible along the DNA of cultured Chinese hamster cells. The arrows indicate the direction of DNA replication at the two ends of each bubble (TEM).



Unwinding of DNA by helicases expose the DNA bases (replication fork) so that replication can tak e place.

Helicase hydrolyzes ATP in order to break the hydrogen bonds Between DNA strands

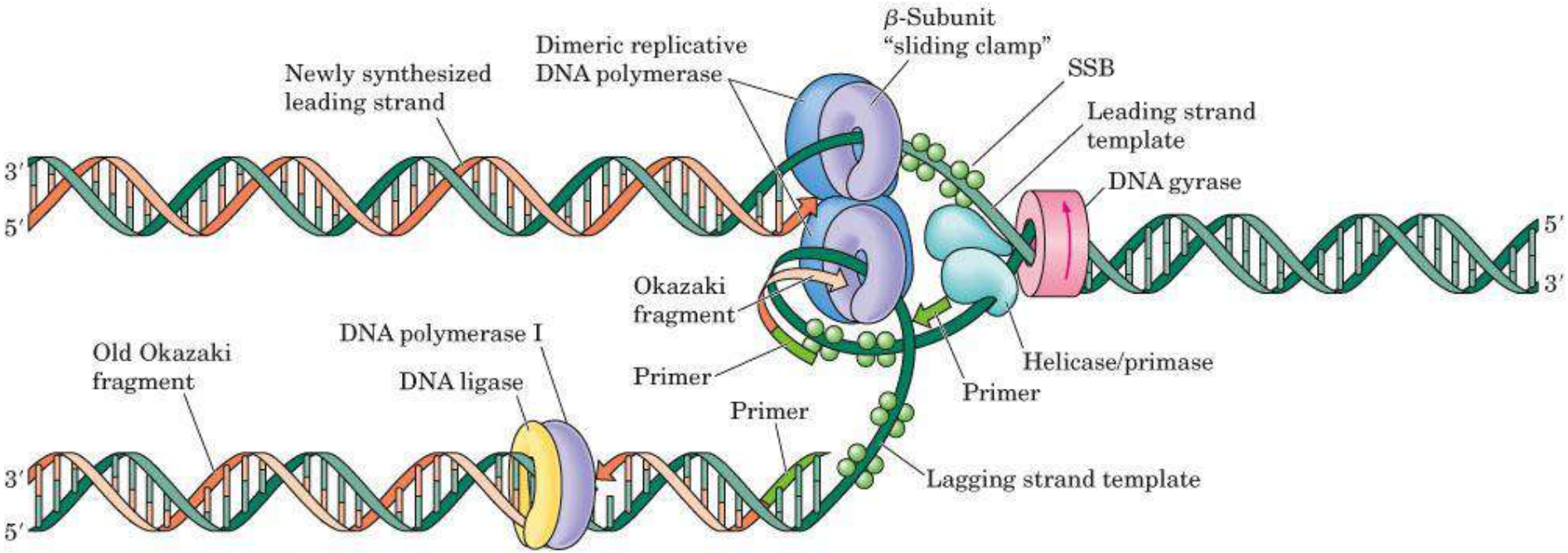
(Fig. 26.8, p. 1192)

animation: http://www.hhmi.org/biointeractive/dna/DNAi_replication_vo2.html

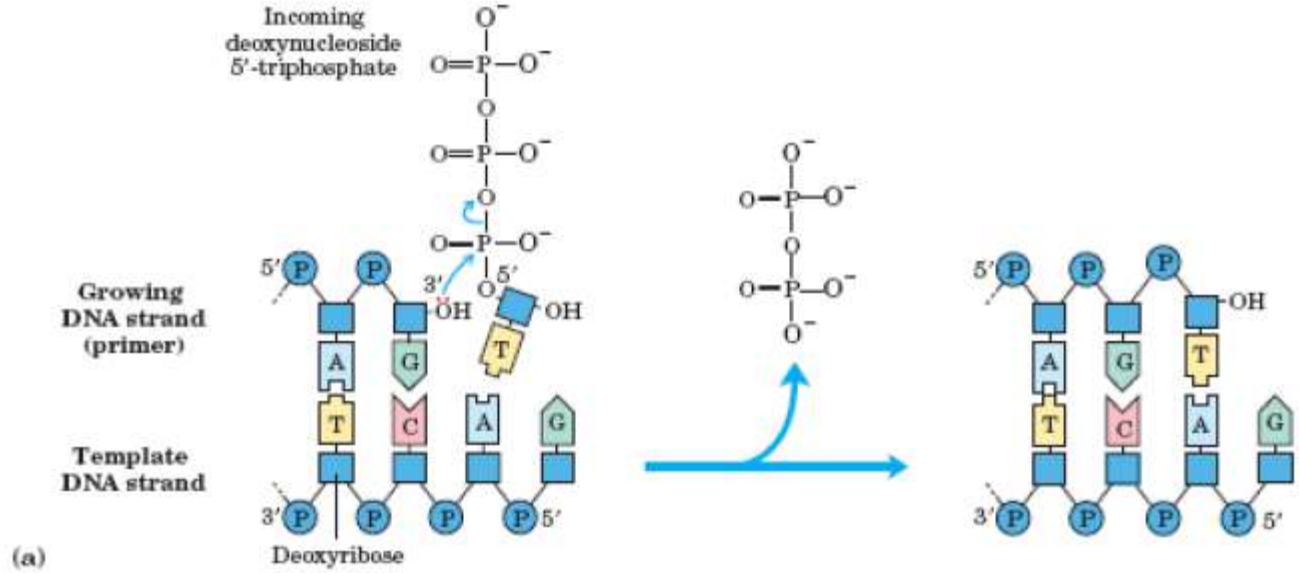
Replication

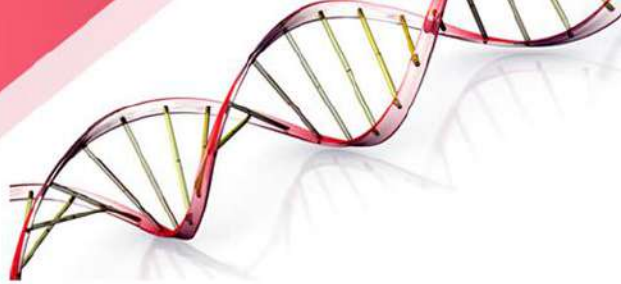
Separation of the two original strands and synthesis
of two new daughter strands using the original strands as templates.

By breaking H-bonds



Penambahan nukleotida baru 5'P menuju 3'OH





DNA Polymerase: the new strand is replicated from the 5'→ 3' (start from the 3' -end of the template)

DNA polymerases are Mg^{2+} ion dependent

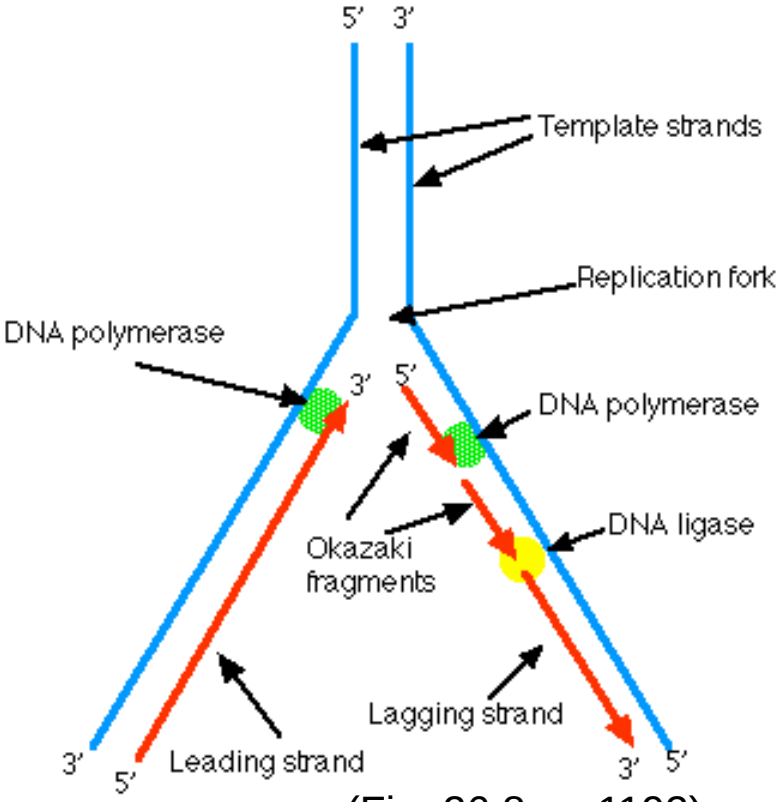
The deoxynucleotide 5'-triphosphate (dNTP) is the reagent for nucleotide incorporation

3' -hydroxyl group of the growing DNA strand acts as a nucleophile and attacks the α-phosphorus atom of the dNTP.

Replication of the *leading strand* occurs continuously in the 5’ → 3’ direction of the new strand.

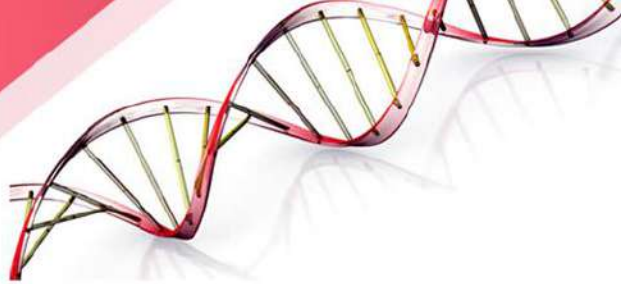
Replication of the *lagging strand* occurs discontinuously. Short DNA fragments are initially synthesized and then ligated together.

DNA ligase catalyzes the formation of the phosphodiester bond between pieces of DNA.



(Fig. 26.8, p. 1192)

Helicases



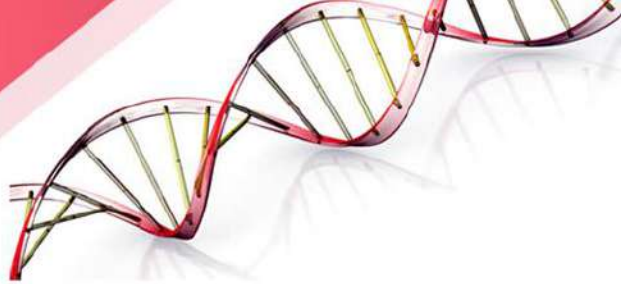
Unwinds the DNA double helix.

