DNA, RNA, & Flow of Genetic Information

DNA & RNA are long linear polymers, called nucleic acids.

Genetic information is stored in a sequence of 4 kinds of bases along the chain, and is passed from one generation to the next

Chapter 5: Outline

5.1 A nucleic acid consists of 4 kinds of bases linked to a sugar-phosphate backbone

5.2 A pair of nucleic acid chains with complimentary sequences can form a double-helical structure

5.3 DNA is replicated by polymerases that take instructions from templates

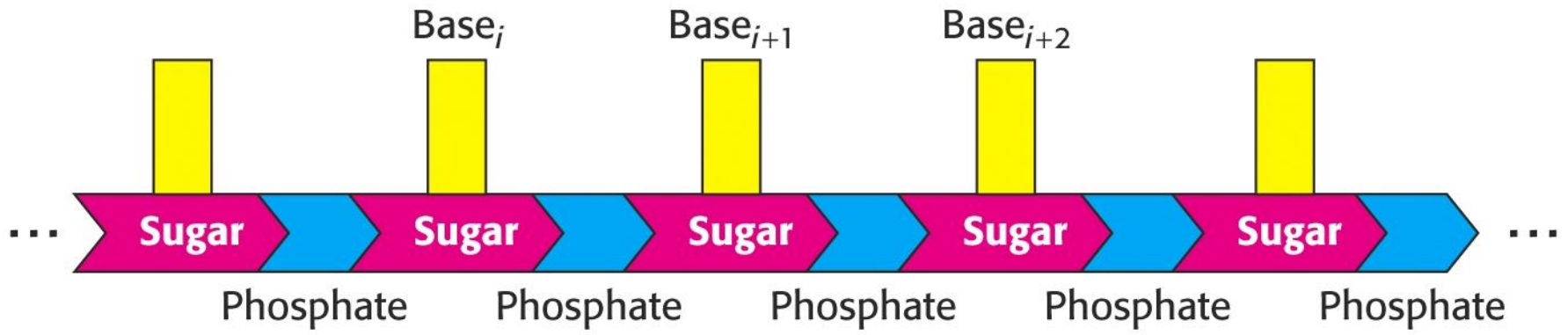
5.4 Gene expression is the transformation of DNA information into functional molecules

5.5 Amino acids are encoded by groups of three bases starting from a fixed point

5.6 Most eucaryotic genes are mosaics of introns & exons

Polymeric structure of nucleic acids

Linear polymers of covalent structures, built from similar units



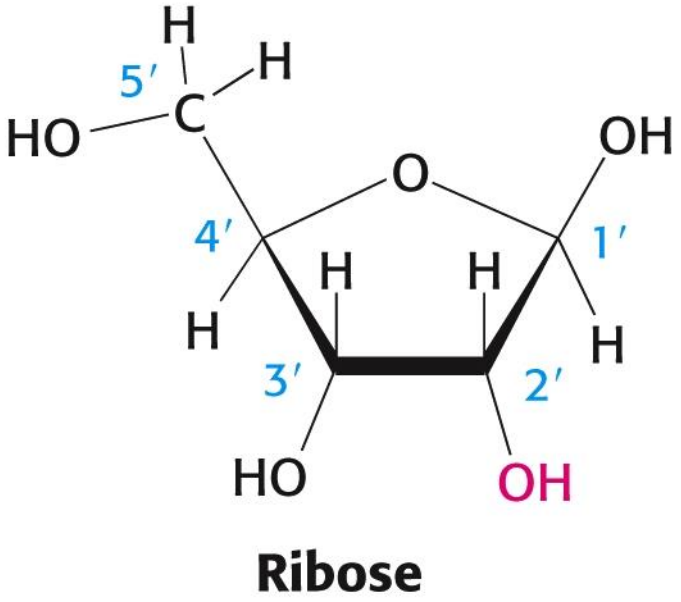
Sequence of bases uniquely characterizes nucleic acids
Represents a form of linear information