- Replication of DNA starts with unwinding of the double helix.

- Unwinding can occur at either end or in the middle.

- Attach themselves to one DNA strand and cause separation of the double helix.

Primases



Catalyze the synthesis of primers.

Primers: are short nucleotides (4 to 15).

- They are required to start the synthesis of both daughter strands.

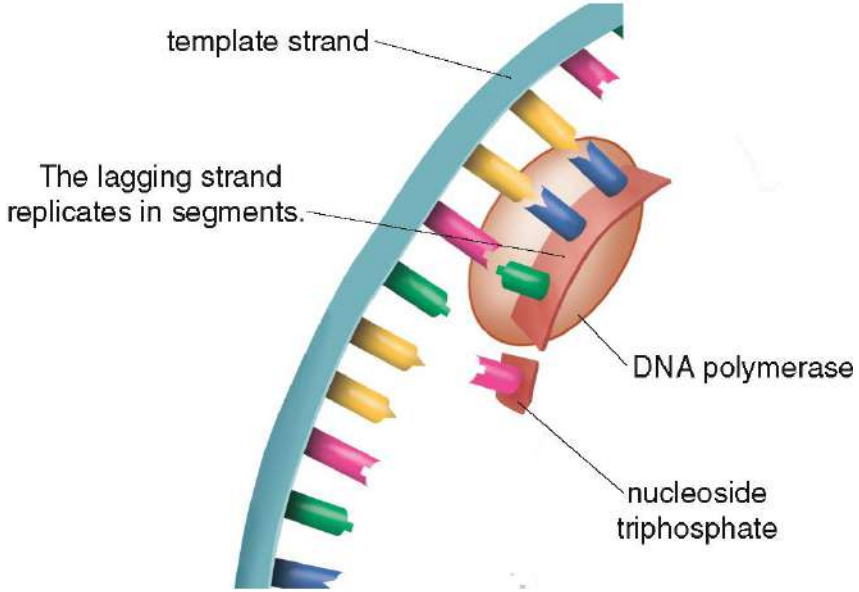
- Primases are placed at about every 50 nucleotides in the lagging strand synthesis.

DNA Polymerase

It catalyzes the formation of the new strands.

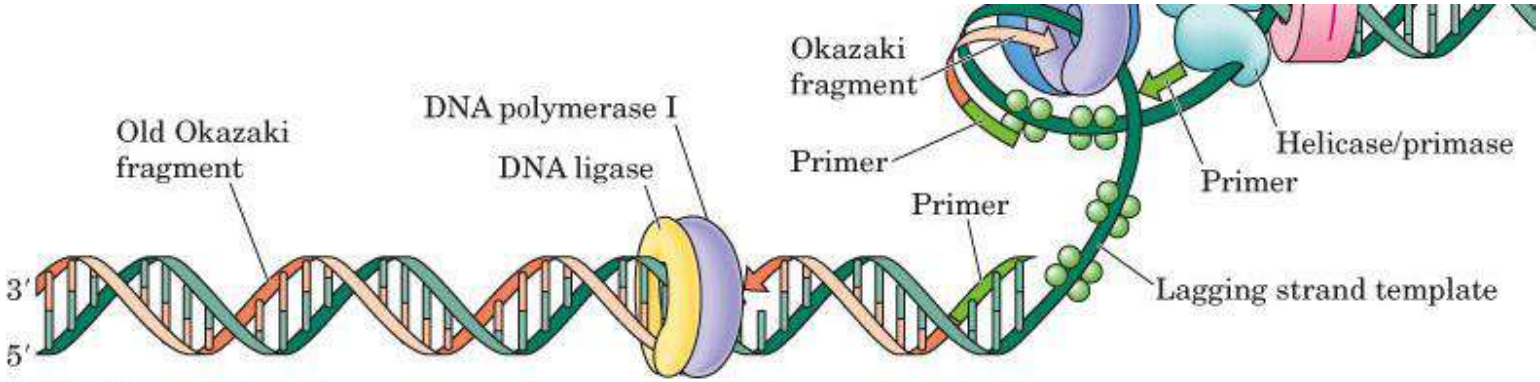
- It joins the nucleoside triphosphates found in the nucleus.

- A new phosphodiester bond is formed between the 5'-phosphate of the nucleoside triphosphate and the 3'-OH group of the new DNA strand.

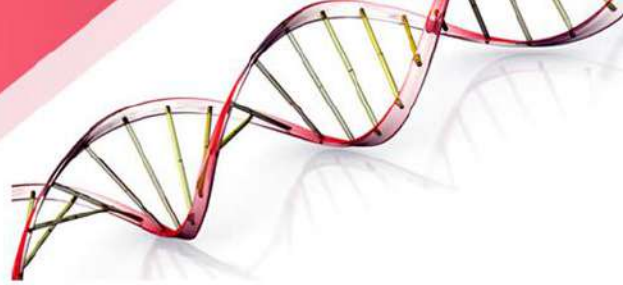


Ligase

In formation of lagging strand, small fragments (Okazaki) are join together by ligase enzyme.



Protein Synthesis

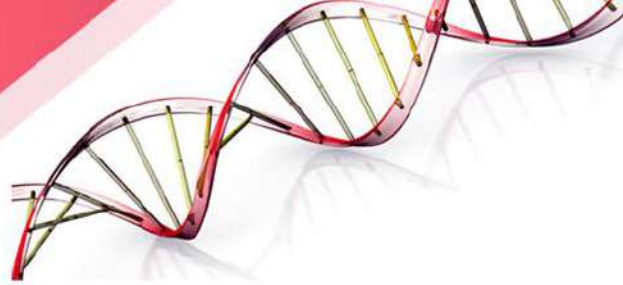


Gene expression: activation of a gene to produce a specific protein.

Only a small fraction (1-2%) of the DNA in a chromosome contains genes.

Base sequence of the gene carries the information to produce one protein molecule.

Change of sequence → New protein

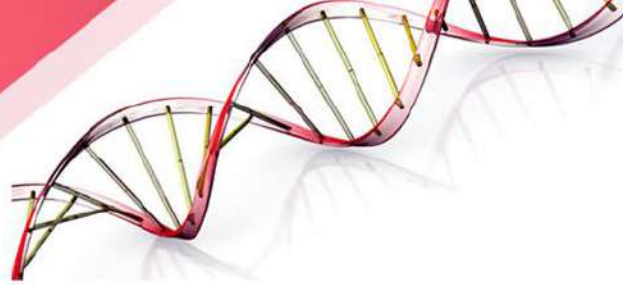


- **Sintesis Protein – Transkripsi dan Translasi**
- Sintesis protein merupakan dasar untuk mempelajari bagaimana informasi genetik di dalam DNA diekspresikan dalam makhluk hidup.

- Dalam istilah genetik sering dikenal dengan yang namanya sentral dogma. Sentral dogma merupakan serangkaian alur informasi dari DNA yang diterjemahkan melalui RNA kemudian menjadi protein di dalam tubuh makhluk hidup.

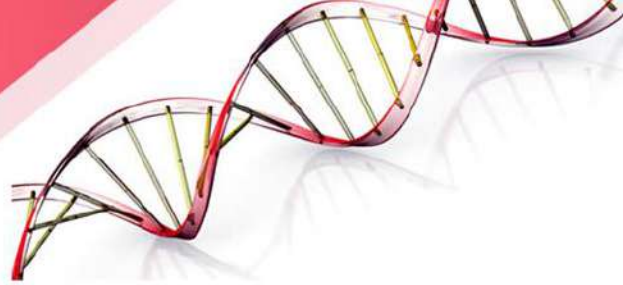
- Sintesis protein memiliki sumber informasi di DNA dalam bentuk gen. Gen tersebut berupa rangkaian kode-kode basa nitrogen. Informasi dalam gen akan diterjemahkan dalam bentuk mRNA. mRNA kemudian akan digunakan untuk merangkai asam amino yang didapatkan dari luar dan dalam tubuh.

- Sintesis protein terjadi pada organel yang dinamakan dengan ribosom. Sintesis protein sangat memerlukan keberadaan RNA, yaitu suatu rantai tunggal basa nitrogen dengan backbone yang sama dengan DNA. Adapun pembagian jenis-jenis RNA secara lengkap adalah sebagai berikut.

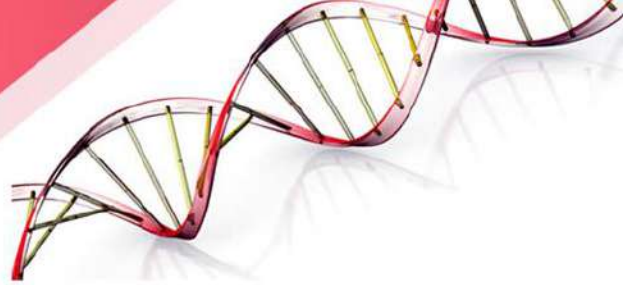


- a. mRNA (messenger RNA / RNA duta)

RNA duta merupakan RNA yang dibuat oleh proses yang dinamakan dengan transkripsi pada inti sel. Peranan mRNA adalah membawa informasi genetik yang ada pada DNA menuju ribosom. Informasi yang terdapat pada mRNA berupa kodon yang tersusun secara triplet, misalkan UCA, UCU, atau AAG. Kodon tersebut dibuat triplet atau tiga-tiga karena 4 pangkat 3 hasilnya 64, yang kombinasi hurufnya diatas 20.

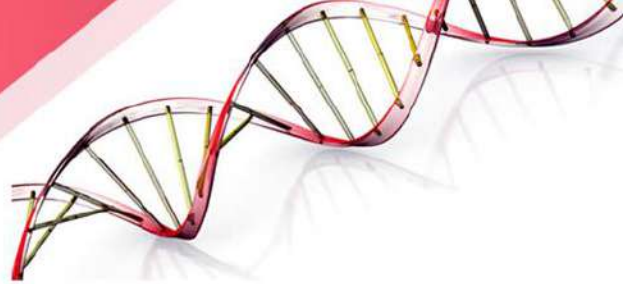


- b. tRNA (transport RNA / RNA transfer)
- RNA transfer merupakan RNA yang berperan untuk membawa asam amino dari sitoplasma menuju ribosom saat terjadi sintesis protein. tRNA disintesis di salah satu bagian inti sel secara langsung. Dalam proses pentransferan asam amino, tRNA memerlukan energi yang berasal dari pemecahan molekul ATP menjadi ADP + Pi.