Backbone is constant: repeating units of sugar-phosphate

Different pentose sugars in RNA & DNA

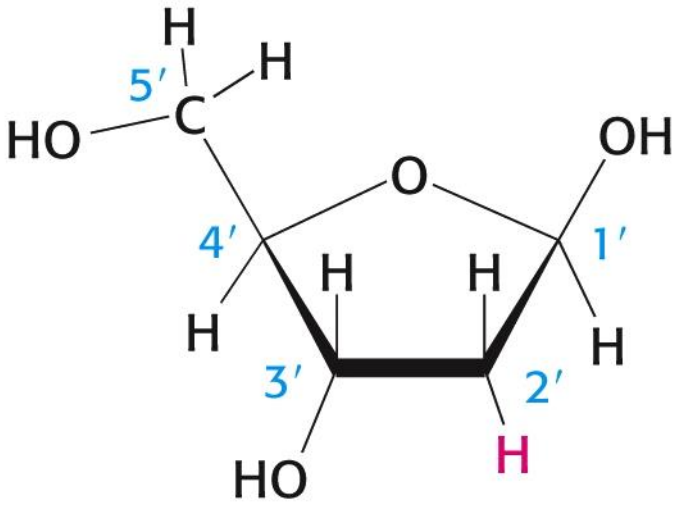
TEST

RNA



Ribose

DNA



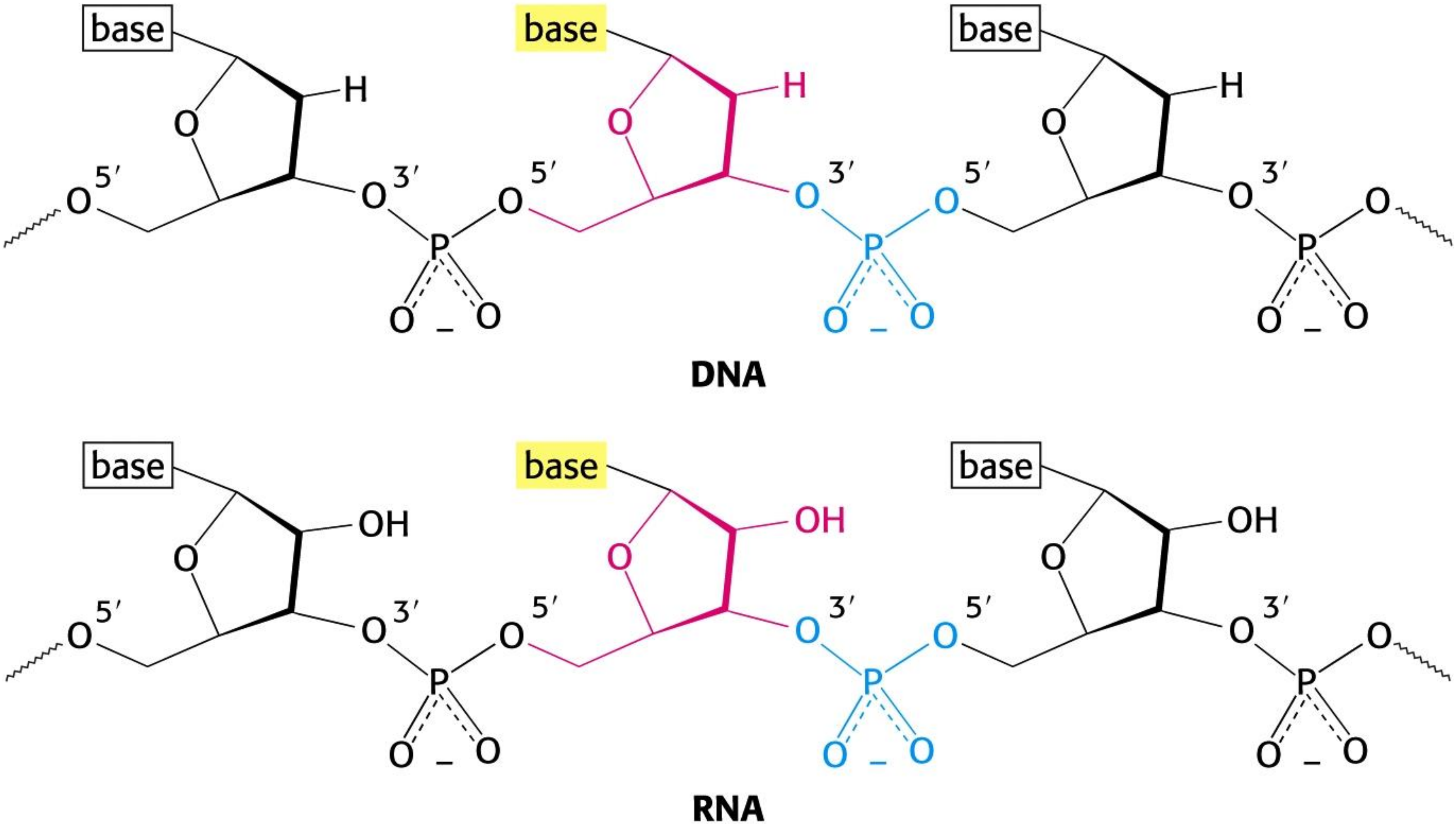
Deoxyribose

Sugar carbons have prime numbers, to distinguish them from atoms in bases

TEST

Backbone of DNA & RNA

3’-to-5’ phosphodiester linkages

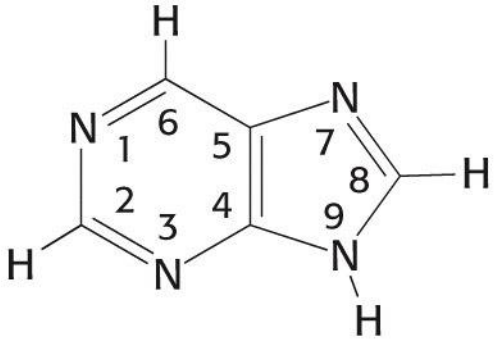


Sugar, red. Phosphate, blue

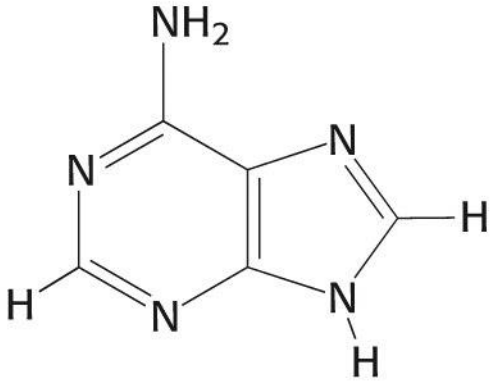
TEST

Purines & Pyrimidines

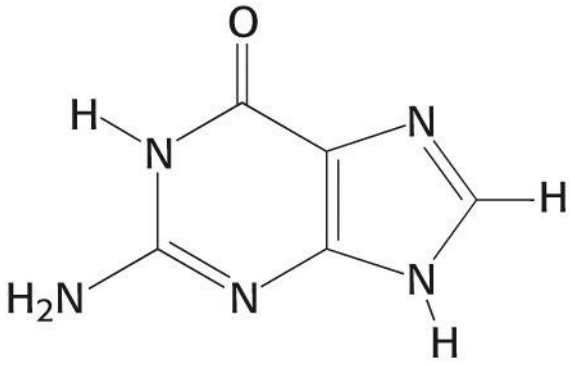
PURINES



Purine

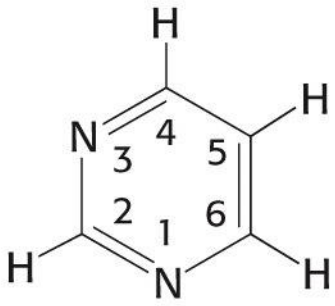


Adenine

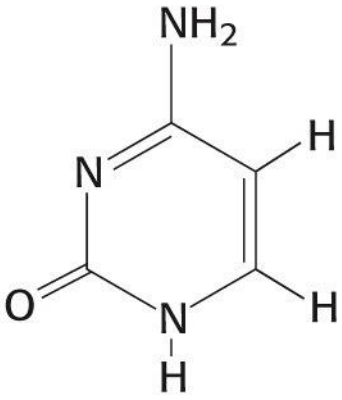


Guanine

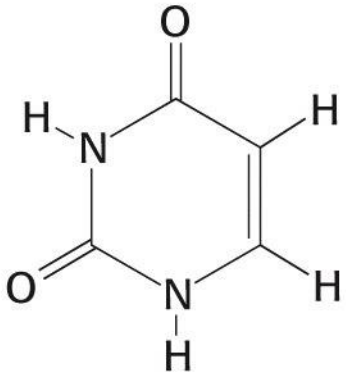
PYRIMIDINES



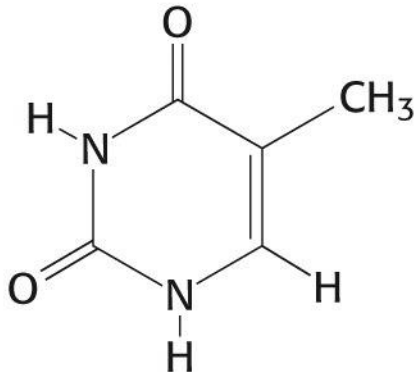
Pyrimidine



Cytosine



Uracil



Thymine

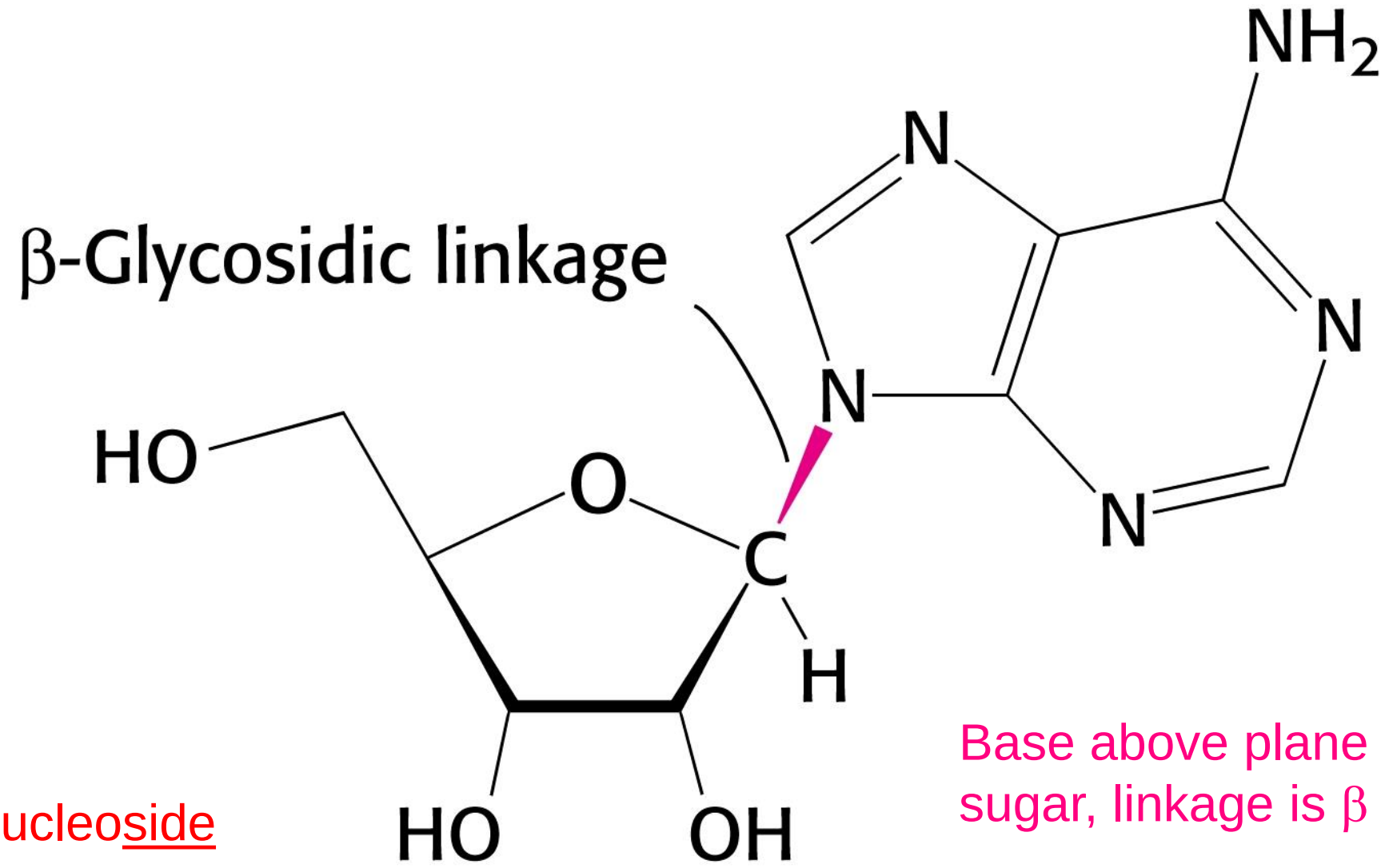
Note: ring atom #s

RNA

DNA

TEST

Sugar - base linkage



Nucleoside

Base above plane of sugar, linkage is β

RNA: adenosine, guanosine, cytidine, & uridine

DNA: deoxyadenosine, deoxyguanosine, deoxycytidine, & thymidine

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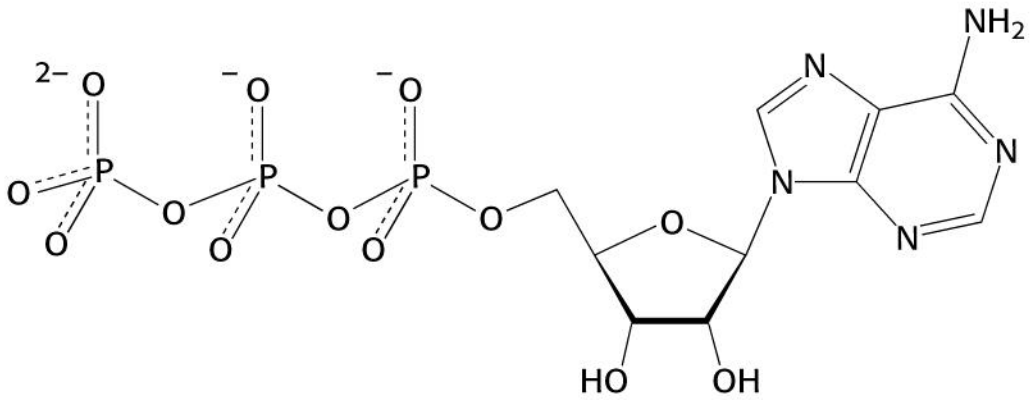
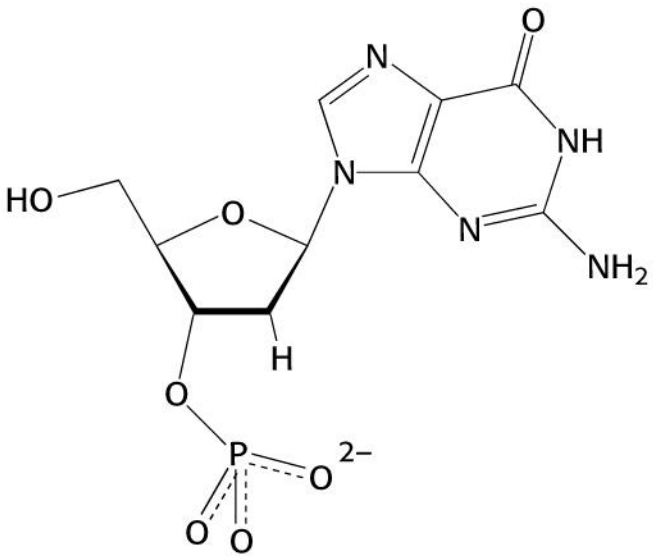
Nucleotides: monomeric units of nucleic acids

Adenosine 5’-triphosphate

Deoxyguanosine
3’ monophosphate



5′-ATP



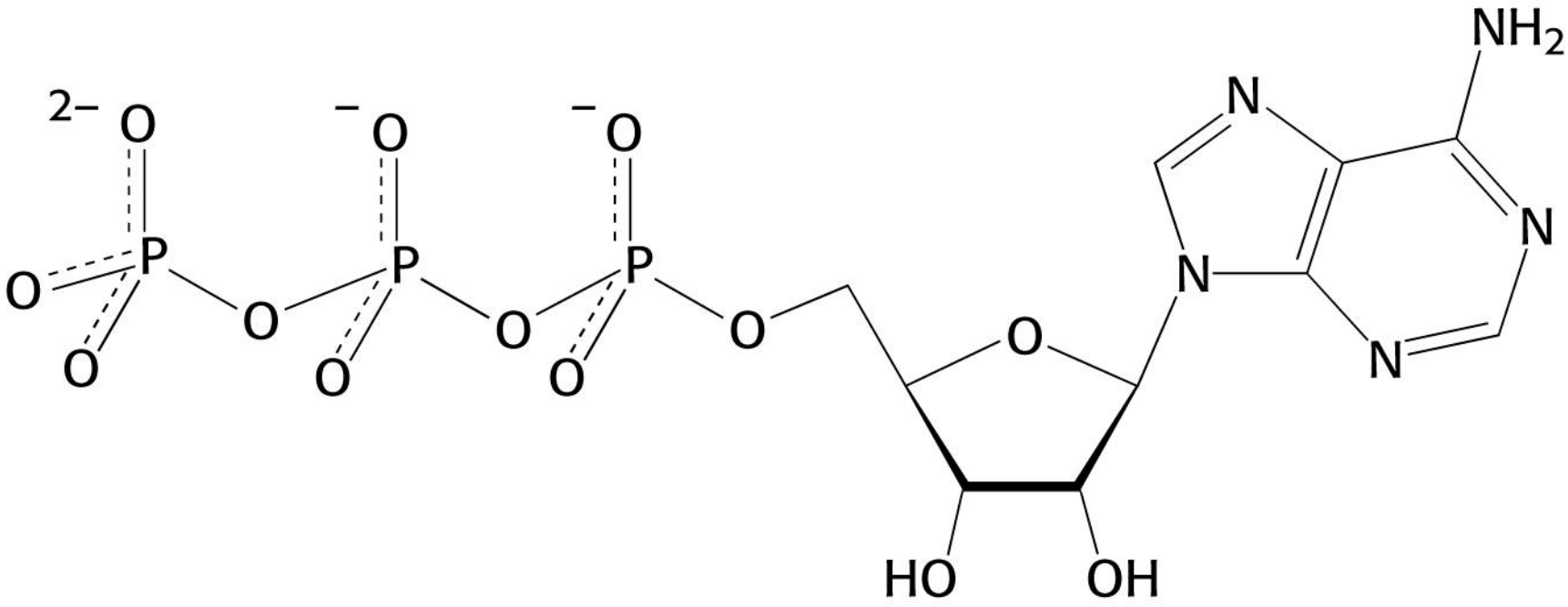
3′-dGMP

5’ nucleotide - most common

3’ nucleotide

Nucleotide: nucleoside joined to one or more phosphate groups by an ester linkage

Adenosine 5’-triphosphate

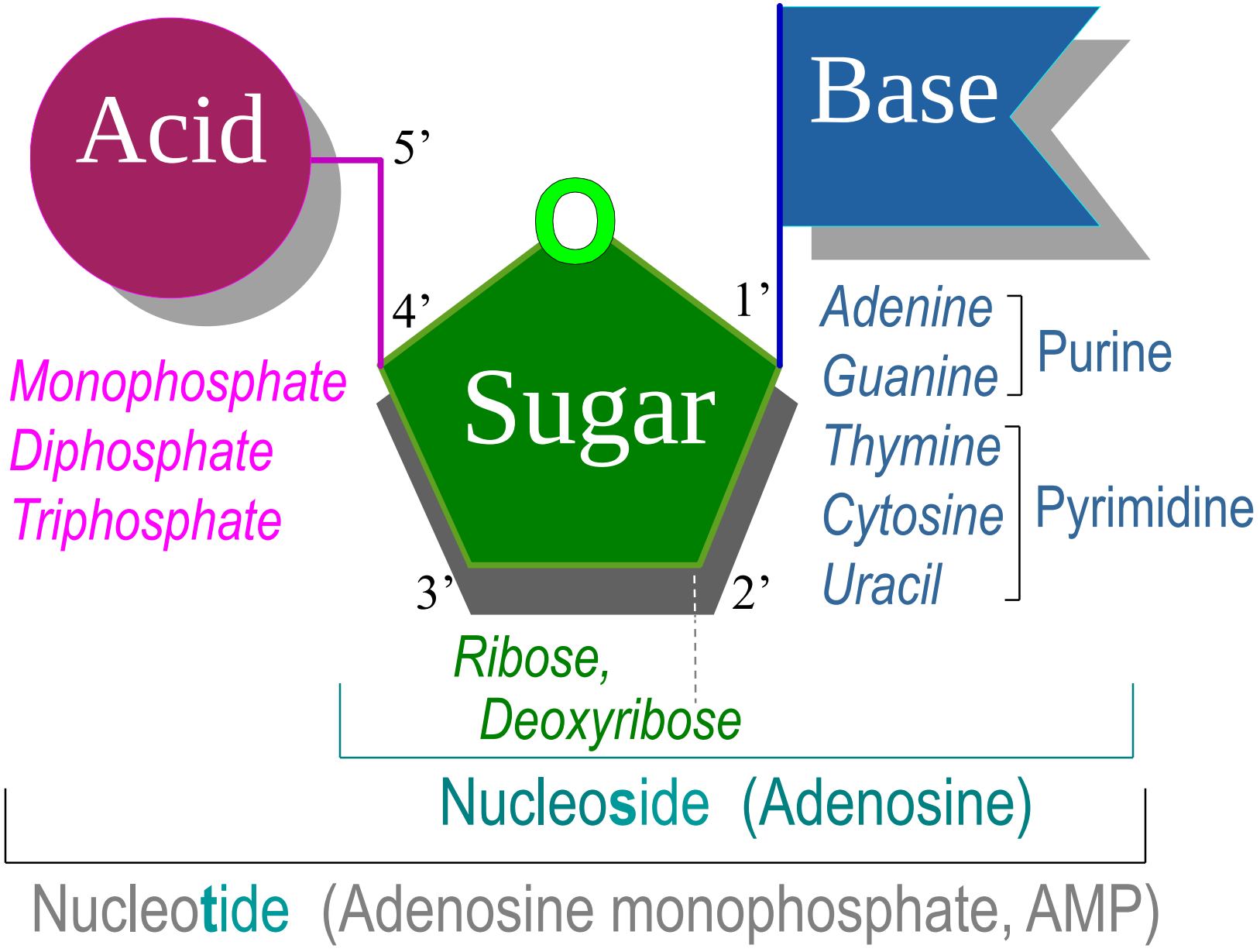


5′-ATP

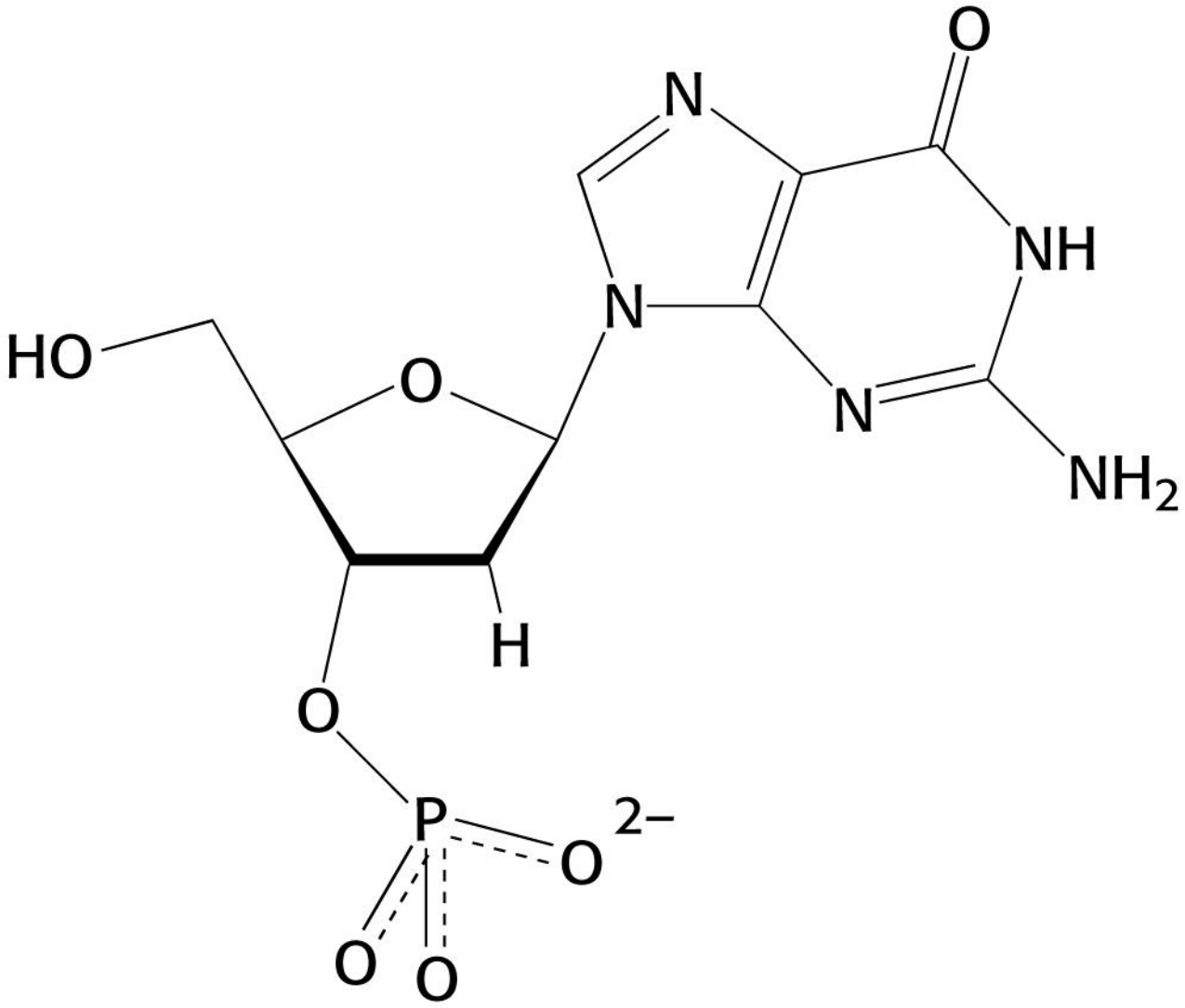
Adenosine linked to sugar C1’

Triphosphate linked to sugar C5’