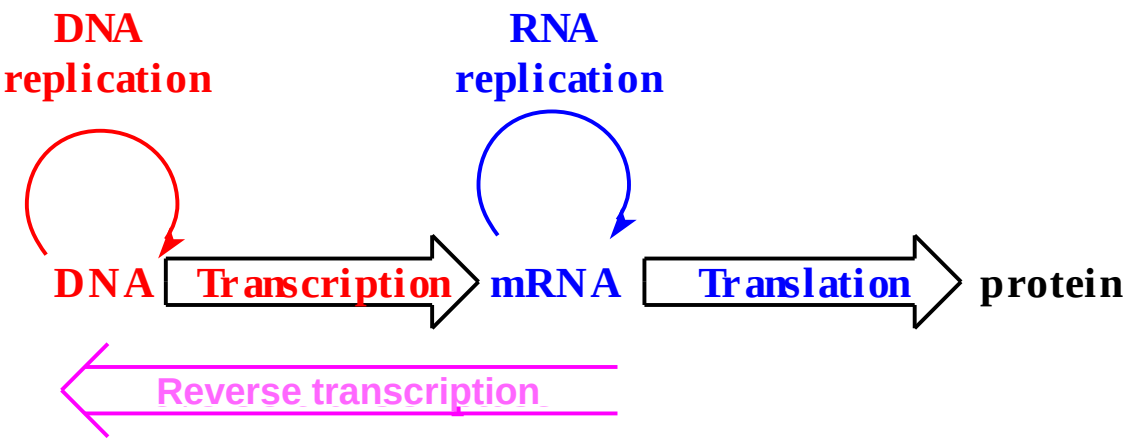


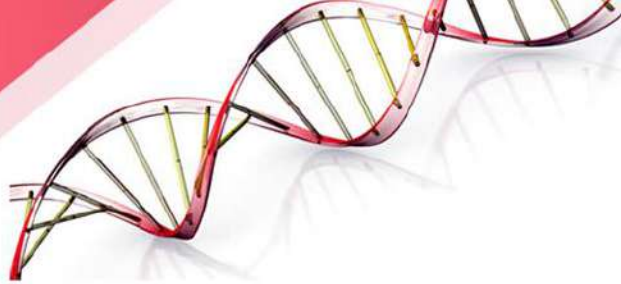
- Tahap-Tahap Sintesis Protein
- Sintesis protein dibagi menjadi dua tahapan utama, yaitu transkripsi dan translasi. Transkripsi secara garis besar merupakan proses pembuatan mRNA dari DNA dalam inti sel. mRNA tersebut lalu bergerak menuju ribosom. Setelah itu, proses translasi, yang meliputi penerjemahan dan perangkaian asam amino, berlangsung di ribosom.

Gene expression



- Transcription: synthesis of mRNA (messenger RNA)
- Translation

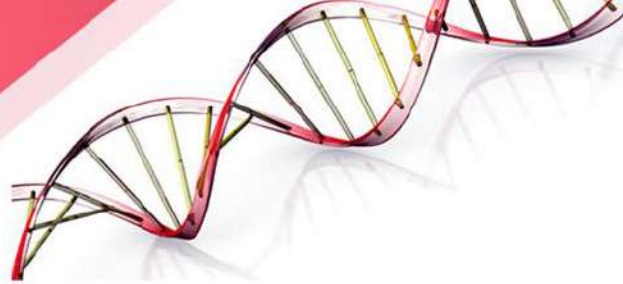




1. Transkripsi – Pemindahan informasi dari DNA ke mRNA

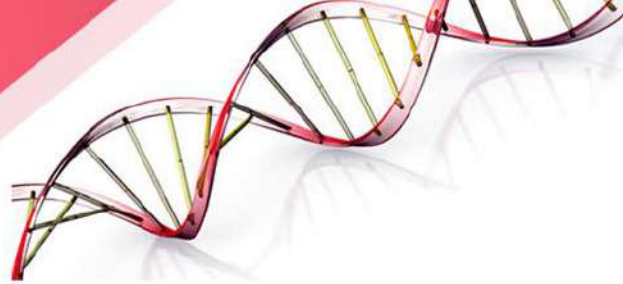
Proses ini sebenarnya merupakan awal mula informasi pada DNA dipindahkan menuju protein pada makhluk hidup.

Transkripsi diawali dari pemutusan ikatan H pada DNA oleh protein-protein pengurai DNA. Proses tersebut mengakibatkan terbukanya rantai DNA pada berbagai tempat. Terbukanya rantai DNA memicu RNA polimerase melekat ke daerah yang dinamakan dengan promotor. RNA polimerase selanjutnya melakukan sintesis molekul mRNA dari arah 3’ DNA, sedangkan pada mRNA dimulai dari ujung 5’ menuju 3’.



Dari kedua rantai DNA, hanya salah satu rantai yang akan diterjemahkan menjadi mRNA.
Rantai DNA yang diterjemahkan menjadi protein → rantai sense atau DNA template atau DNA cetakan,
Rantai pasangannya → DNA antisense.

Dari DNA template inilah mRNA akan membentuk rantai berpasangan dengan basa-basa yang ada pada DNA sense.



Komponen untuk pembuatan mRNA t→ nukleotida trifosfat, seperti ATP, GTP, UTP, dan CTP.

Fungsi RNA polimerase → katalis reaksi penempelan nukleotida triposfat sehingga terbentuk rantai. Energi yang digunakan untuk menjalankan reaksi tersebut berasal dari masing-masing nukleotida trifosfat yang kaya akan energi.

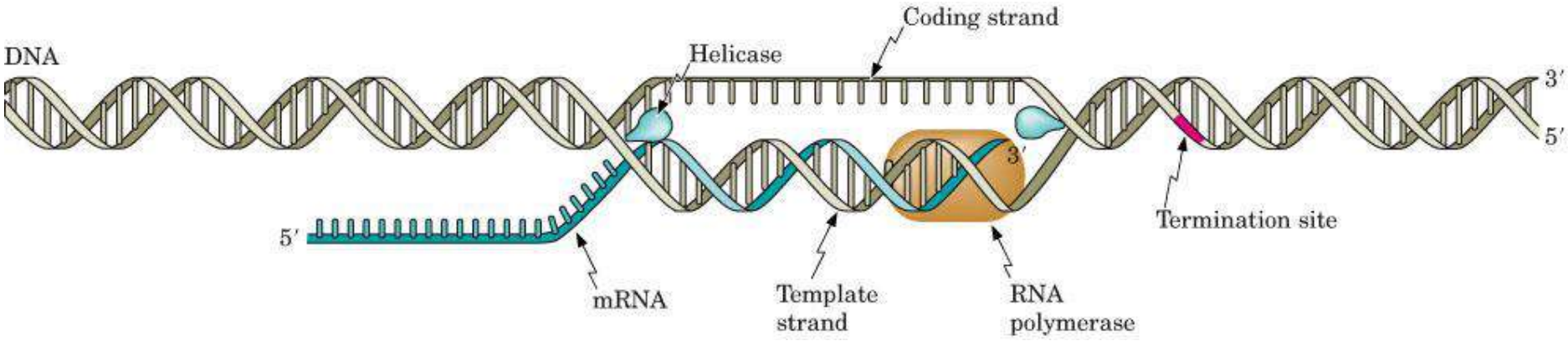
Ketika sintesis mRNA berakhir → mRNA yang terbentuk selanjutnya akan dipindahkan dari **inti menuju ribosom**, kemudian diterjemahkan menjadi protein di ribosom.

Transcription

Genetic information is copied from a gene in DNA to make a mRNA.

Begins when the section of a DNA that contains the gene to be copied unwinds.

Polymerase enzyme identifies a starting point to begin mRNA synthesis.



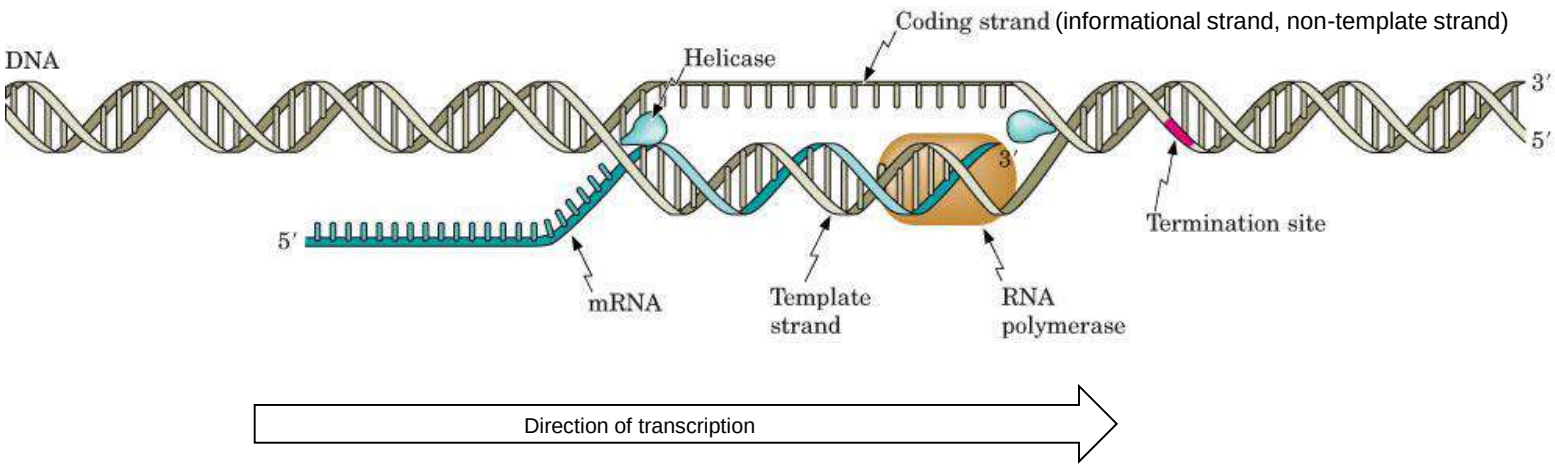
Transcription

The DNA splits into two strands:

Template strand: it is used to synthesize RNA.

Coding Strand (Informational strand): it is not used to synthesize RNA.

- Transcription proceeds from the 3’ end to the 5’ end of the template.



- When mRNA is released, the double helix of the DNA re-forms.

Transcription

C is paired with G, T pairs with A
But A pairs with U (not T).

Polymerase enzyme moves along the unwound DNA, forming bonds between the bases.