Basic Structure of Nucleic Acids

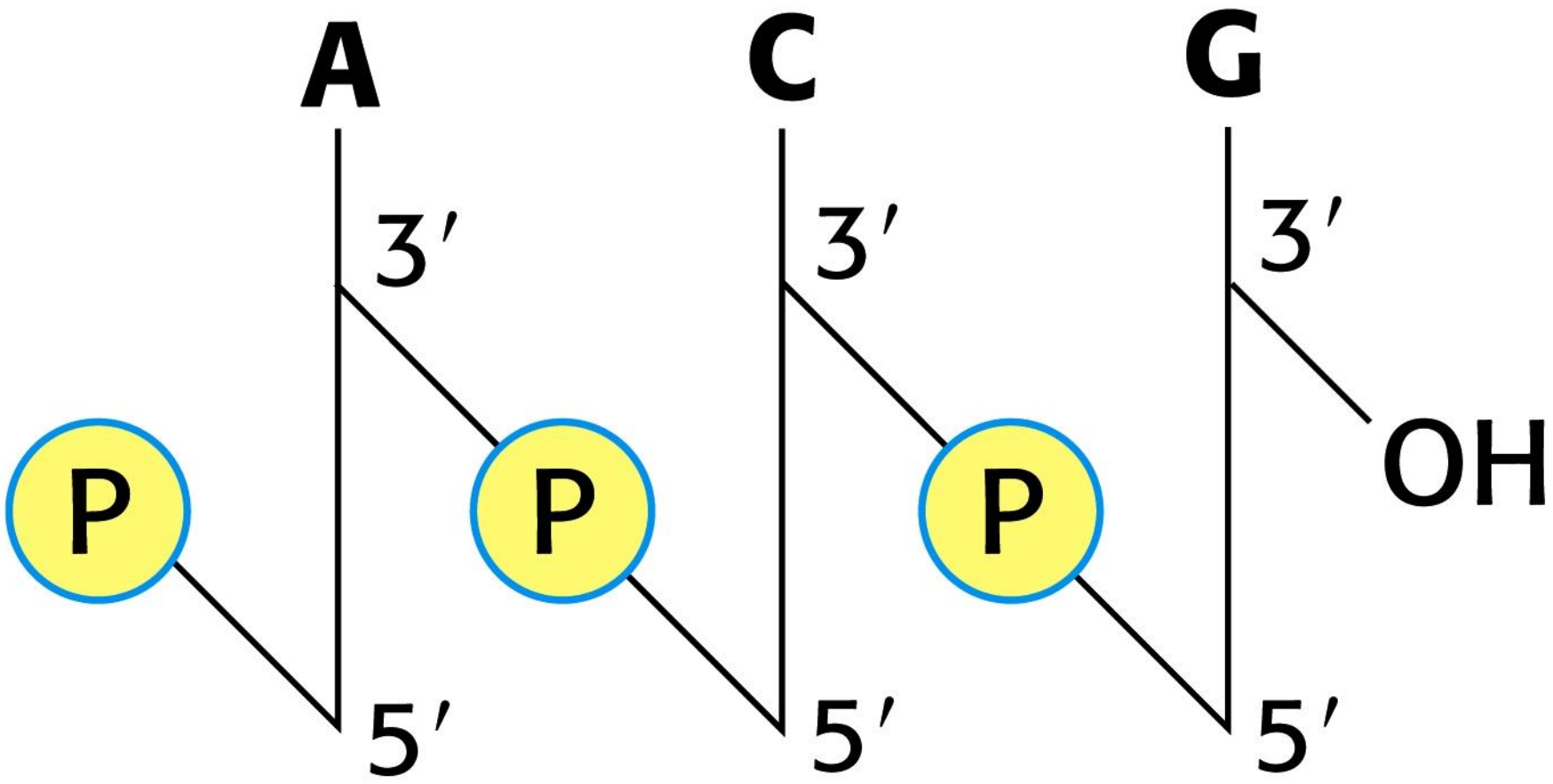


Deoxyguanosine 3’-monophosphate



3′-dGMP

Structure of DNA chain



5’ end, phosphate attached

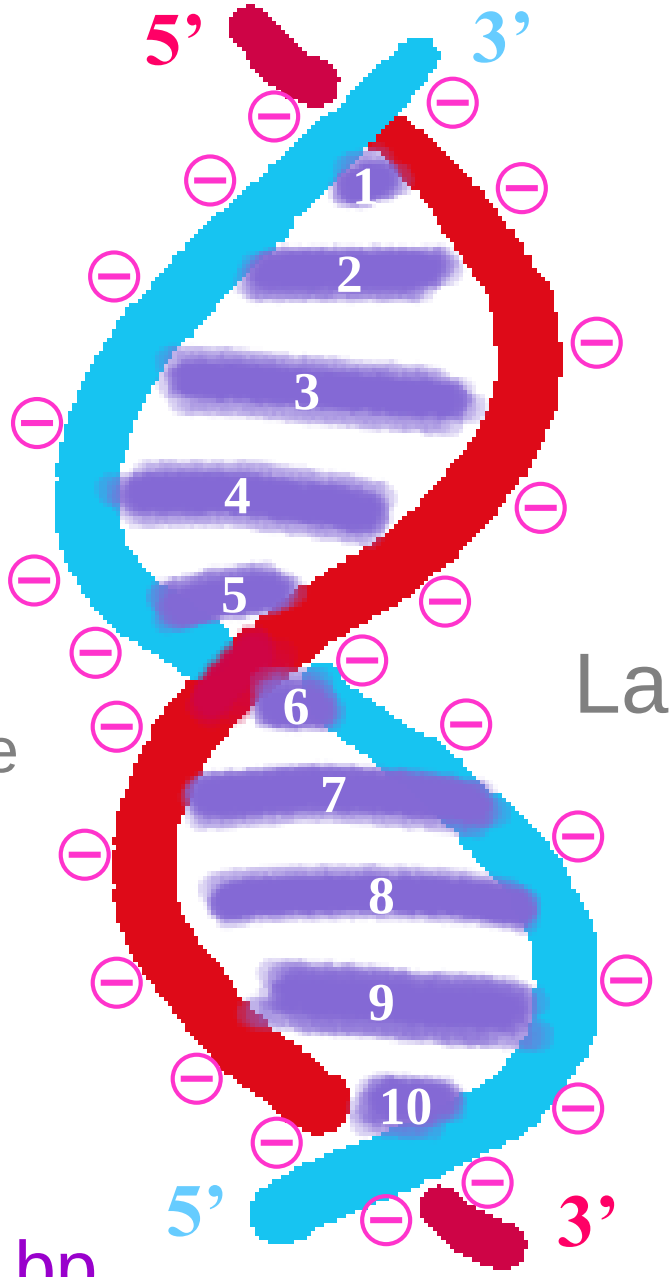
3’ end, free hydroxyl group

The Two Chains of DNA Are Antiparallel

5’ pApTpCpGpApTpCpG-OH 3’

3’ HO-TdAdGdCdTdAdGdC 5’

Characteristics of Double Helix



Small groove

Large groove

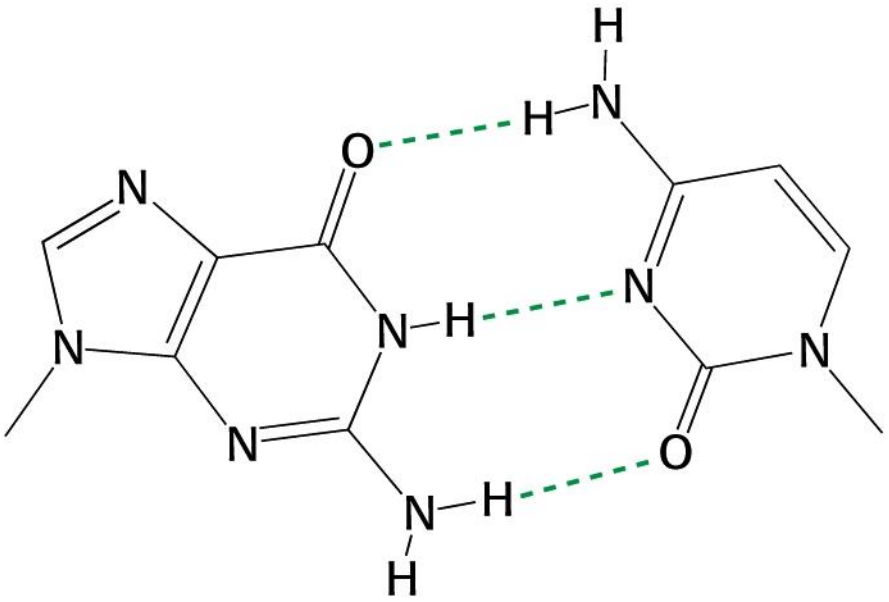
36 Å

1 Twist = 10.5 bp

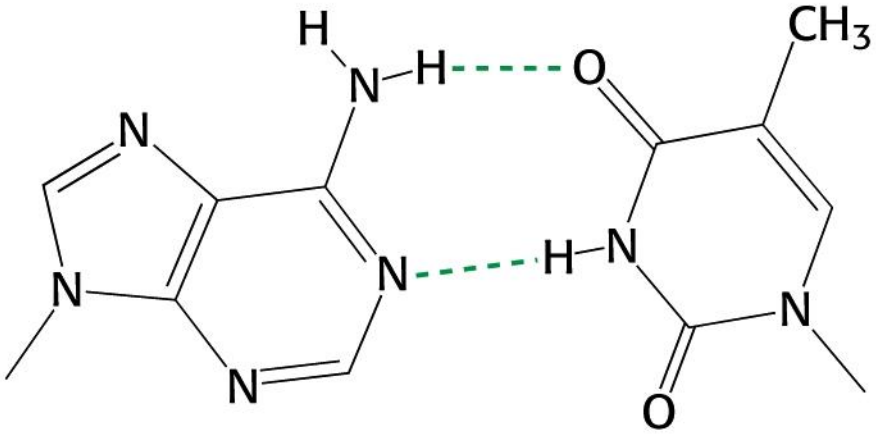
Juang RH (2004) BCbasics

Watson and Crick base pairs

Essentially the same shape



Guanine **Cytosine**



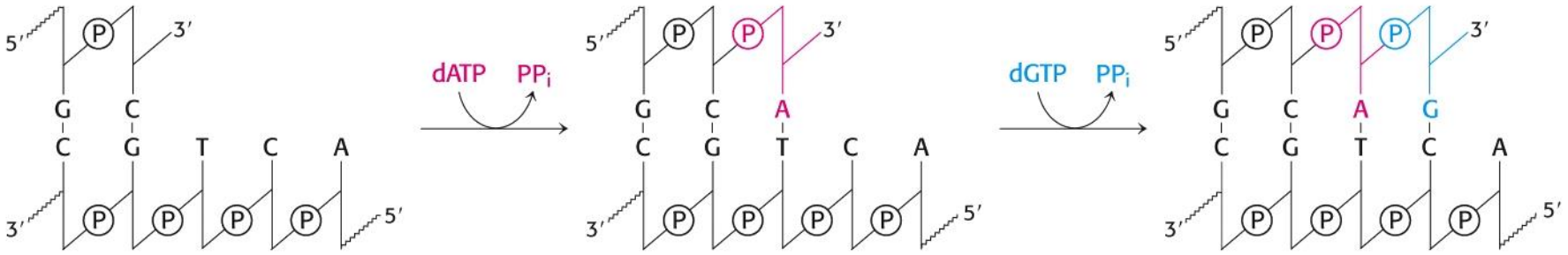
Adenine **Thymine**

DNA polymerization reaction

By DNA polymerase

Step by step addition of deoxyribonucleotide units to a DNA chain

New DNA chain assembled directly on a preexisting DNA template



Primer & template required

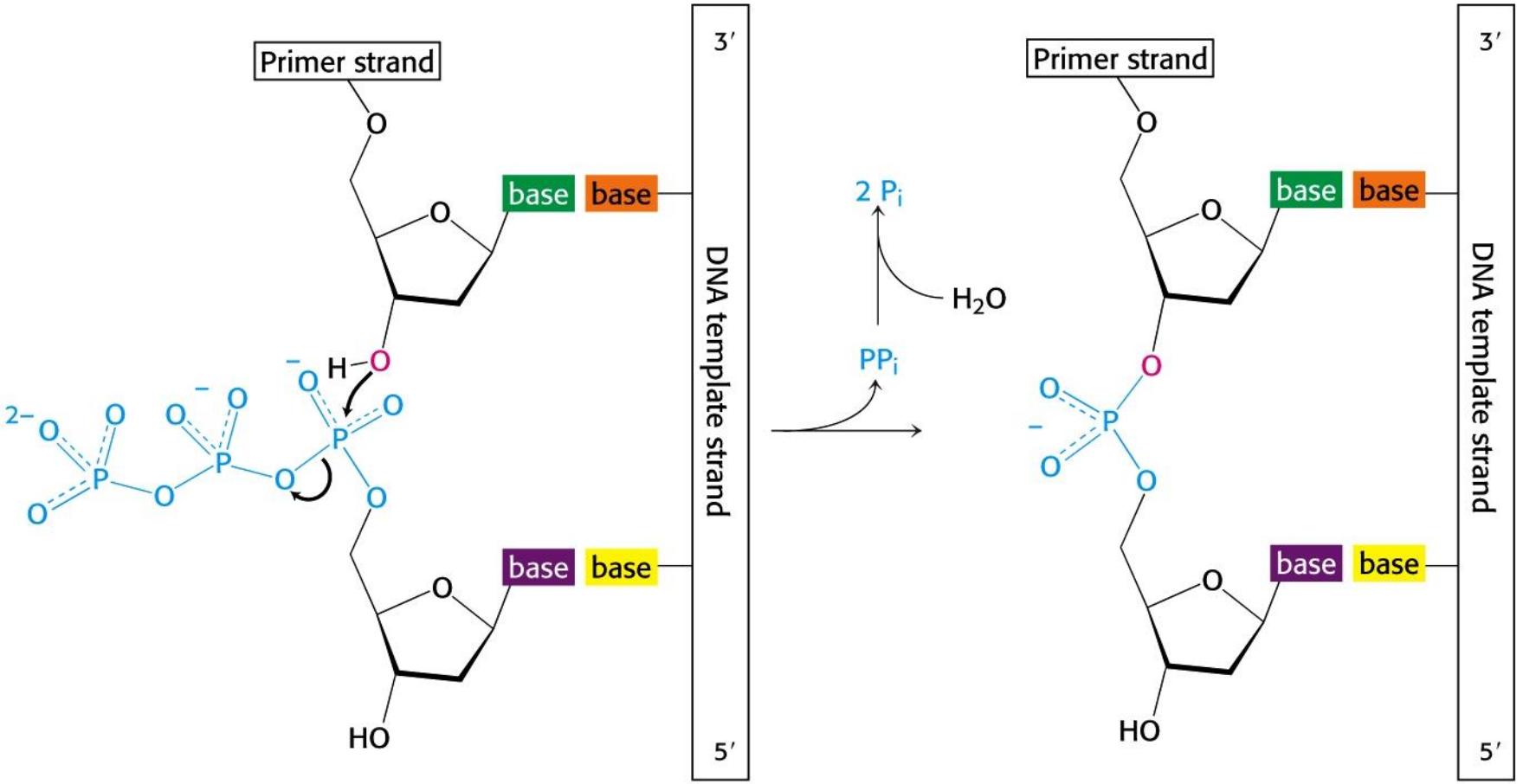
Activated precursors required: dATP, dGTP, TTP, dCTP

Also required: Mg^{2+} ion

DNA replication, phosphodiester bridge

Nucleophilic attack by 3’ -hydroxyl group of primer on innermost phosphorus atom of deoxynucleotide triphosphate (dNTP)

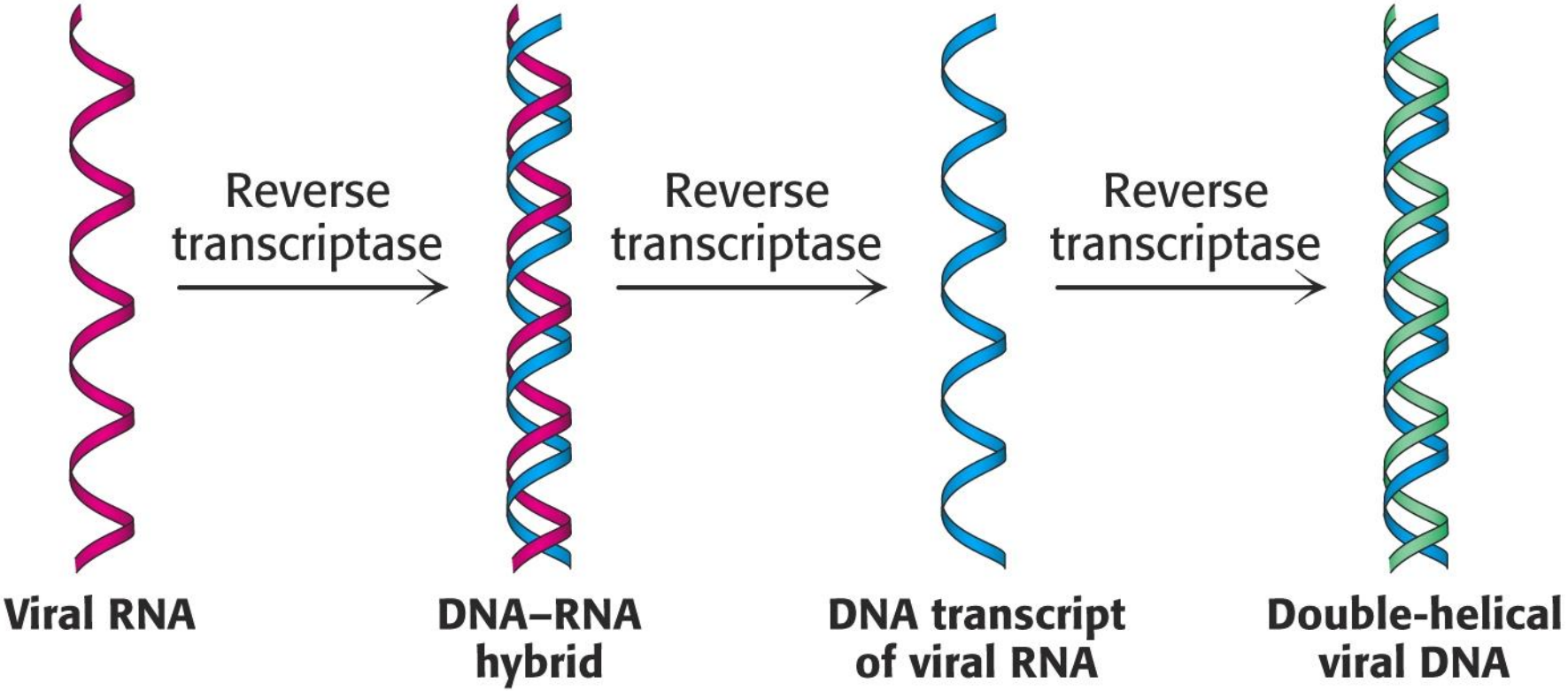
Elongation proceeds, 5’ -to- 3’



Hydrolysis of pyrophosphate (PP_i) helps drive polymerization

Retroviruses reverse flow of information

Reverse transcriptase brought into cell by the virus (eg. HIV-1)



ssRNA genome

Incorporated into host DNA

Roles of RNA in gene expression

TABLE 5.2 **RNA molecules in *E. coli***

Type	Relative amount (%)	Sedimentation coefficient (S)	Mass (kd)	Number of nucleotides
Ribosomal RNA (rRNA)	80	23	1.2×10^3	3700
		16	0.55×10^3	1700
		5	3.6×10^1	120
Transfer RNA (tRNA)	15	4	2.5×10^1	75
Messenger RNA (mRNA)	5		Heterogeneous	

Messenger RNA: template for translation (protein synthesis)

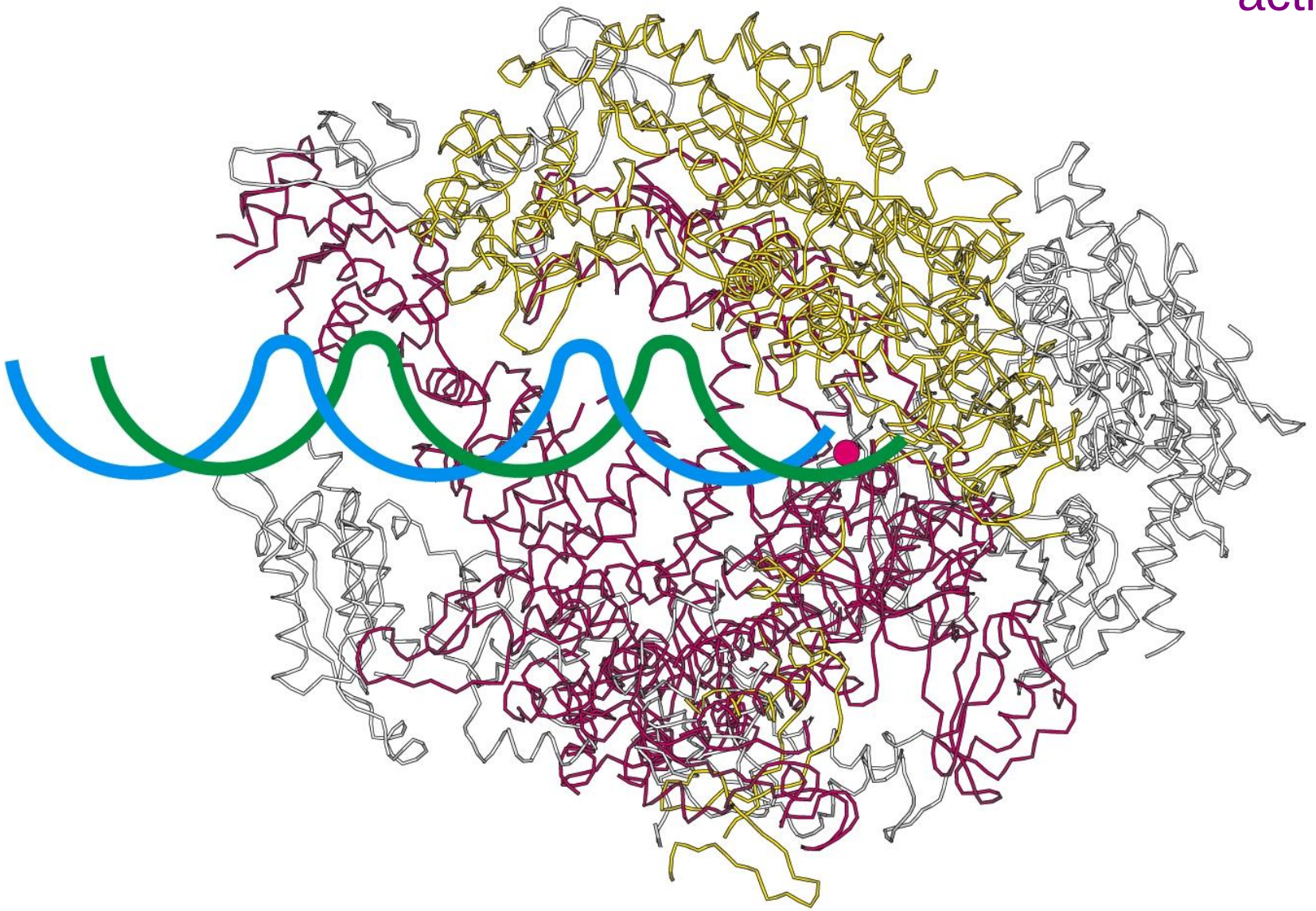
Transfer RNA: carriers of activated AAs to ribosomes
(at least one kind for each of 20 AAs)

Ribosomal RNA: major component of ribosomes
(play structural and catalytic roles)

RNA polymerase

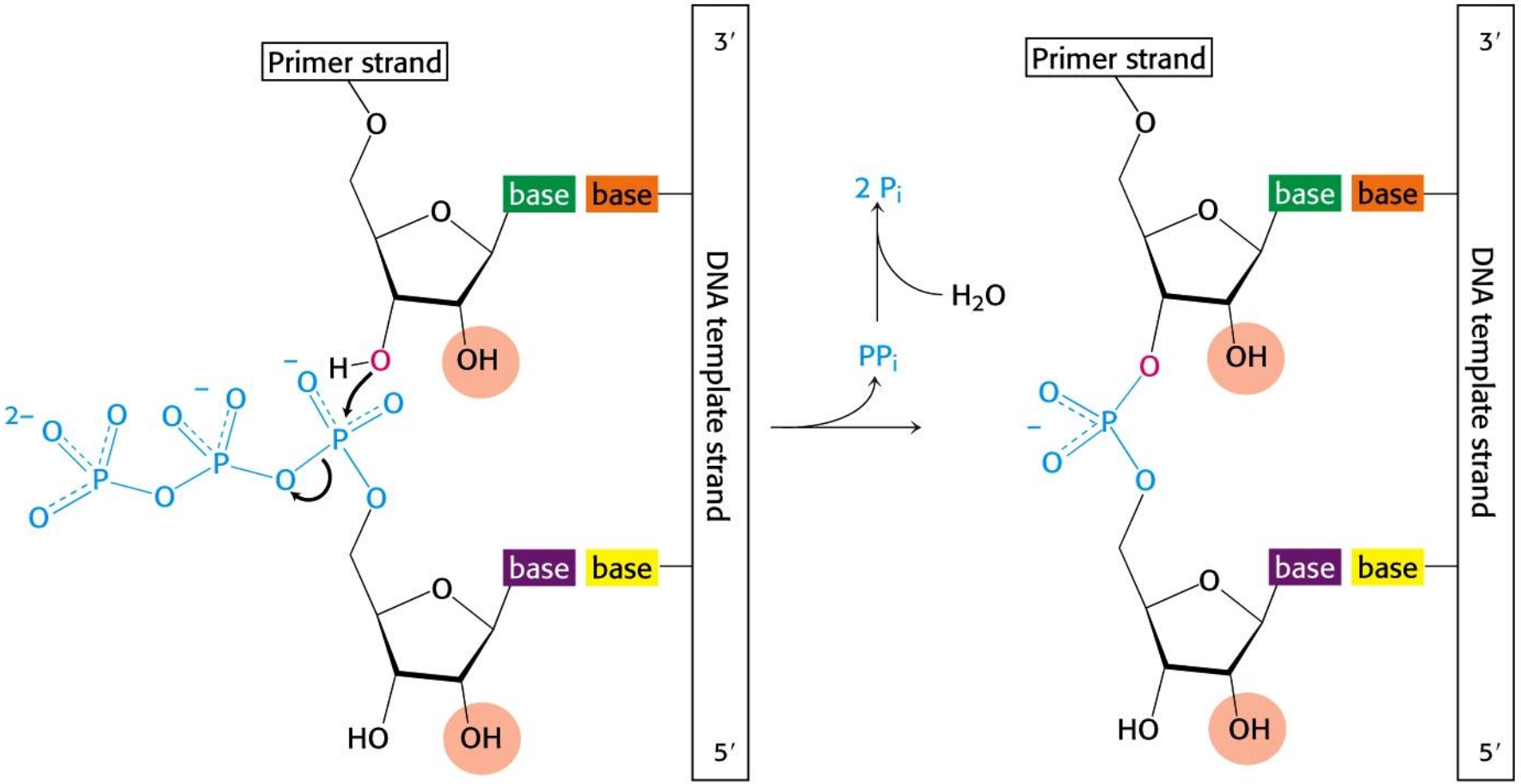
"claw" shape to hold DNA to be transcribed

Mg^{2+} ion at active site



Transcription reaction - RNA polymerase

Nucleophilic attack by 3’ hydroxyl group



Requirements: a template, activated precursors (NTPs), &
Divalent metal ion, Mg^{2+} or Mn^{2+}

RNA polymerase: instructions from DNA templates

TABLE 5.3 Base composition (percentage) of RNA synthesized from a viral DNA template

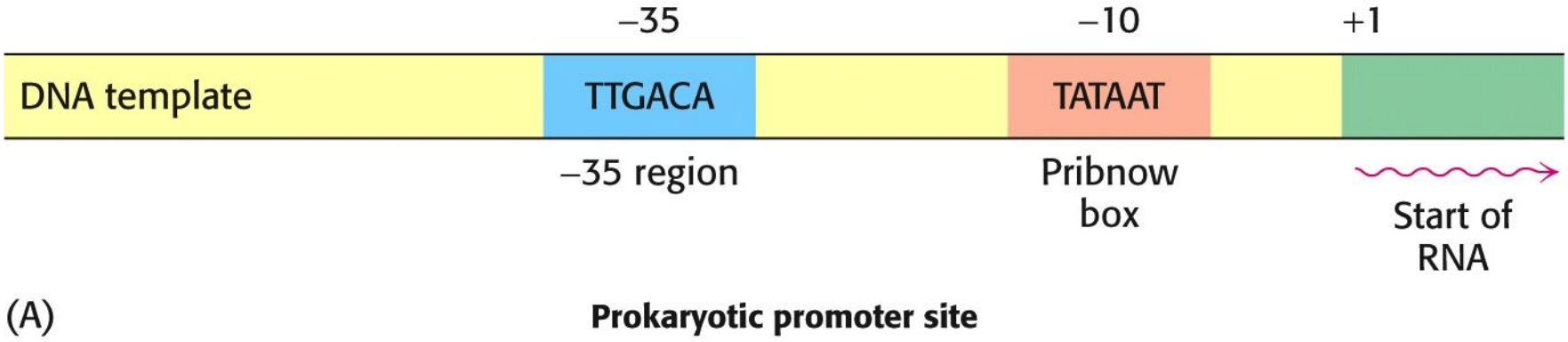
DNA template (plus strand of ϕX174)		RNA product	
A	25	25	U
T	33	32	A
G	24	23	C
C	18	20	G

mRNA & DNA complementarity

mRNA sequence is the compliment of that of the DNA template & is the same as that of the coding DNA strand, except for T in place of U

Prokaryotic promoter

Consensus sequences centered at -10, & -35

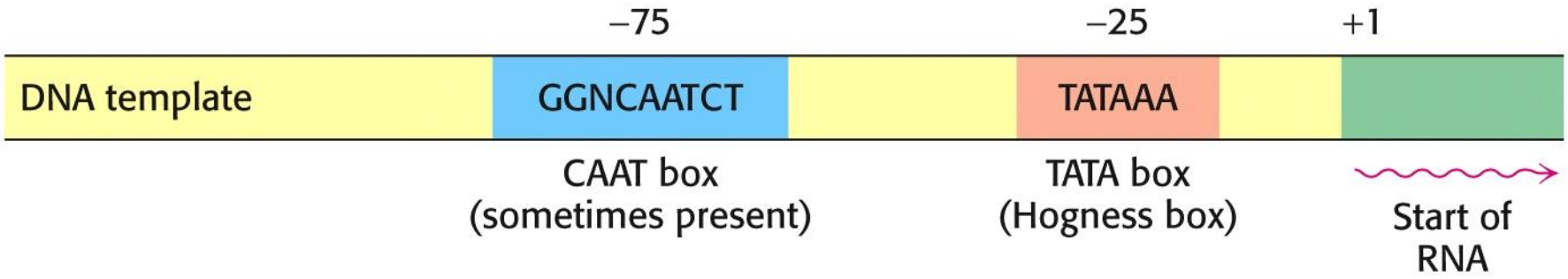


(A) **Prokaryotic promoter site**

Promoter sites specifically binds RNA polymerase, & determine where transcription begins

Eukaryotic promoter

Consensus sequences centered at -25 & -75



(B) **Eukaryotic promoter site**

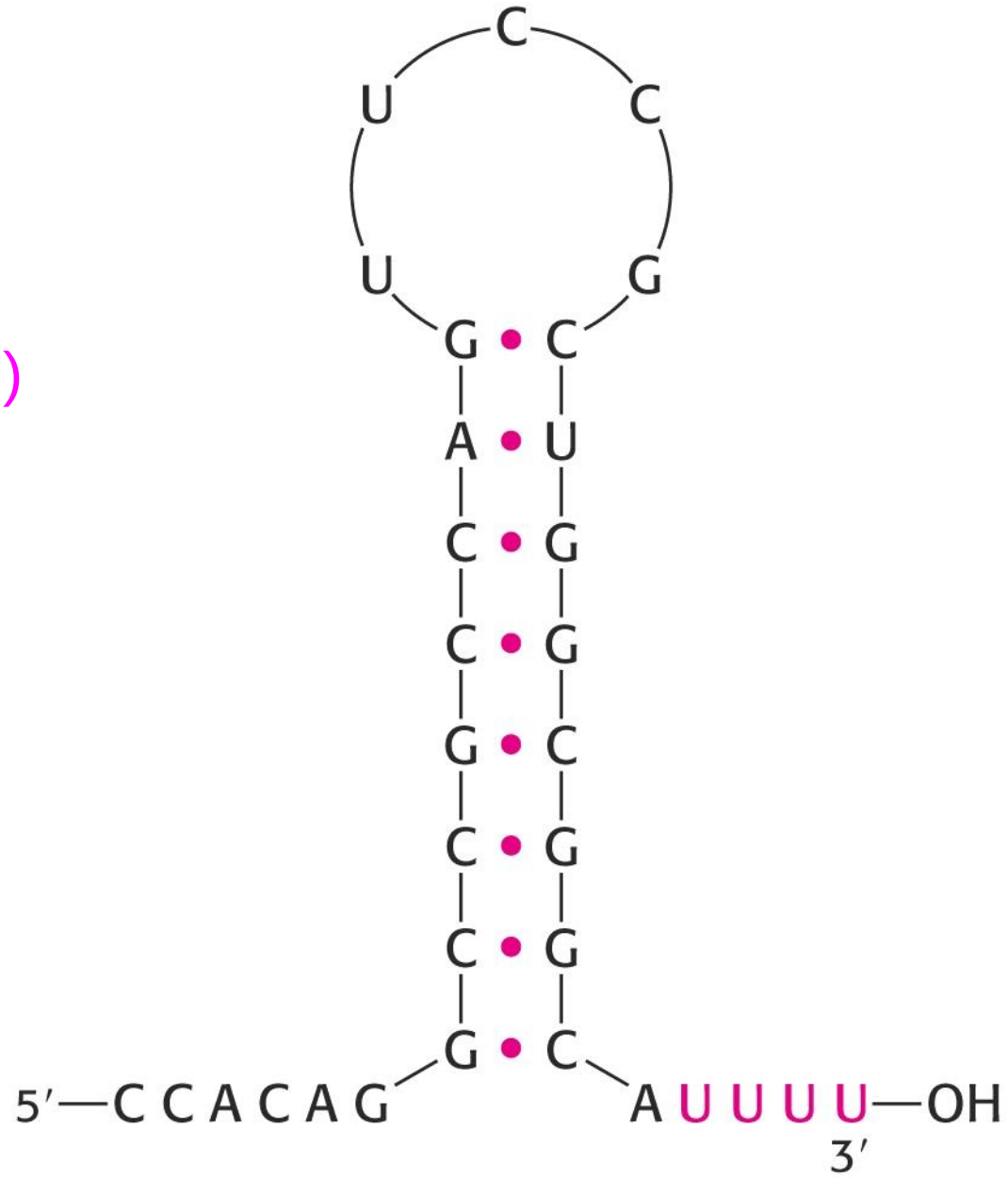
Eukaryotic promoters are further stimulated by enhancer sequences (can be at a distance of several kb from start site on either its 5' or 3' side

Termination signal in E. coli

Sequence at 3' end of mRNA:

Hairpin loop followed by a string of uridines (U)