RNA Polymerase →

Section of bases on DNA (template strand): - G – A – A – C – T -

↓ ↓ ↓ ↓ ↓

Complementary base sequence in mRNA: - C – U – U – G – A -

Transcription

Sample Problem 22.6

From the template strand of DNA below, write out the mRNA and informational strand of DNA sequences:

Template strand:	3'—C T A G G A T A C—5'
mRNA:	5'—G A U C C U A U G—3'
Informational strand:	5'—G A T C C T A T G—3'

Translation

mRNA (as a carrier molecule) moves out of the nucleus and goes to ribosomes.

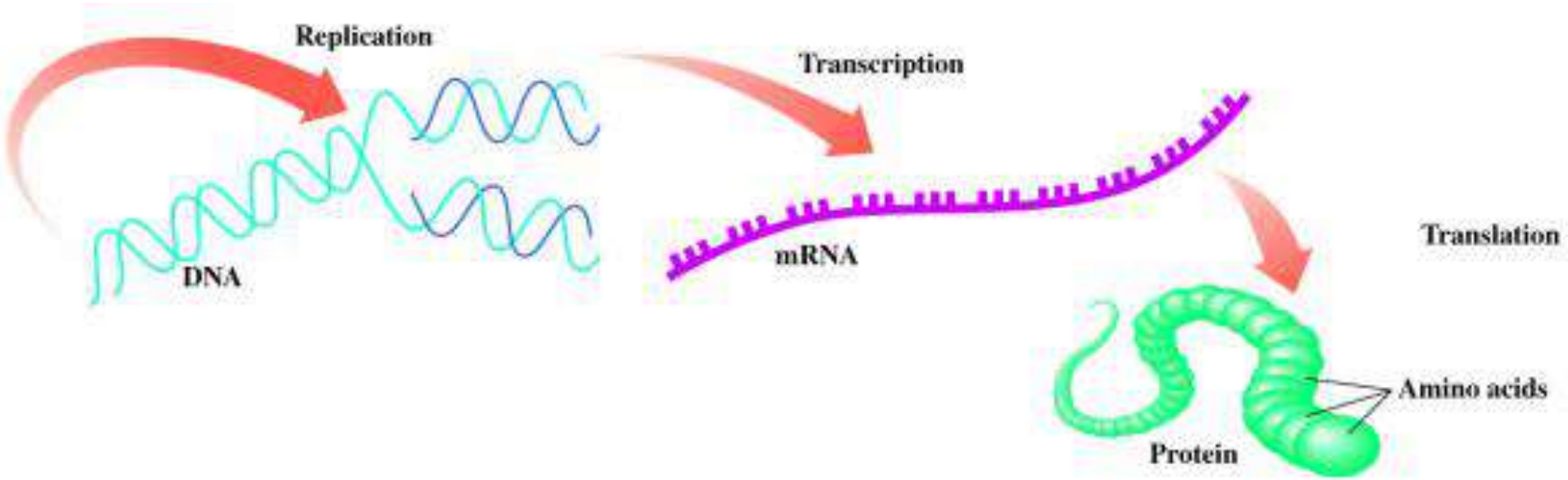
tRNA converts the information into amino acids.

Amino acids are placed in the proper sequence.

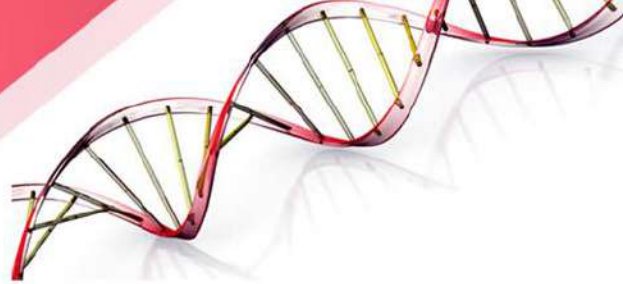
Proteins are synthesized.

Gene expression

Overall function of RAN's in the cell: **facilitate the task of synthesizing protein.**



Genetic code



Genetic code: language that relates the series of nucletides in mRNA to the amino acids specified.

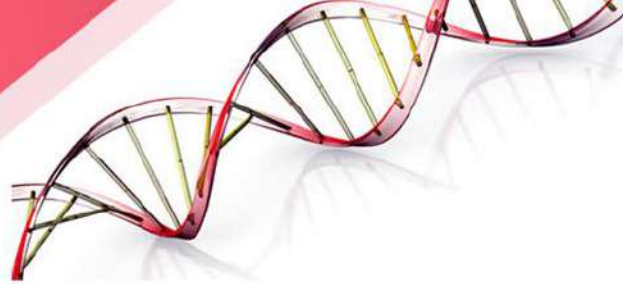
- The sequence of nucleotides in the mRNA determines the amino acid order for the protein.
- Every three bases (triplet) along the mRNA makes up a **codon**.
- Each codon specifies a particular amino acid.
- Codons are present for all 20 amino acids.

Genetic code

5'	U		C		A		G		3'
U	UUU	Phe	UCU	Ser	UAU	Tyr	UGU	Cys	U
	UUC	Phe	UCC	Ser	UAC	Tyr	UGC	Cys	C
	UUA	Leu	UCA	Ser	UAA	Stop	UGA	Stop	A
	UUG	Leu	UCG	Ser	UAG	Stop	UGG	Trp	G
C	CUU	Leu	CCU	Pro	CAU	His	CGU	Arg	U
	CUC	Leu	CCC	Pro	CAC	His	CGC	Arg	C
	CUA	Leu	CCA	Pro	CAA	Gln	CGA	Arg	A
	CUG	Leu	CCG	Pro	CAG	Gln	CGG	Arg	G
A	AUU	Ile	ACU	Thr	AAU	Asn	AGU	Ser	U
	AUC	Ile	ACC	Thr	AAC	Asn	AGC	Ser	C
	AUA	Ile	ACA	Thr	AAA	Lys	AGA	Arg	A
	AUG	Met*	ACG	Thr	AAG	Lys	AGG	Arg	G
G	GUU	Val	GCU	Ala	GAU	Asp	GGU	Gly	U
	GUC	Val	GCC	Ala	GAC	Asp	GGC	Gly	C
	GUA	Val	GCA	Ala	GAA	Glu	GGA	Gly	A
	GUG	Val	GCG	Ala	GAG	Glu	GGG	Gly	G

*AUG signals translation initiation as well as coding for Met

Genetic code



- 64 condons are possible from the triplet combination of A, G, C, and U.

- Codons are written from the 5’ end to the 3’ end of the mRNA molecule

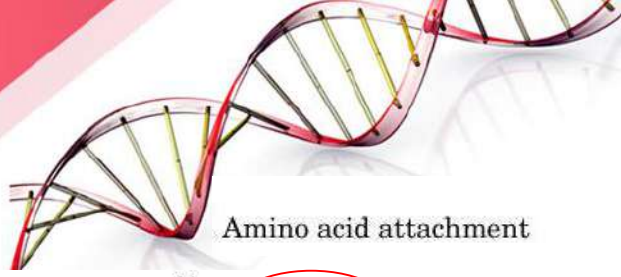
- UGA, UAA, and UAG, are stop signals.
(code for termination of protein synthesis).

- AUG has two roles:

1. Signals the start of the proteins synthesis (at the beginning of an mRNA).

2. Specifies the amino acid methionine (Met) (in the middle of an mRNA).

tRNA (transfer RNA)

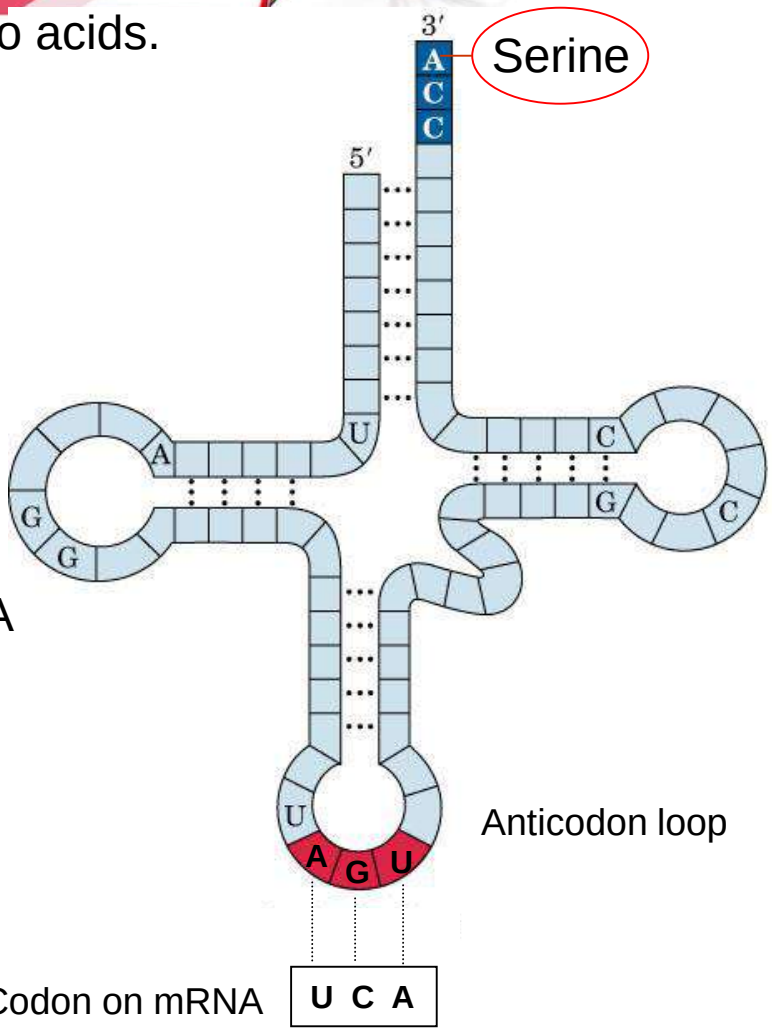


tRNA translates the codons into specific amino acids.