Alternatively, transcription ended by action of Rho protein



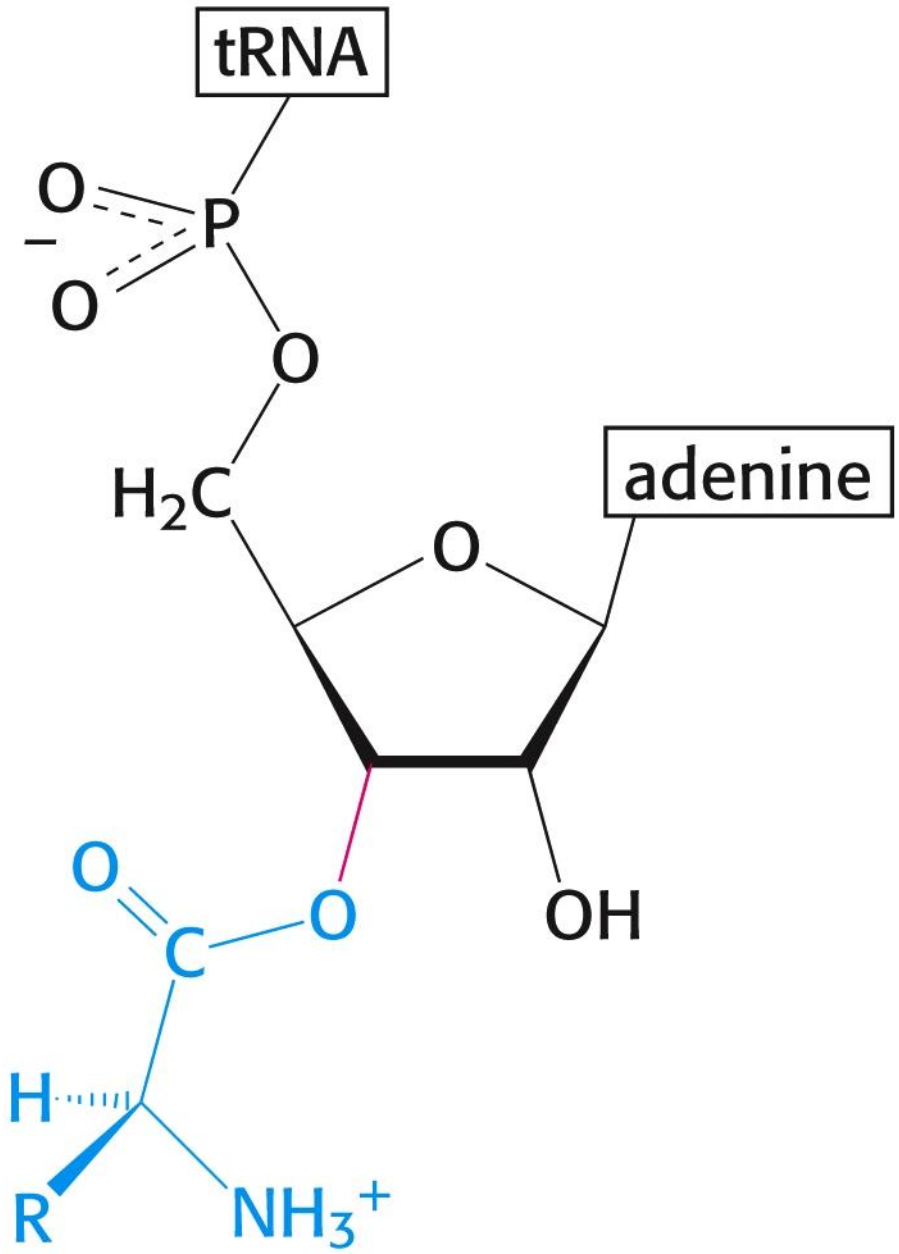
mRNA modification in eukaryotes

(Less known about transcription termination in eukaryotes)

mRNA is modified after transcription

A “cap” structure is attached to 5’ end & a sequence of adenylates, the poly(A) tail, is added to the 3’ end

Amino acid attached to tRNA



Amino acid esterified to 3’-hydroxyl group of terminal adenosine of tRNA

Amino acid is in an activated form

Whole molecule is, *aminoacyl* - tRNA

Aminoacyl - tRNA, symbolic diagram

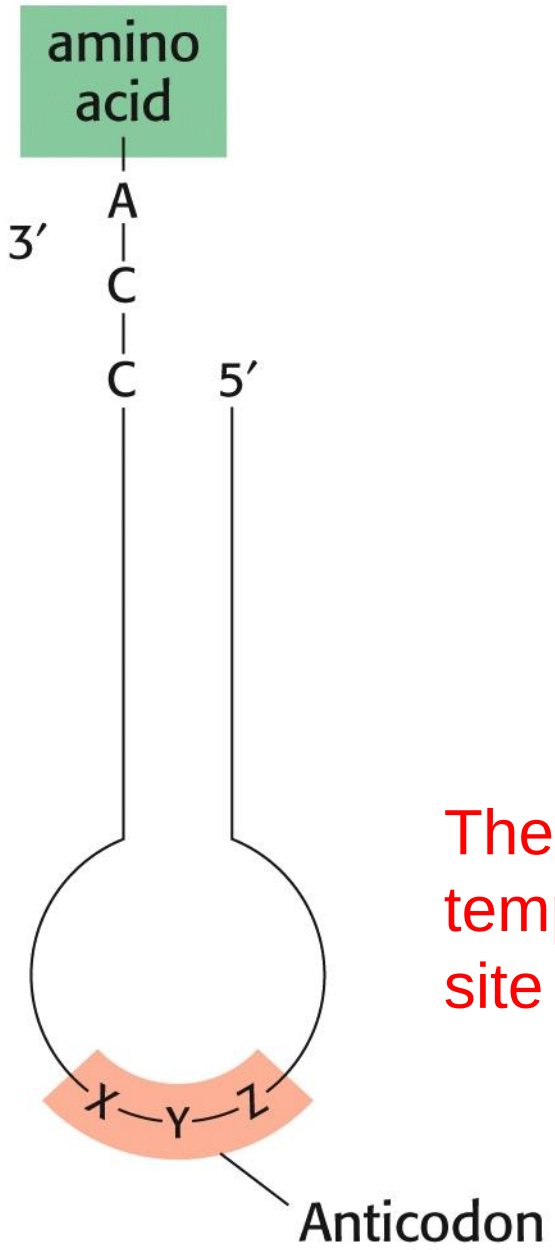
1961, Crick & Brenner,
the genetic code:

3 nucleotides encode
an amino acid,

Code is nonoverlapping,

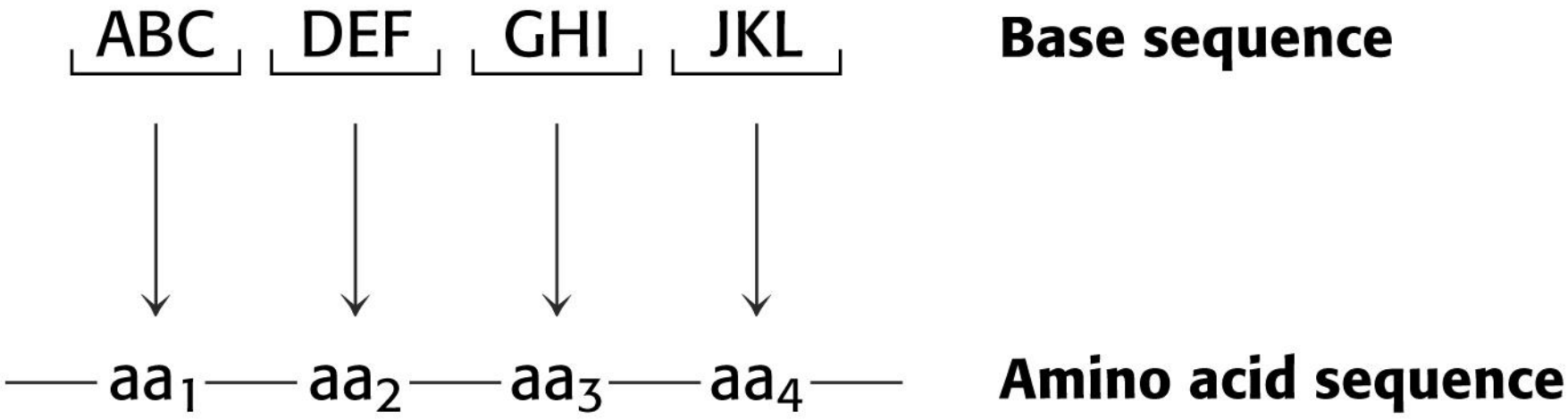
Code has no punctuation,

Code is degenerate,
(some AAs encoded by
more than one codon)



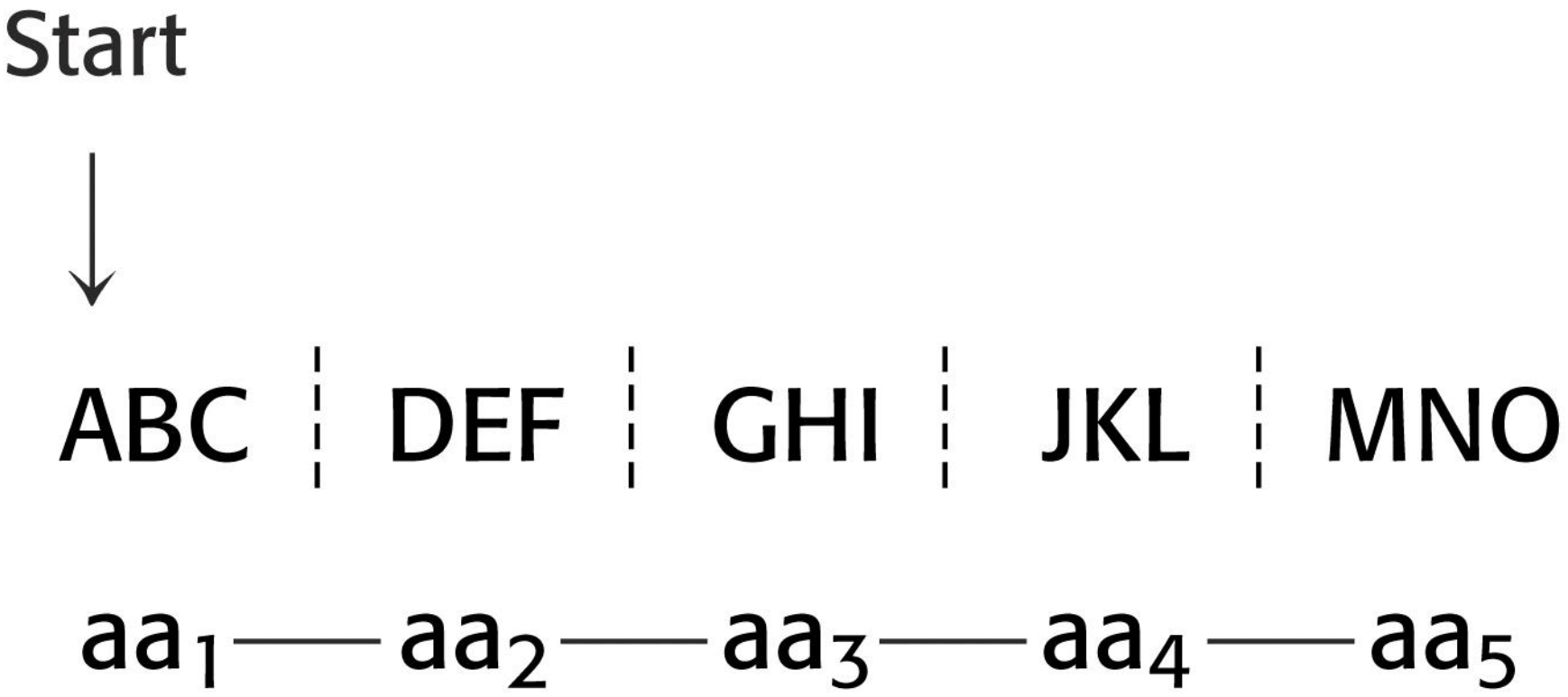
The anticodon is the template-recognition site

Genetic code, nonoverlapping



Genetic code, no punctuation

Sequence of bases is read in blocks of 3 bases from a fixed starting point



Genetic code, degenerate (64 codons, 20 aa_s)

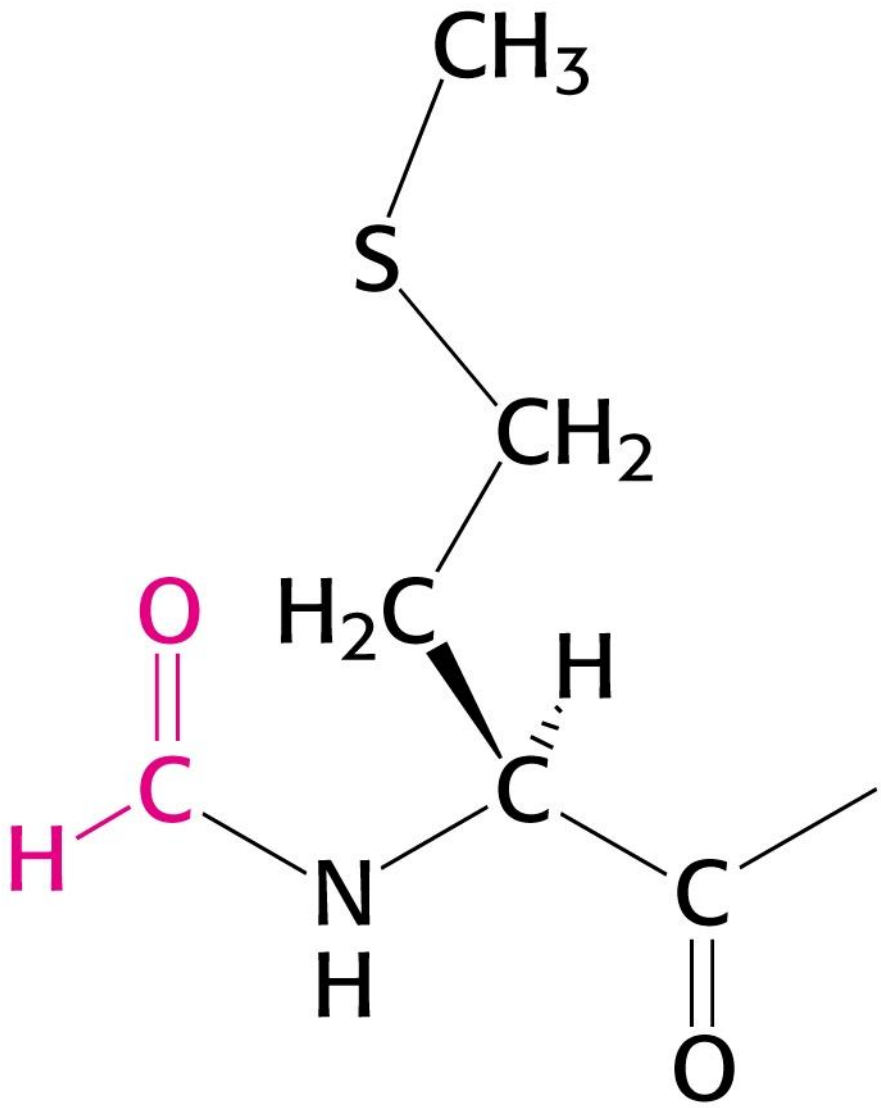
TABLE 5.4 The genetic code

First position (5′ end)	Second position				Third position (3′ end)
	U	C	A	G	
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	Stop	Stop	A
	Leu	Ser	Stop	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

Note: This table identifies the amino acid encoded by each triplet. For example, the codon 5′ AUG 3′ on mRNA specifies methionine, whereas CAU specifies histidine. UAA, UAG, and UGA are termination signals. AUG is part of the initiation signal, in addition to coding for internal methionine residues.

Trp & Met, one codon each,
other 18 aa_s, two or more codons,
Leu, Arg, & Ser, six codons each,
Synonyms, codons for same aa,
Synonyms differ in last base,
3 stop codons, designate translation termination

Translation initiation: start codon

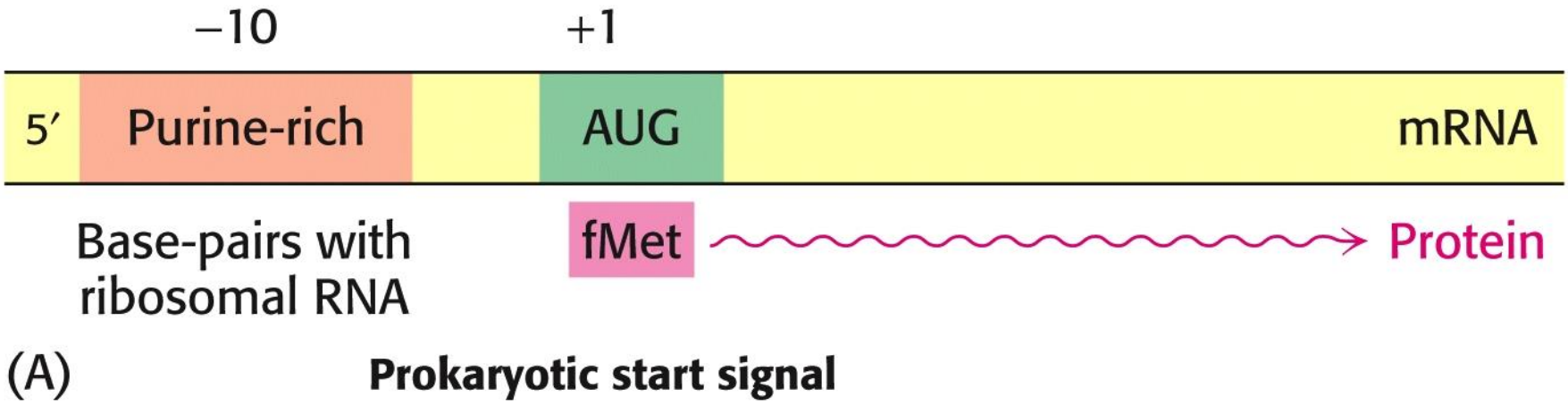


fMet

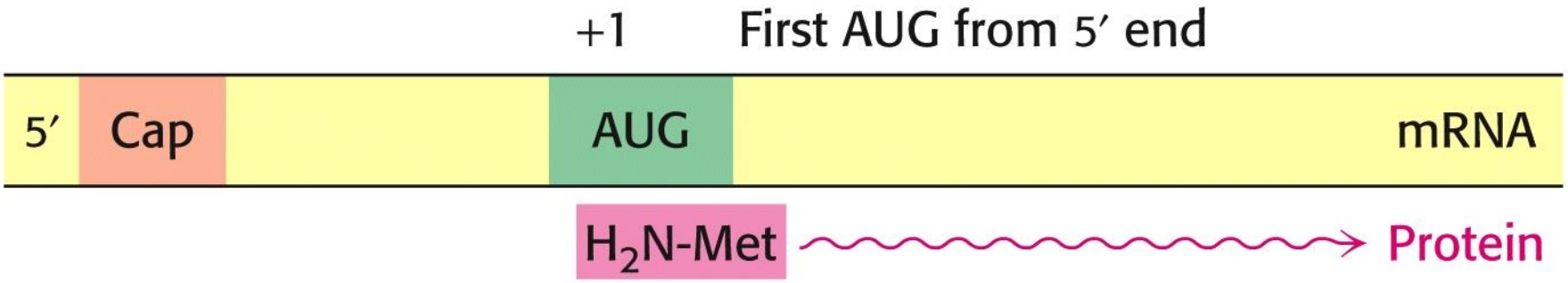
Messenger RNA is translated into proteins on ribosomes, large molecular complexes of proteins & ribosomal RNA

Initiator tRNA carries fMet (formylmethionine) to AUG (& sometimes GUG) in prokaryotes, but initiation is more complex

Prokaryotic translation start



Eukaryotic translation start



(B) **Eukaryotic start signal**

Genitic code, universal, except…

Nearly but not absolutely universal

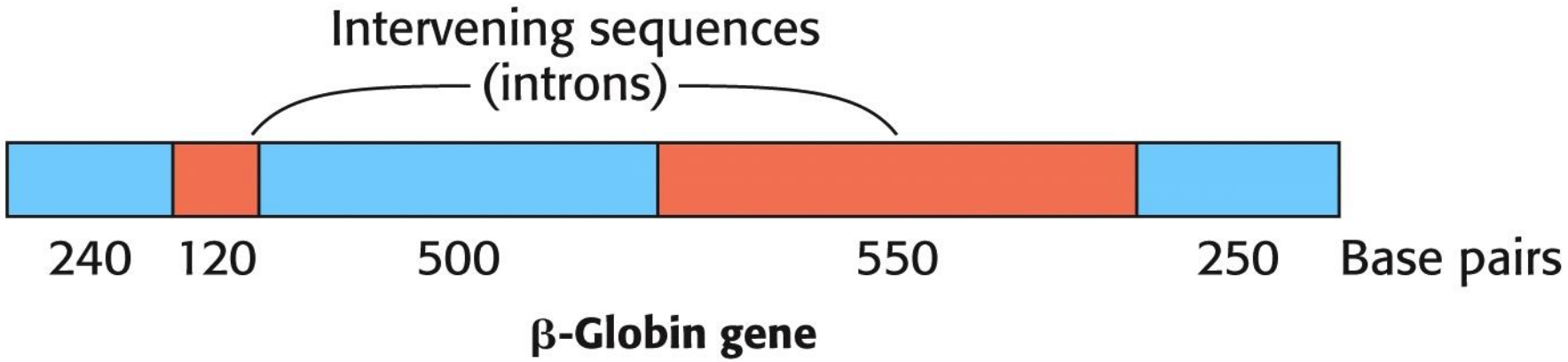
TABLE 5.5 Distinctive codons of human mitochondria

Codon	Standard code	Mitochondrial code
UGA	Stop	Trp
UGG	Trp	Trp
AUA	Ile	Met
AUG	Met	Met
AGA	Arg	Stop
AGG	Arg	Stop

Ciliated protozoa read UAA & UAG as codons for aa_s instead of stop signals. UGA is their only stop

Eukaryotic genes: mosaic of introns & exons

Introns (intervening sequences), brown

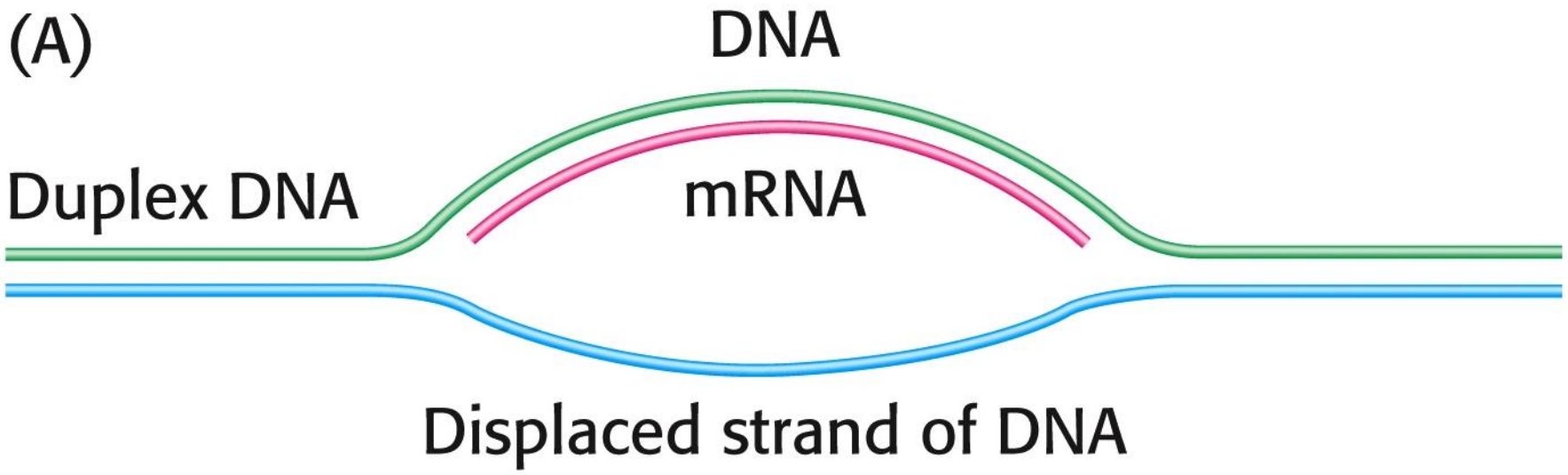


Exons (expressed sequences), blue

Detecting introns by EM

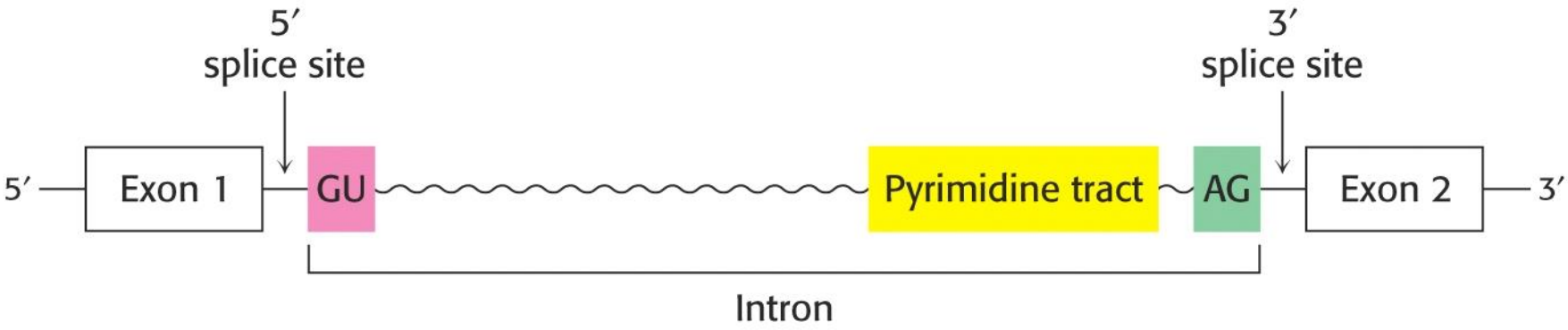
mRNA hybridized to corresponding genomic DNA

Single loop indicates gene is continuous



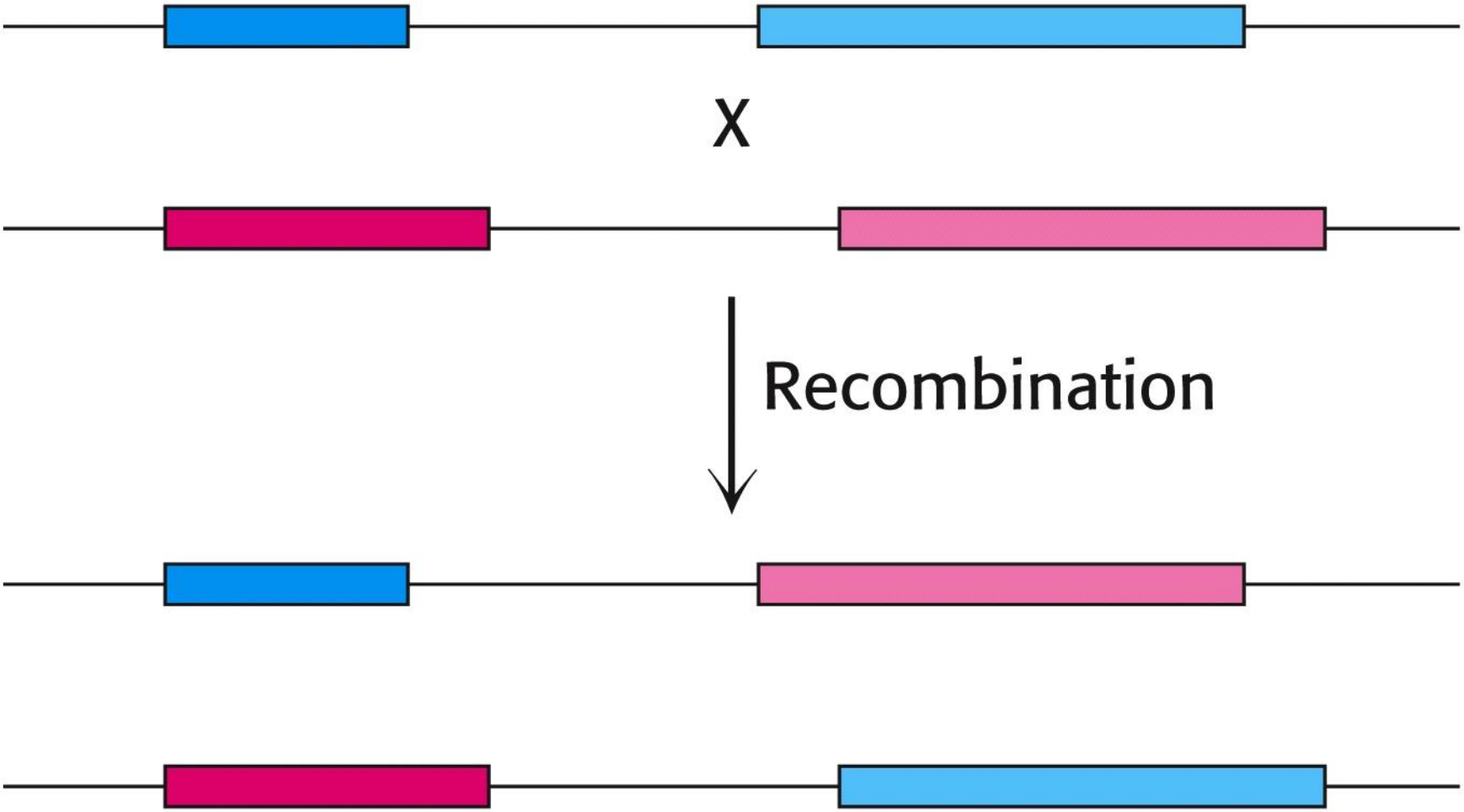
Splicing at consensus sequences

Introns excised by *spliceosomes* (assemblies of proteins & small RNAs)