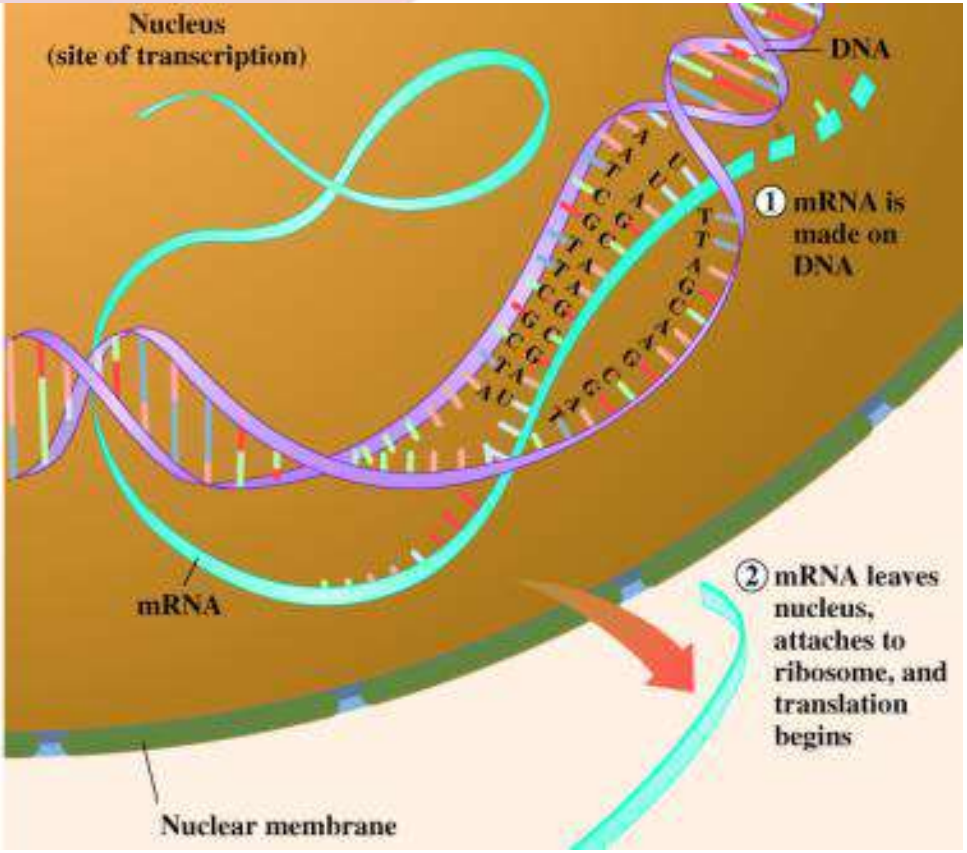
- It contains 70-90 nucleotides.

- The 3’ end, called the acceptor stem and always has the nucleotide ACC and a free OH group that binds a specific amino acid.

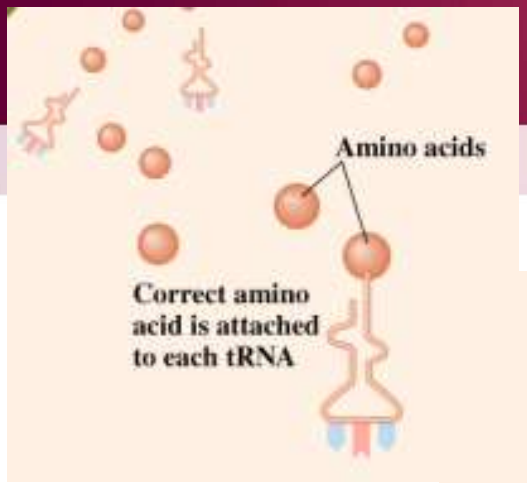
- Anticodon: a sequence of three nucleotides at the bottom of tRNA, which is complementary to three bases in an mRNA and it can identify the needed amino acid.

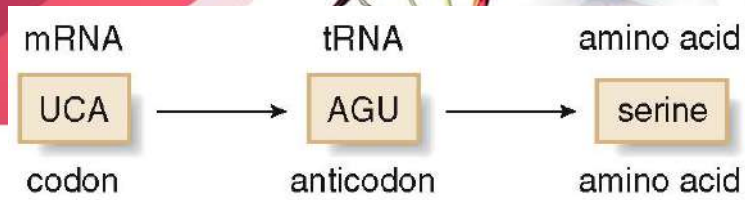


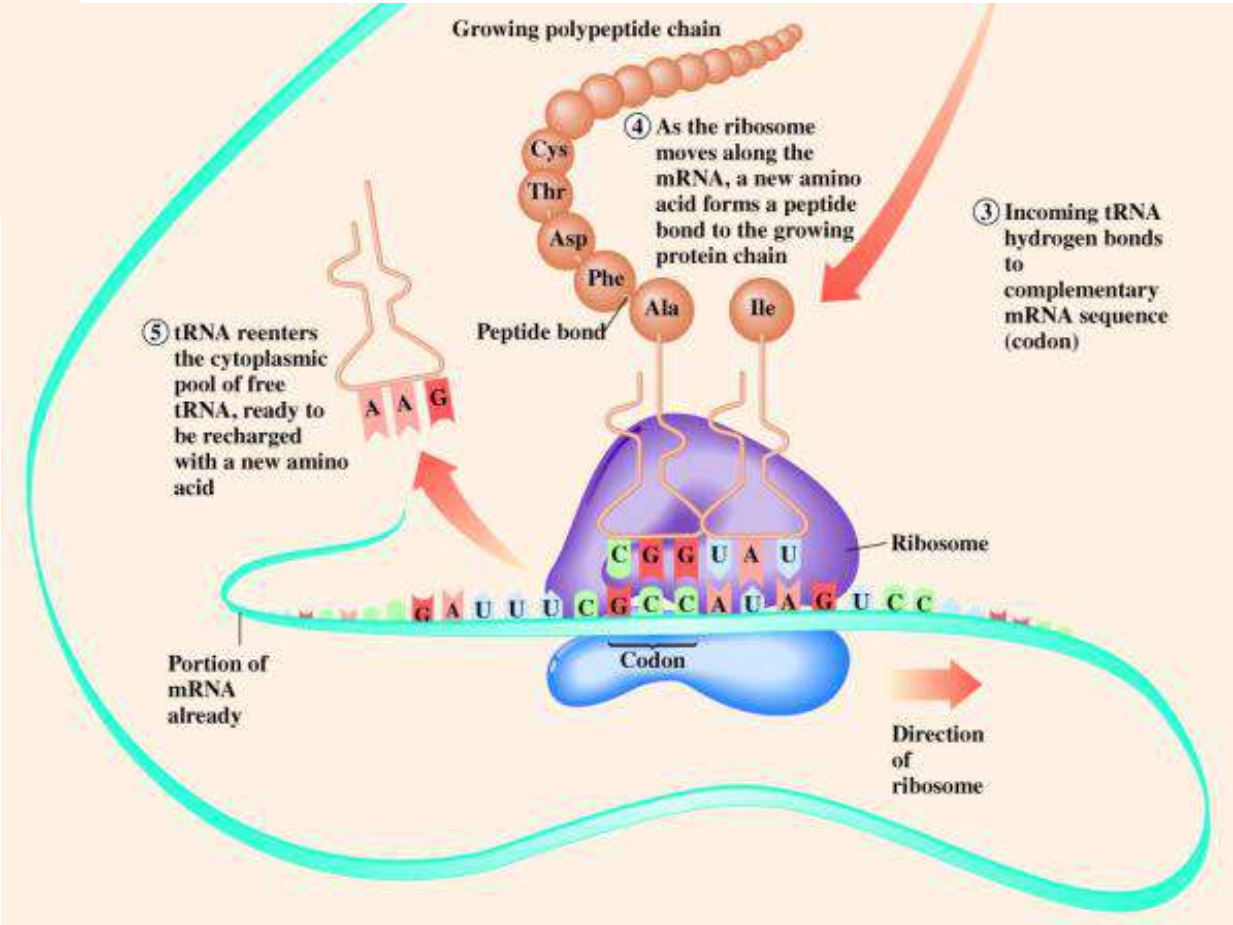
Transcription



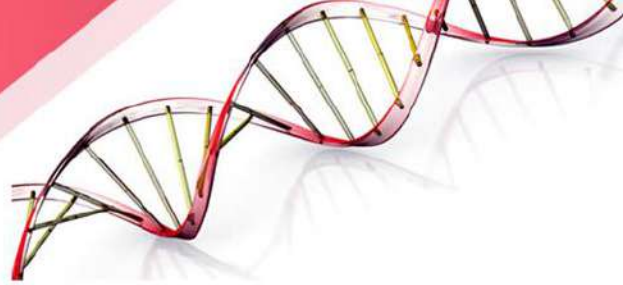
Translation







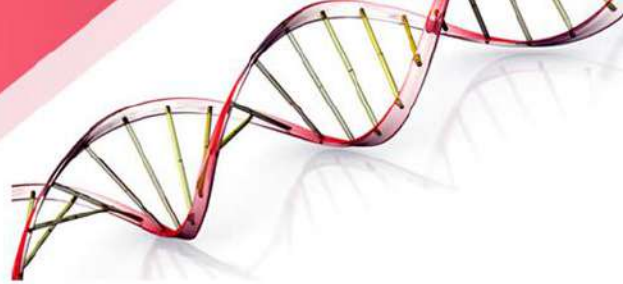
Protein synthesis



- mRNA attaches to smaller subunit of a ribosome.
- tRNA molecules bring specific amino acids to the mRNA.
- Peptide bonds form between an amino acid and the end of the growing peptide chain.
- The ribosome moves along mRNA until the end of the codon (translocation).
- The polypeptide chain is released from the ribosome and becomes an active protein.

Sometimes several ribosomes (polysome) translate the same strand of mRNA at the same time to produce several peptide chains.

Termination



Ribosome encounters a stop condon.

UAA Stop **UGA Stop** **UAG Stop**

No tRNA to complement the termination codon.

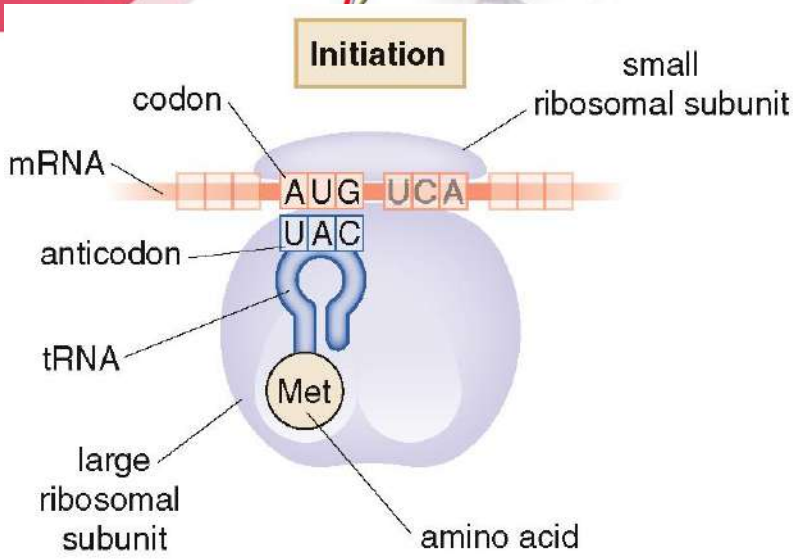
An enzyme releases the complete polypeptide chain from the ribosome.