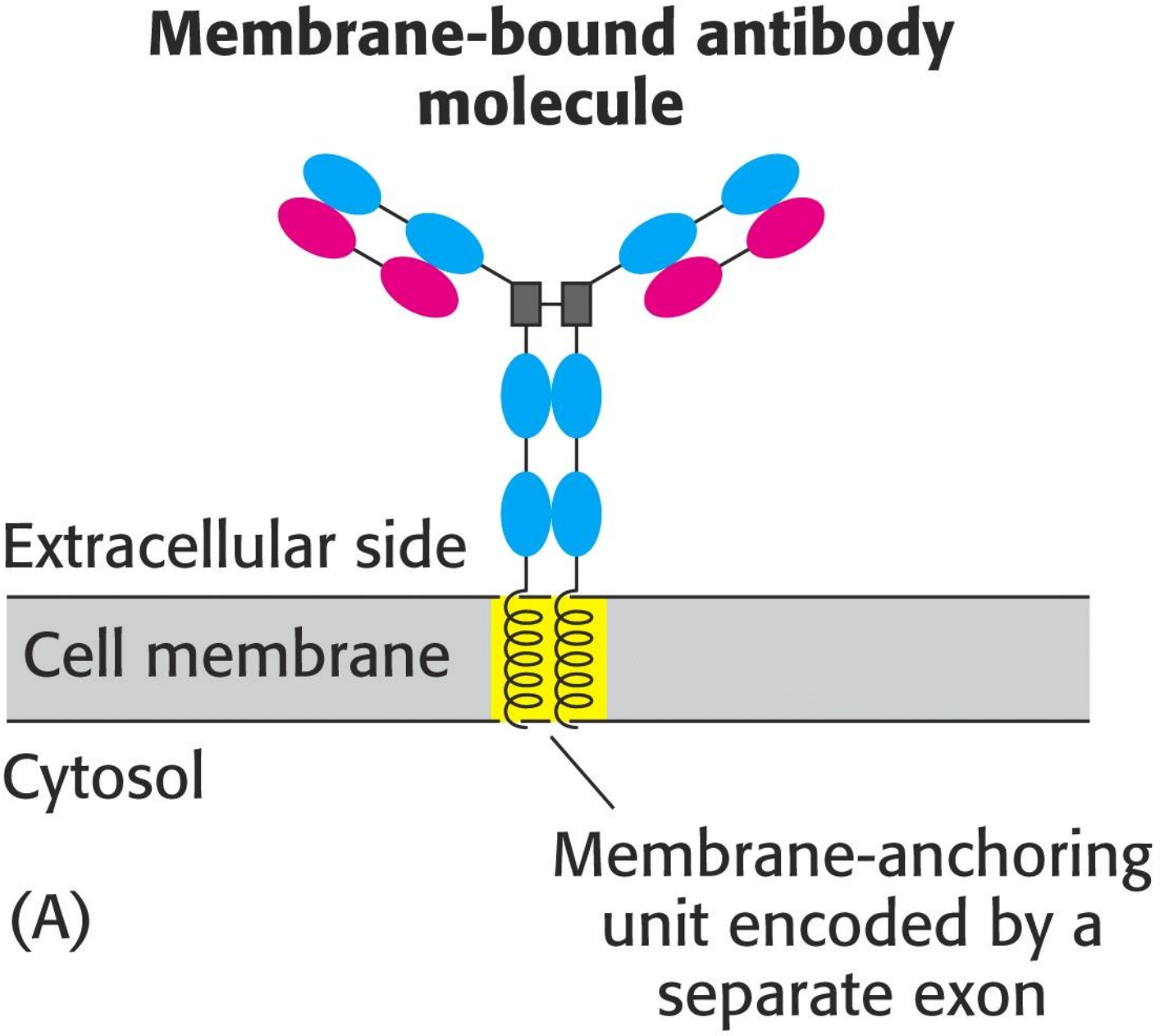


Exon shuffling

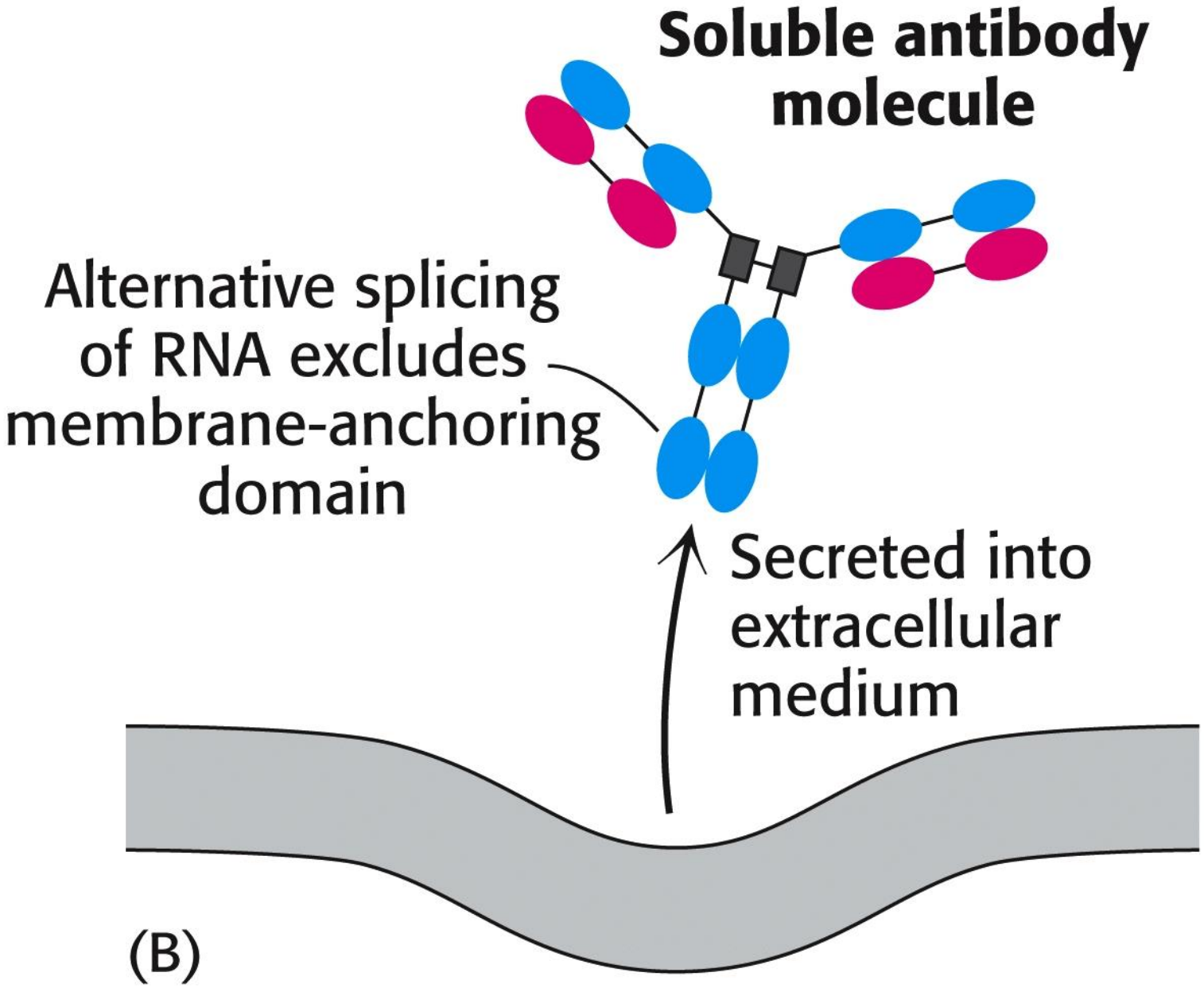
Shuffling expands genetic repertoire



Alternative splicing, 1st variation



Alternative splicing, 2nd variation



TEST

Biosynthesis of purine and pyrimidine nucleotides

• all cells needs ribonucleosides, deoxyribonucleosides and their phosphates

• **purine and pyrimidine** basis from food are not used

• **Synthesis of purine and pyrimidine nucleotides are coordinated**

TEST

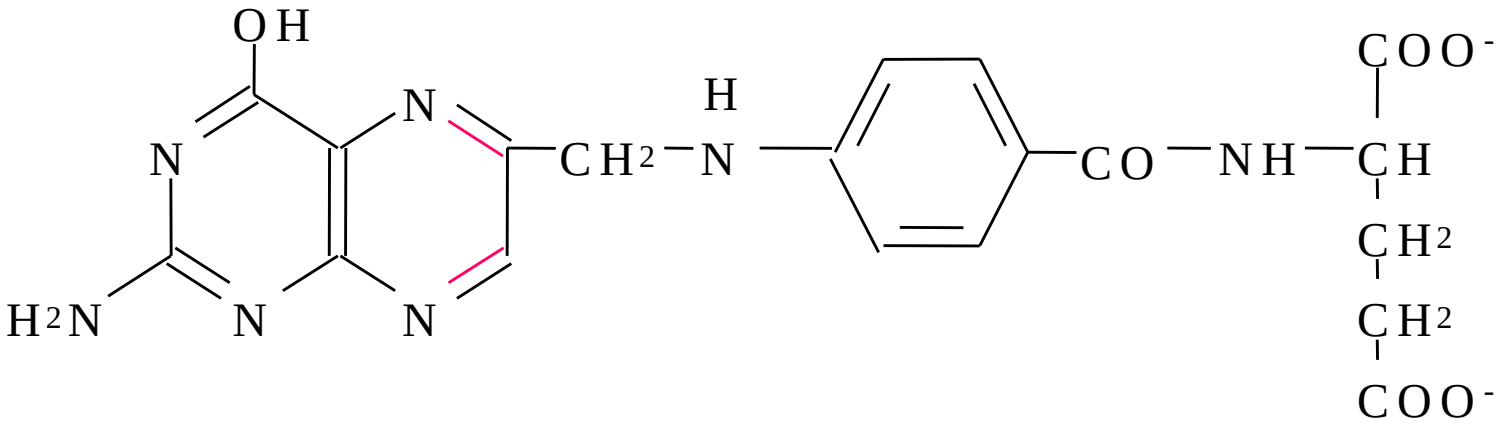
Precursore molecules

- 3 main compounds:
- **tetrahydrofolate**
- **glutamine**
- **PRPP – 5-phosphoribosyl-1-pyrophosphate**

Importance of folic acid for biosynthesis of NA bases

Green leafy vegetables, liver, whole grains, yeast, k

Folate



Used form in human is tetrahydrofolate

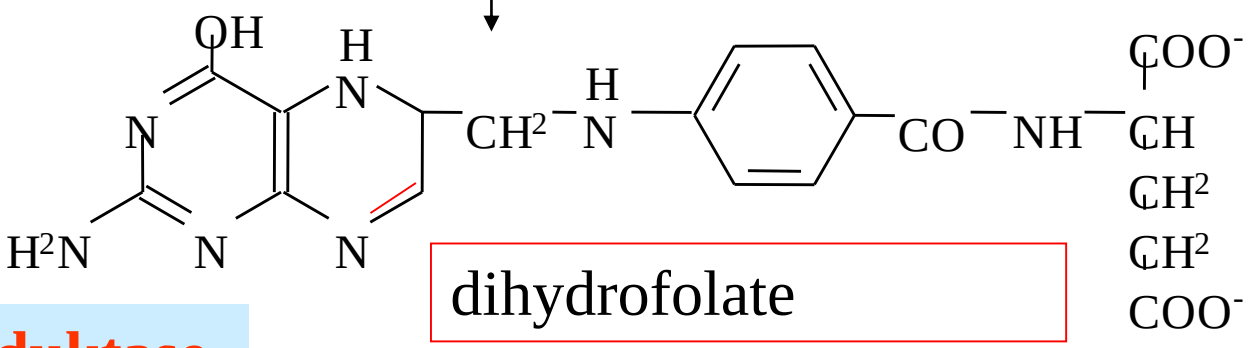
Formation of tetrahydrofolate

folate

NADPH + H^+

NADP +

DEHYDOGENATION

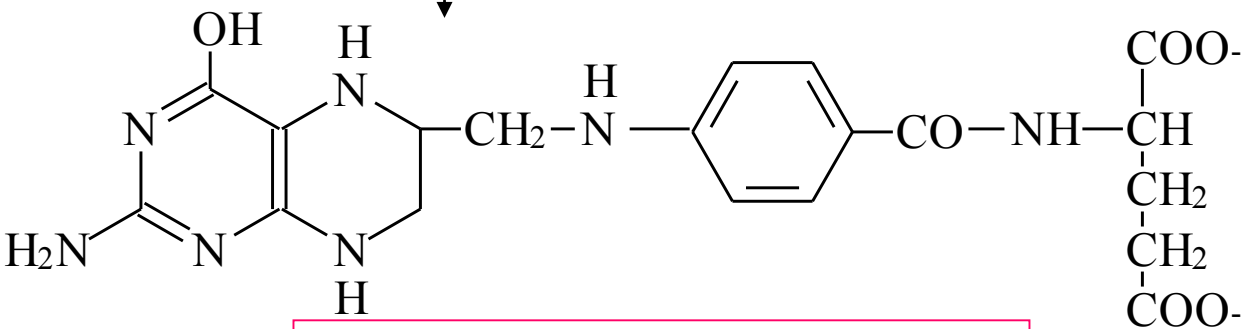


dihydrofolate

(dihydro)folatereduktase

NADPH + H^+

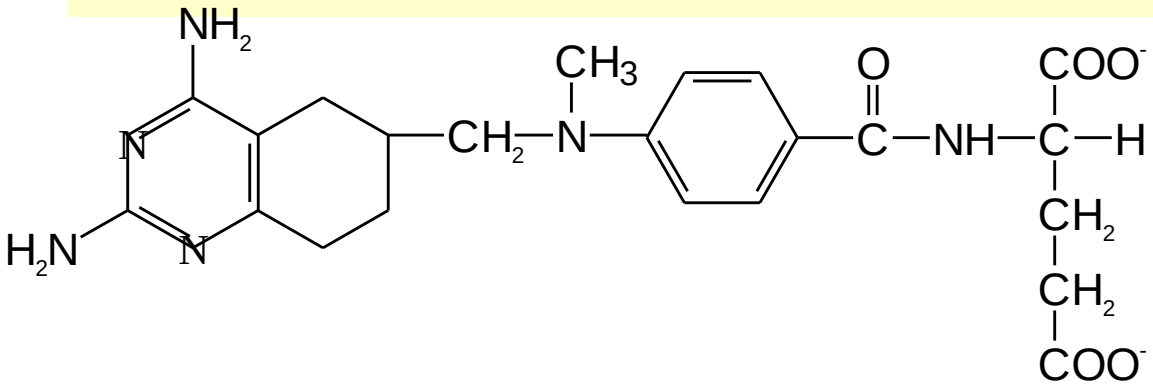
NADP +



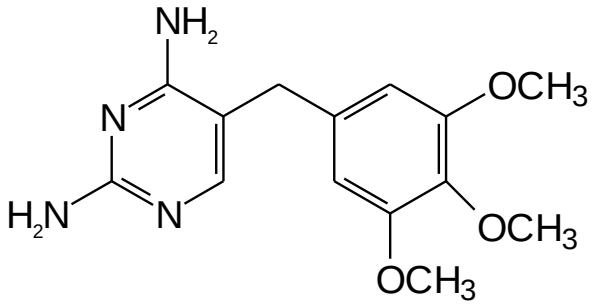
tetrahydrofolate

Inhibitors (dihydro)folatereductase:

Methotrexate (anticancer agent)

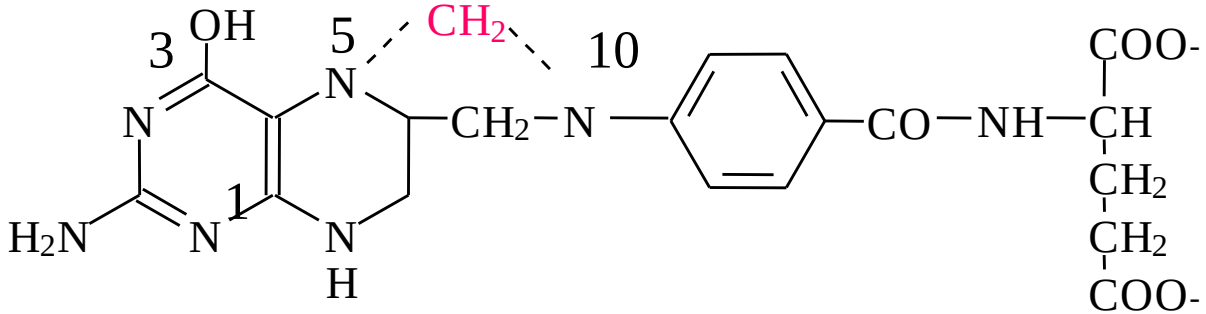


Trimethoprim (bacteriostaticum)

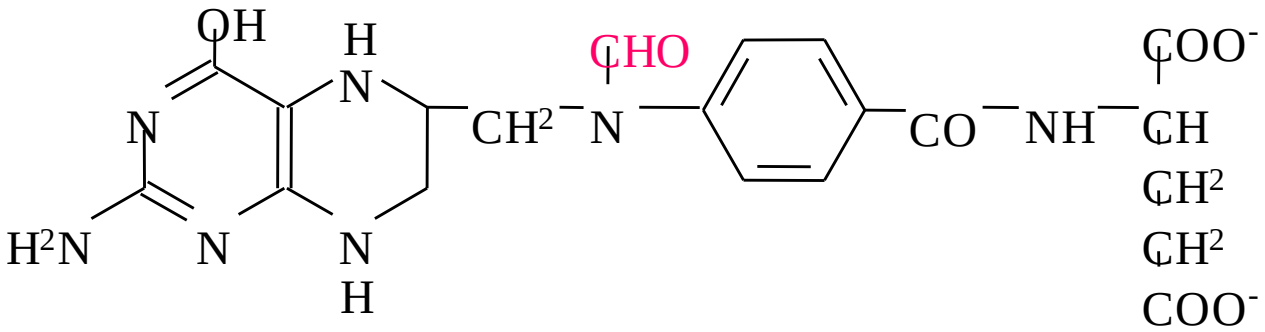


Using of tetrahydrofolate

N-5,N-10- methylen H_4F – synthesis of thymin

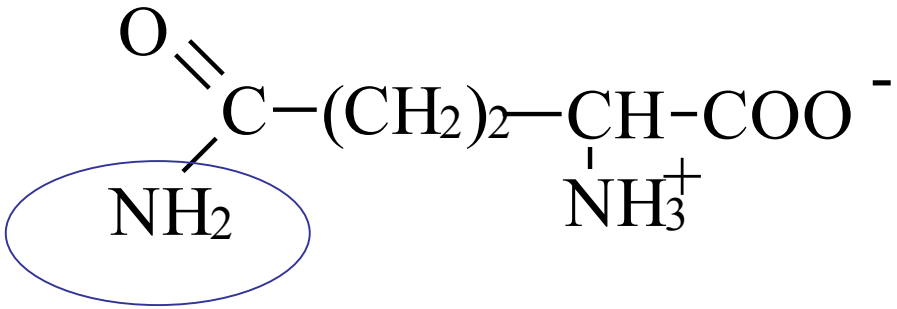


N-10-formyl H_4F – synthesis of purins

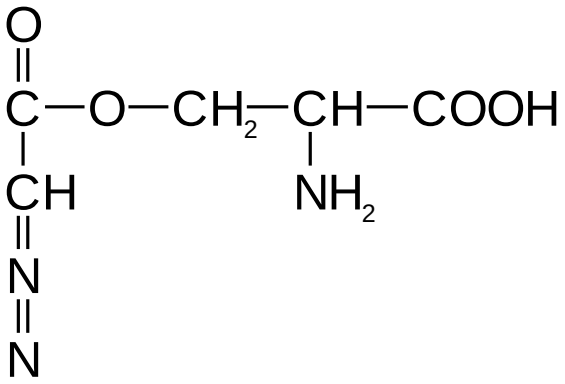


Importance of glutamin for purine and pyrimidine biosynthesis

- Donor of aminogroup



Glutmine antagonists inhibits synthesis of purines and pyrimidines



azaserin

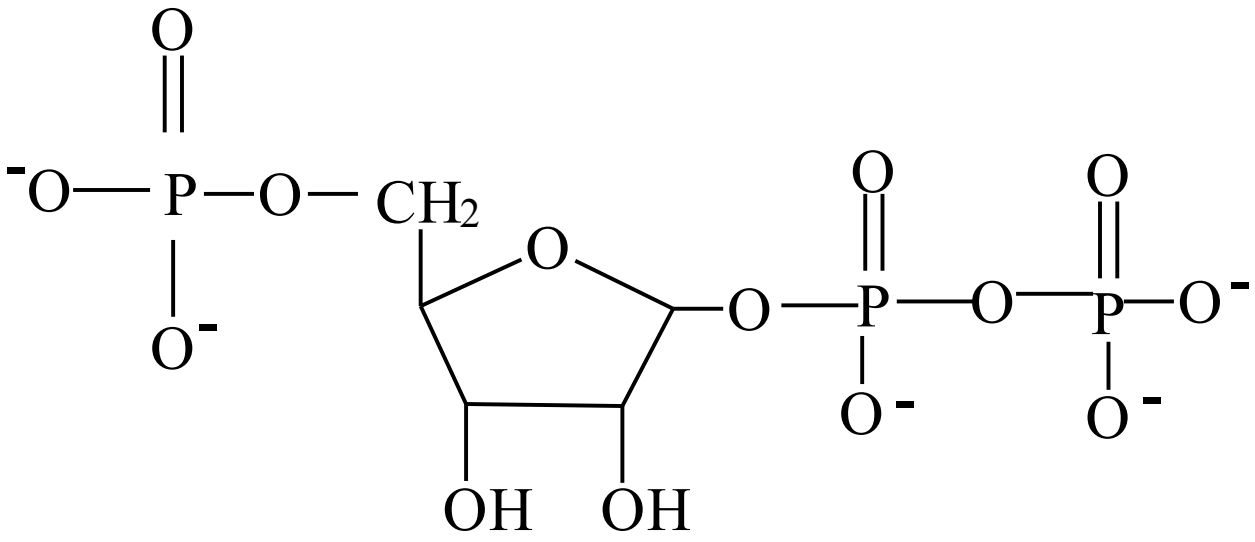
PRPP - phosphorybosylphyridoxalphosphate