Amino acids form the three-dimensional structure (active protein).

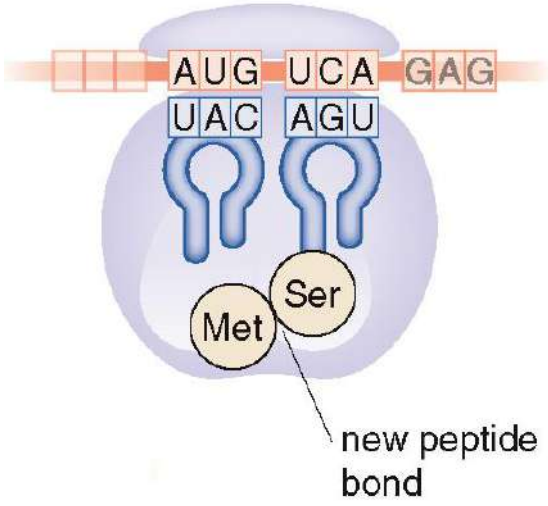
Translation

There are 3 stages in translation:

1. Initiation begins with mRNA binding to the ribosome.

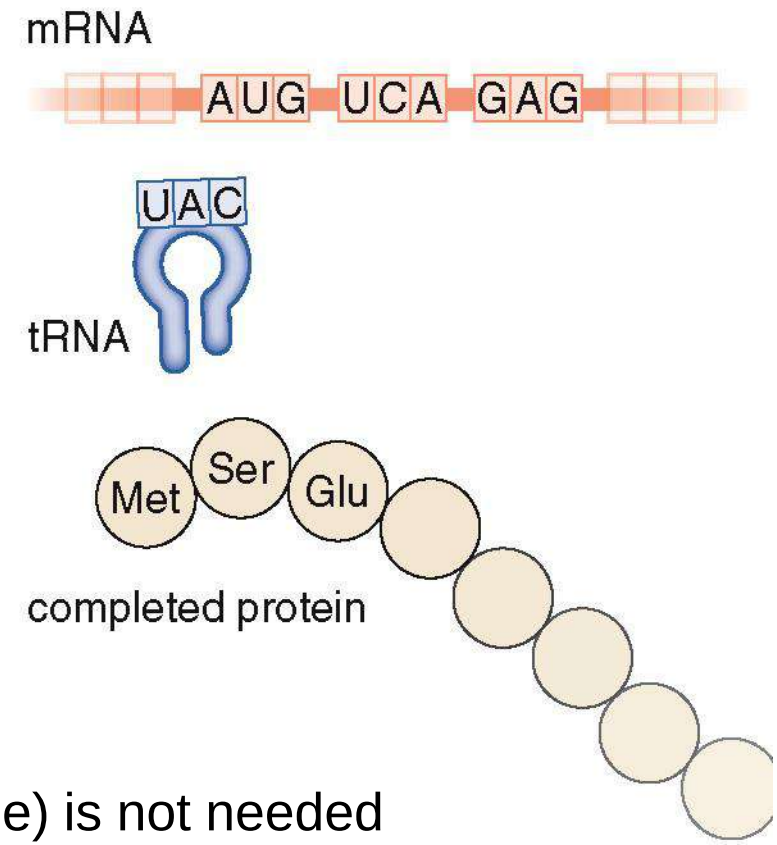


2. Elongation proceeds as the next tRNA molecule delivers the next amino acid, and a peptide bond forms between the two amino acids.

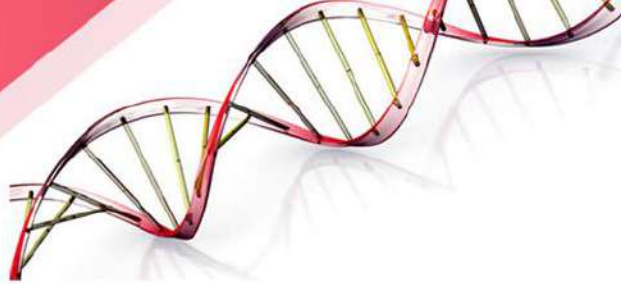


Translation

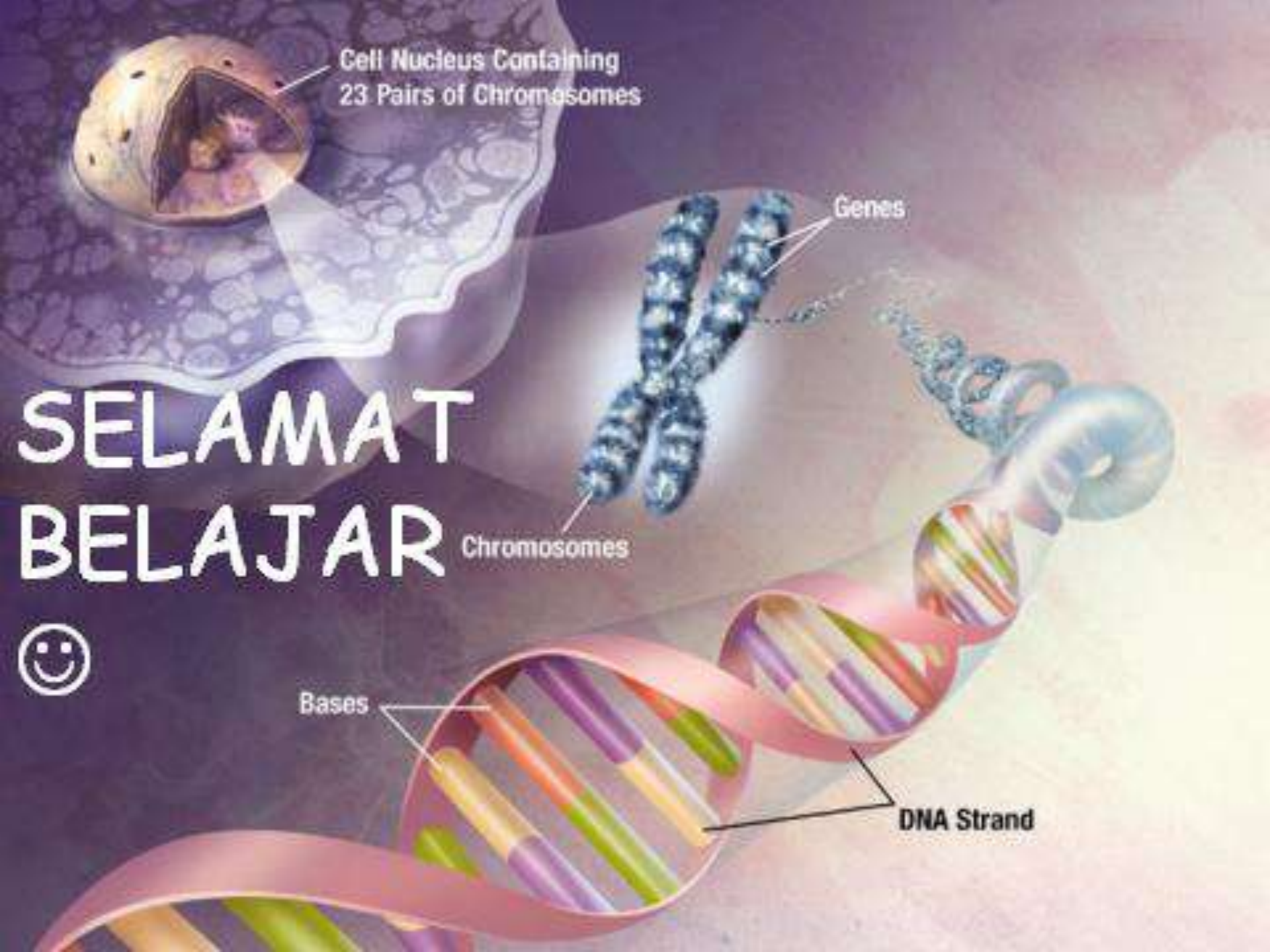
3. Termination: Translation continues until a stop codon (UAA, UAG, or UGA) is reached and the completed protein is released.



Often the first amino acid (methionine) is not needed and it is removed after protein synthesis is complete.

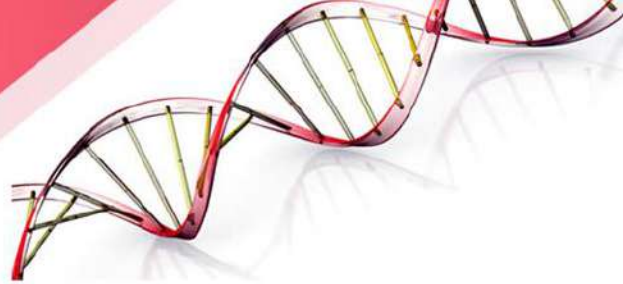


	Transkripsi	Tranlasi
a	Proses mengikat basa nitrogen	Menyusun basa nitrogen menjadi tiga titik berupa kodon
b	Melibatkan rantai antisense pada DNA menjadi RNad	Terjadi pengikatan asam amino yang larut dalam plasma
c	Proses mencetak RNad	Proses menerjemahkan urutan basa molekul RNad dalam urutan asam amino polipeptida
d	Berperan dalam pembentukan protein dari asam amino	Berperan dalam membentuk enzim-enzim
e	Memerlukan basa nitrogen adenine, timin, guanine dan sitosin	Memerlukan basa nitrogen adenine, timin, guanine, dan sitosin


Cell Nucleus Containing
23 Pairs of Chromosomes
Genes
SELAMAT
BELAJAR
Chromosomes
Bases
DNA Strand

End of slide show, click to exit.

MUTASI, REKOMBINAN, PCR, DNA FINGERPRINT



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Mutation

A heritable change in DNA nucleotide sequence.

⇩

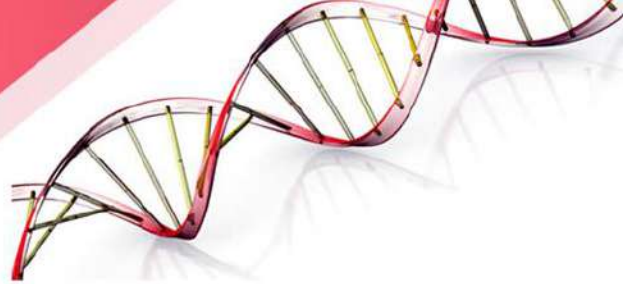
It changes the sequence of amino acids (structure and function of proteins).