<u>Necessary for synthesis:</u>

Purine nucleotides

Pyrimidine nucleotides

NAD^+, $NADP^+$

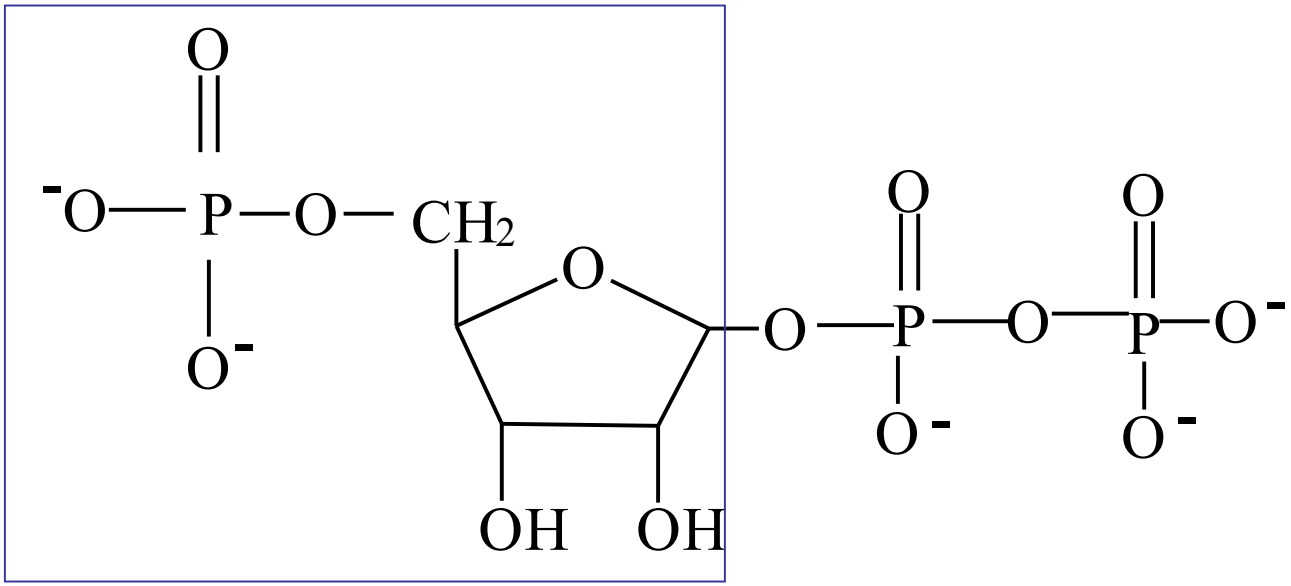


Synthesis of PRPP

PRPP-synthetase

ribose-5-phosphate (pentose cycle), activeted penthose

Ribosa-5P + ATP → PRPP + AMP



Differences in purine and pyrimidine synthesis

Synthesis - *puzzle* – one part to others.

TEST

Diffrence in the beginning :

–purines : first PRPP and than is form base

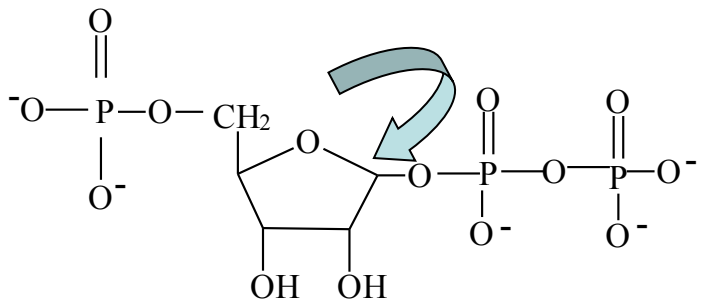
- Pyrimidines : first base and than ribosa-5-P from PRPP.

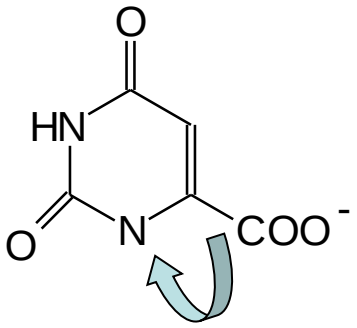
Purins

First PRPP…

Pyrimidins

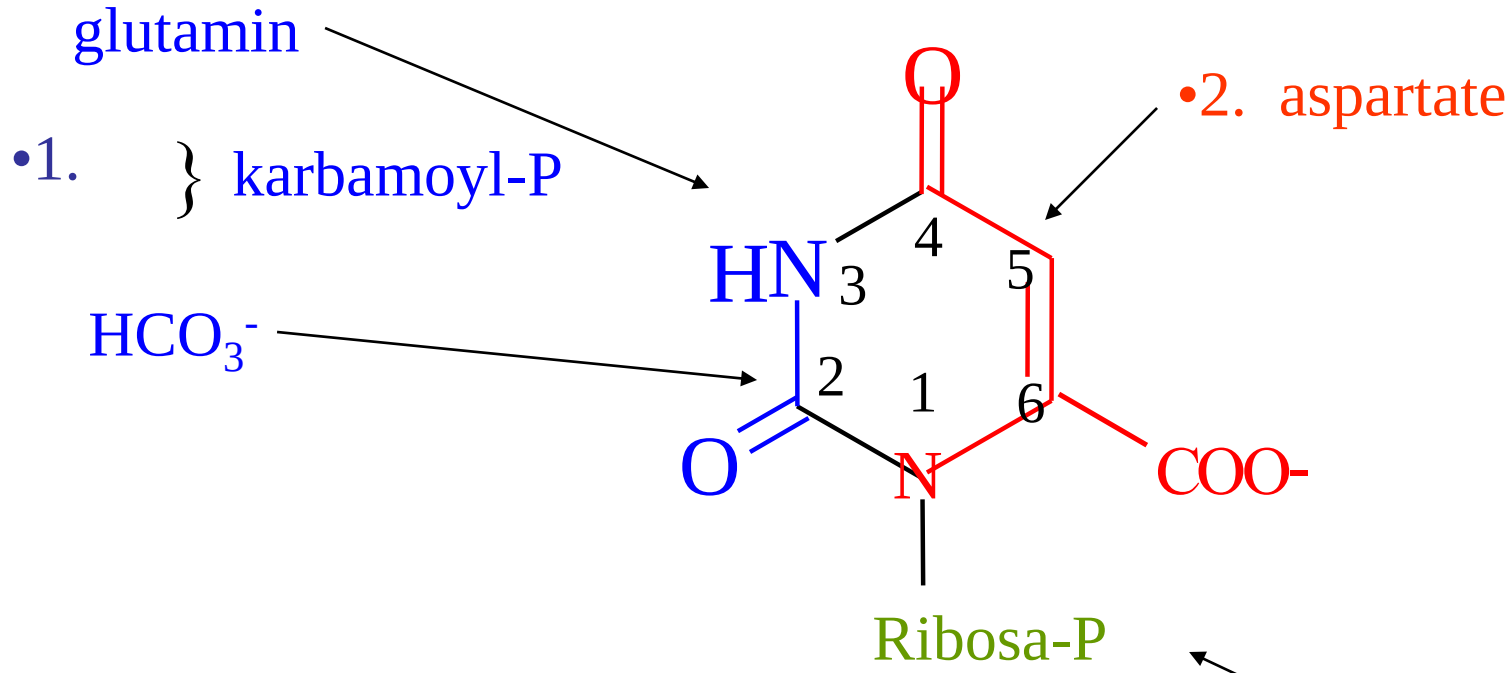
First heterocycle ribose-P from PRPP





BIOSYNTESIS of PYRIMIDINS

Origin of atoms in pyrimidines



Orotidinmono-P

Decarboxylation – uridin mono-P

• syntesis of karbamoyl -P

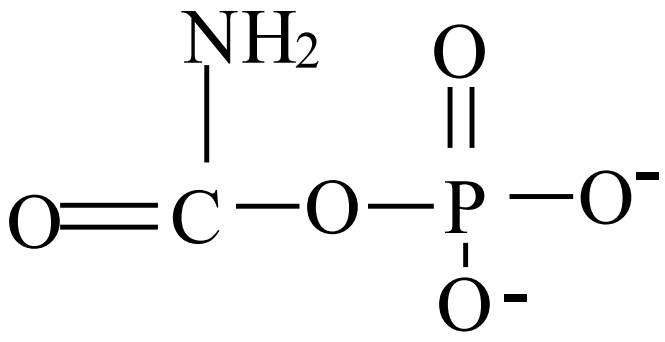
CYTOPLASM

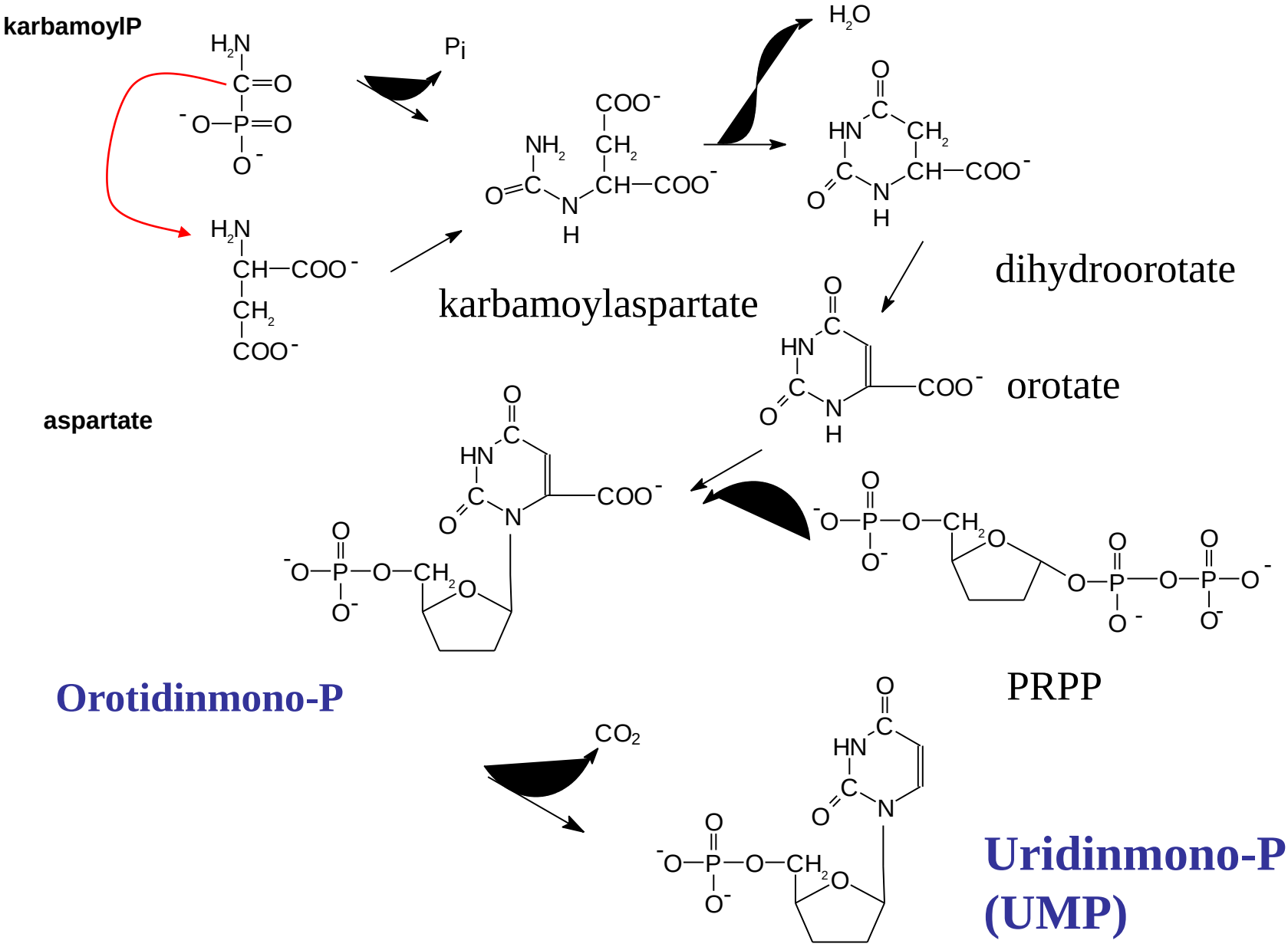
Karbamoyl-P-synthetase

-energy, enzym **karbamoylphosphatesynthetase II**
Inhibition by UTP („inhibition by product“) and
aktivation by ATP.

• 1 Glutamine + 2 ATP + HCO_3^-

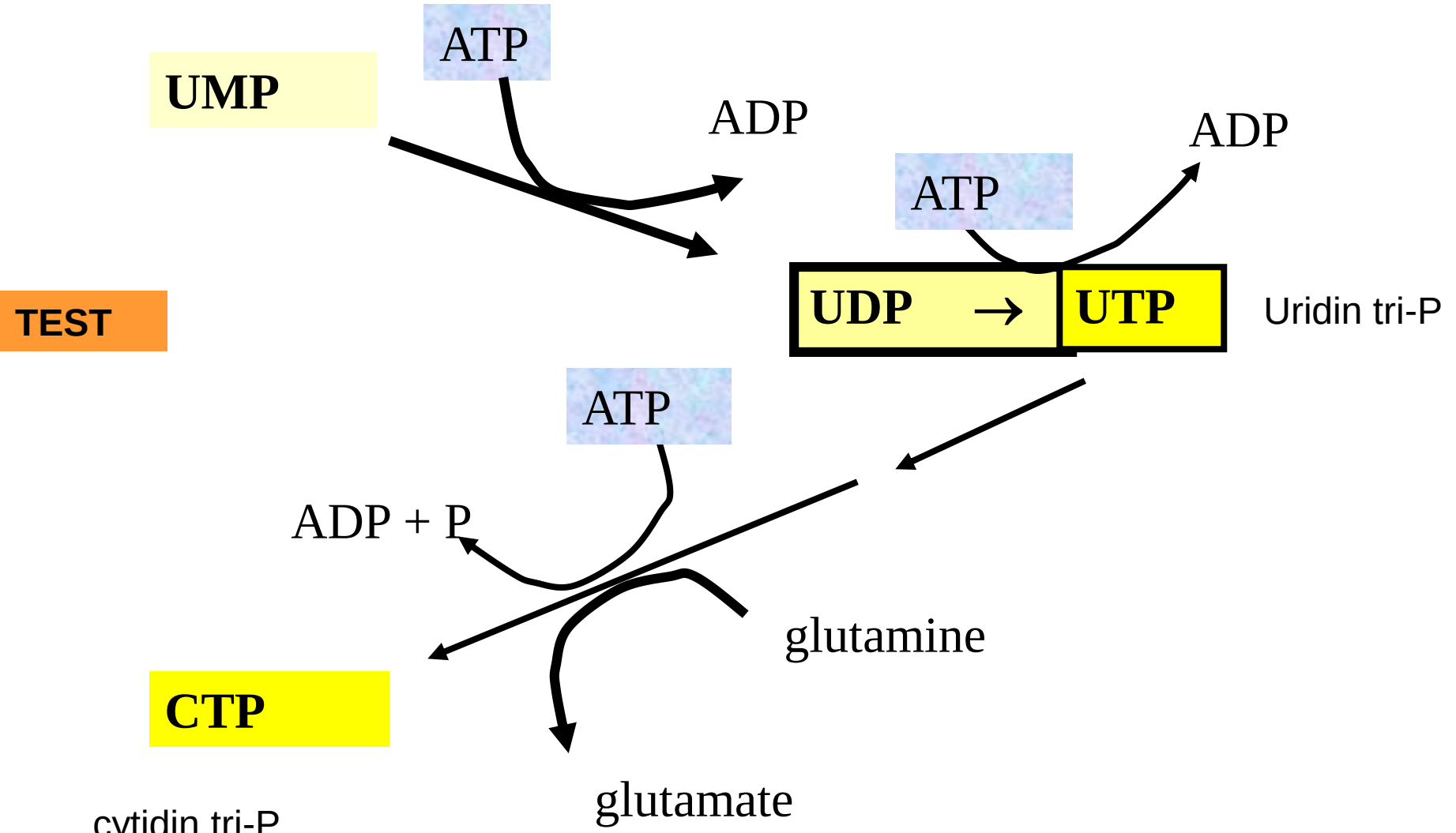
→ karbamoyl-P + glutamate + 2 ADP + P_i




karbamoylP
Pi
H2O
karbamoylaspartate
dihydroorotate
orotate
aspartate
PRPP
Orotidinmono-P
CO2
Uridinmono-P
(UMP)