Enzyme cannot catalyze.

X rays, Overexpose to sun (UV light), Chemicals (mutagens), or Viruses

However, some mutations are random events.

Effect of Mutation



Somatic cell (nonreproductive cell):

Altered DNA will be limited to that cell and its daughter cells. → Cancer

Germ cell (reproductive cell like an egg or sperm):

All new DNA will contain the same default and it is passed on to the next generation. → Genetic diseases

Type of Mutations

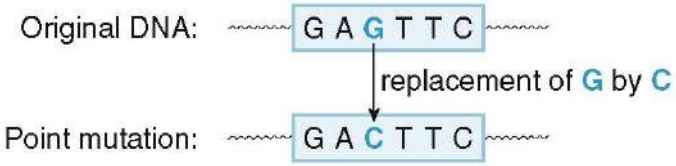
Point (substitution) Mutation

The most common

Replacement of one base in the coding strand of DNA with another.

⇩

Different amino acid



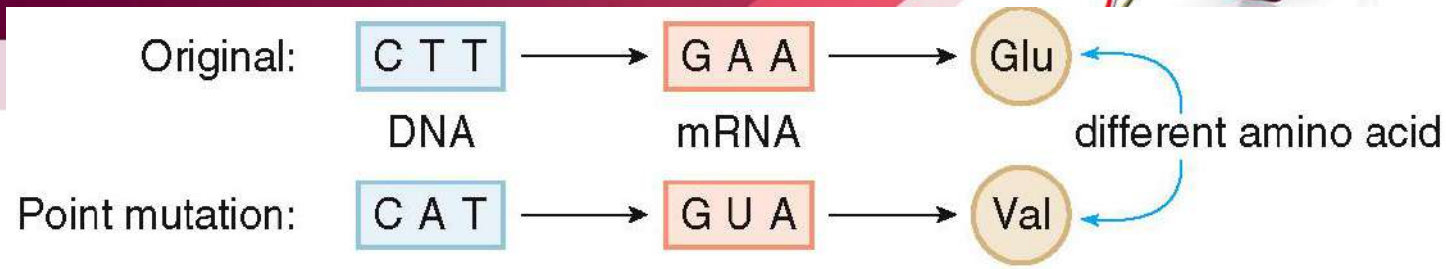
Frameshift Mutation

One or more bases is/are added to or deleted from the normal order of bases in DNA.

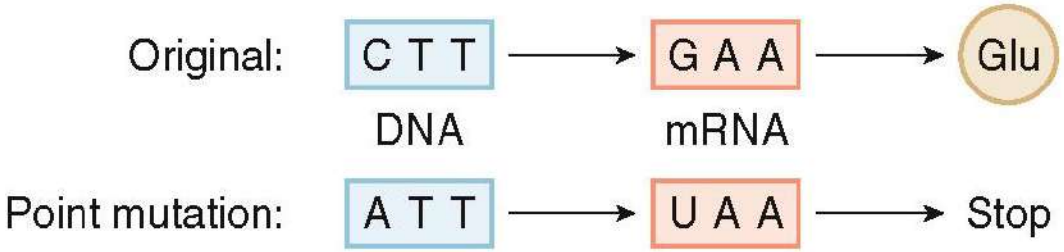
⇩

All the triplets shift over by one base.
Different sequence of amino acids

Point Mutation



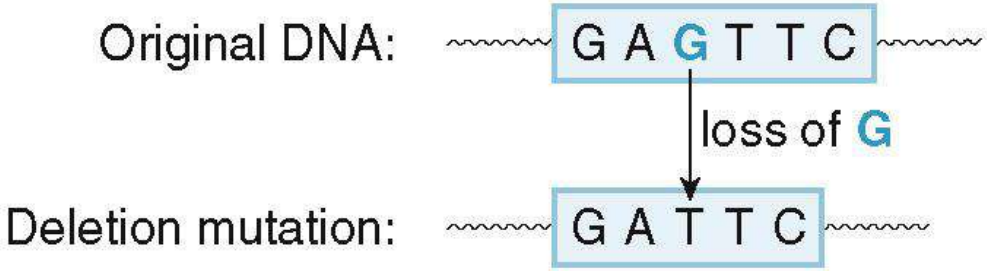
In hemoglobin, substitution of just one amino acid can result in the fatal disease sickle cell anemia.



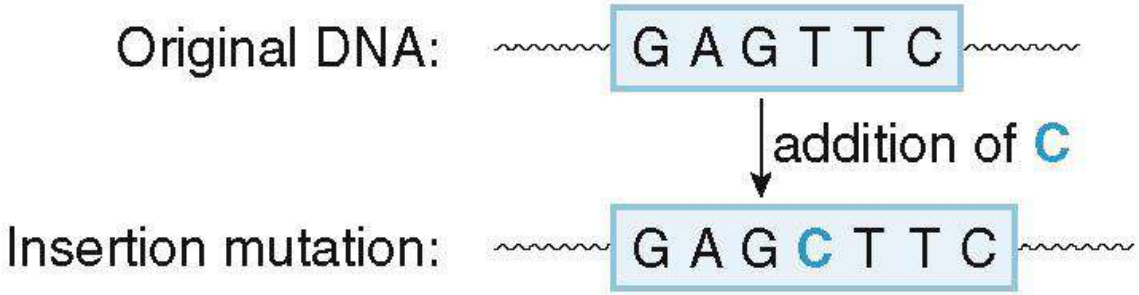
No more amino acids are added. A need protein is not synthesized. The organism may die.

Frameshift Mutation

1. A deletion mutation occurs when one or more nucleotides is/are lost from a DNA molecule.

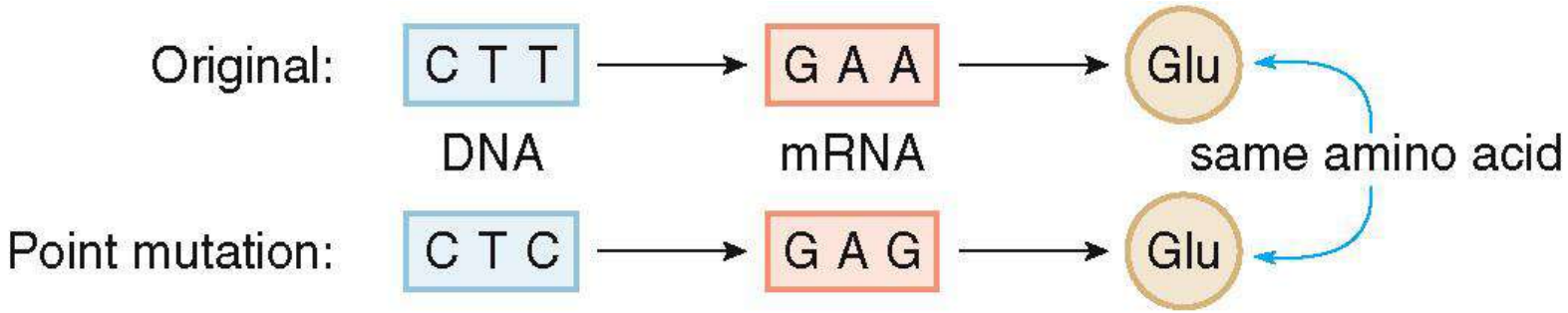


2. An insertion mutation occurs when one or more nucleotides is/are added to a DNA molecule.



Silent Mutation

A silent mutation has a negligible effect to the organism, because the resulting amino acid is identical.



The mutation has no effect.

Recombinant DNA

Recombinant DNA is synthetic DNA that contains segments from more than one source.

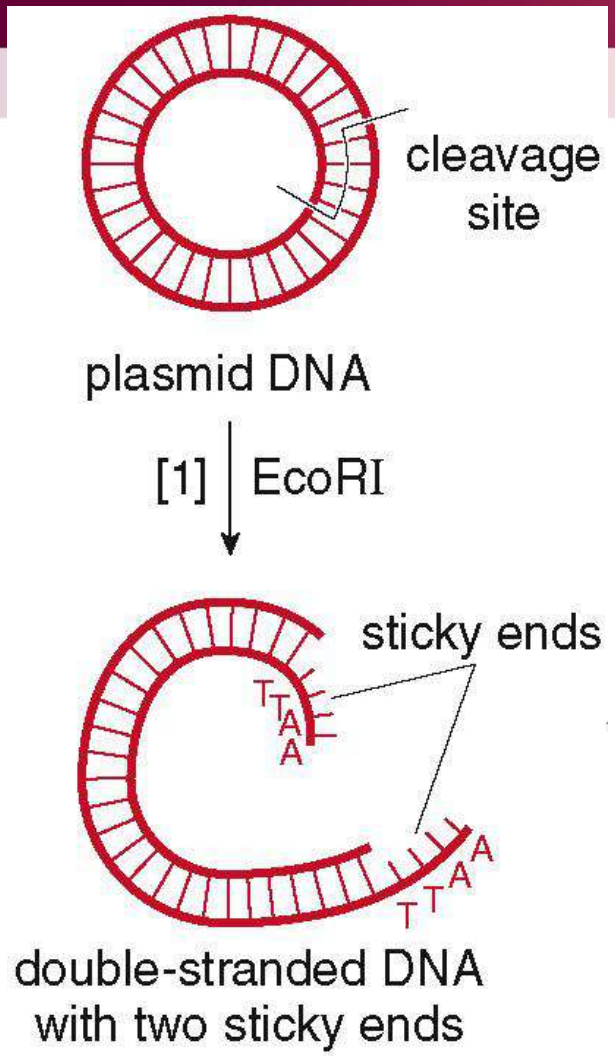
Three key elements are needed to form recombinant DNA:

1. A DNA molecule into which a new DNA segment will be inserted.

2. An enzyme that cleaves DNA at specific locations.

3. A gene from a second organism that will be inserted into the original DNA molecule.

Recombinant DNA



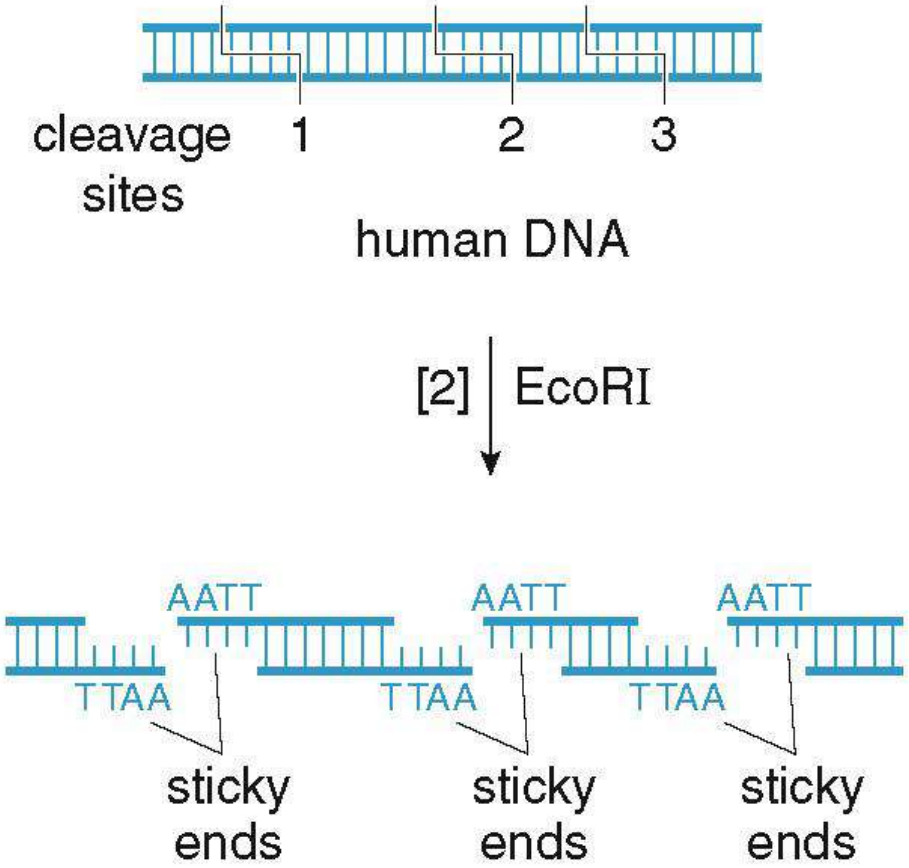
First, bacterial plasmid DNA is cut by the restriction endonuclease EcoRI, which cuts in a specific place.

This gives a double strand of linear plasmid DNA with two ends ready to bond, called sticky ends.

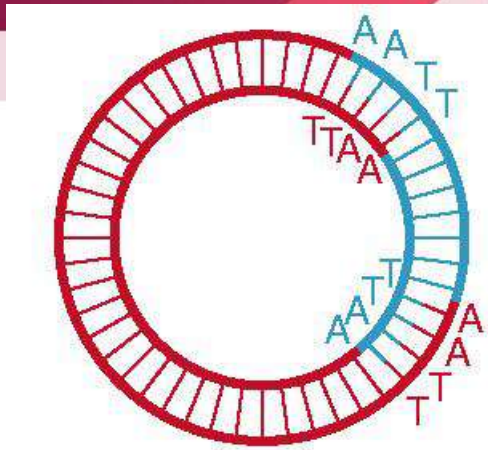
Recombinant DNA

Then, a second sample of human DNA is cut with the same EcoRI.

This forms human DNA segments with sticky ends that are complimentary to the plasmid DNA.



Recombinant DNA



recombinant DNA

Combining the two pieces of DNA (with DNA ligase enzyme) forms DNA containing the new segment.

This DNA chain is slightly larger because of its additional segment.

This new DNA is re-inserted into a bacterial cell. Large amounts of needed proteins can be synthesized by bacteria.

Polymerase Chain Reaction (PCR)

Polymerase chain reaction (PCR) amplifies a specific portion of a DNA molecule, producing millions of exact copies.

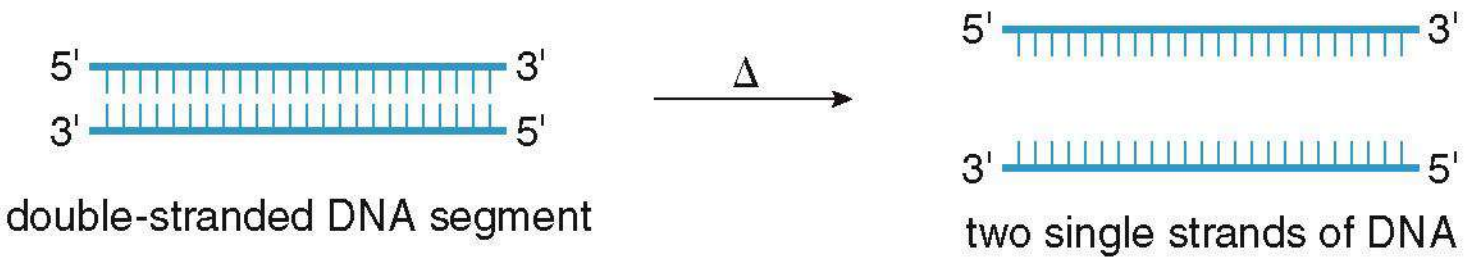
Four elements are needed to amplify DNA by PCR:

1. The segment of DNA that must be copied.
2. Two primers—short polynucleotides that are complementary to the two ends of the segment to be amplified.
3. A DNA polymerase enzyme to catalyze the synthesis of a complementary strand.
4. Nucleoside triphosphates—the source of the A, T, C, and G needed to make the new DNA.

HOW TO Use the Polymerase Chain Reaction to Amplify a Sample of DNA

Step [1]

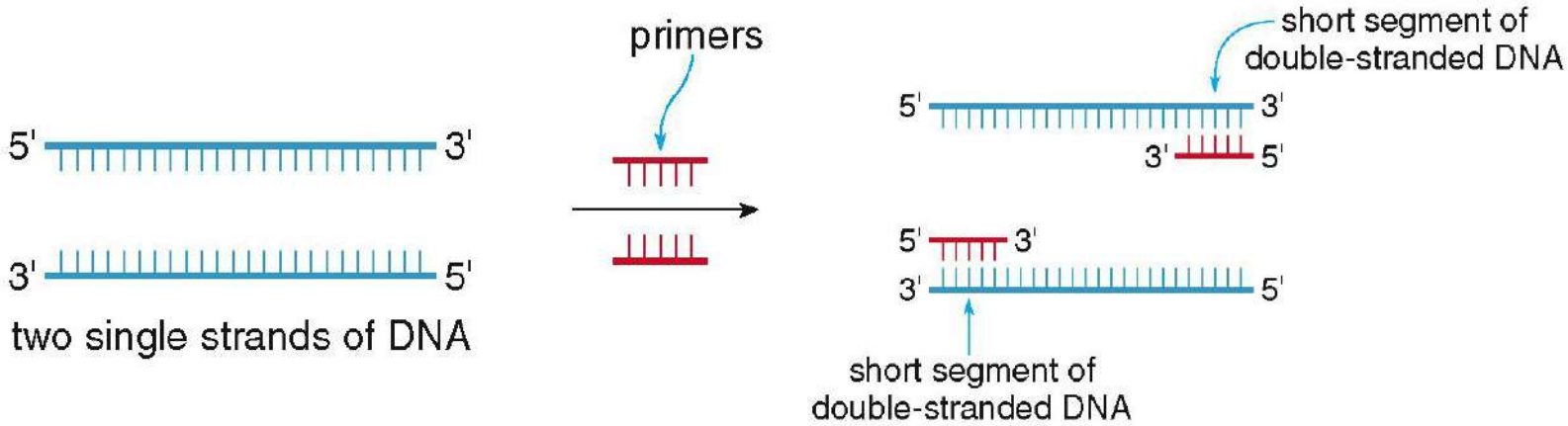
Heat the DNA segment to unwind the double helix to form single strands.



HOW TO Use the Polymerase Chain Reaction to Amplify a Sample of DNA

Step [2]

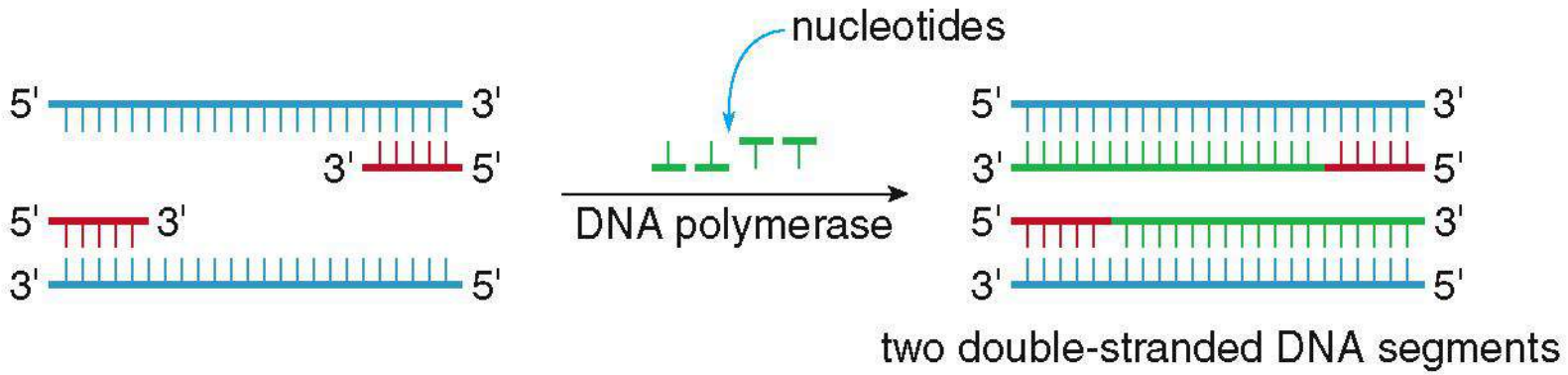
Add primers that are complementary to the DNA sequence at either end of the DNA segment.



HOW TO Use the Polymerase Chain Reaction to Amplify a Sample of DNA

Step [3]

Use a DNA polymerase and added nucleotides to lengthen the DNA segment.



After each cycle the amount of DNA is doubled, so after 20 cycles, 1,000,000 copies have been made.

DNA Fingerprinting

The DNA of each individual person is unique, so DNA can be used as a method of identification.

- Any type of cell (skin, saliva, semen, blood, etc.) can be used to obtain a DNA fingerprint.

- The DNA is first amplified by PCR, and then cut into fragments by restriction enzymes.

- The DNA fragments are then separated by size by gel electrophoresis.

DNA Fingerprinting

DNA fragments can be visualized on X-ray film after they have been separated: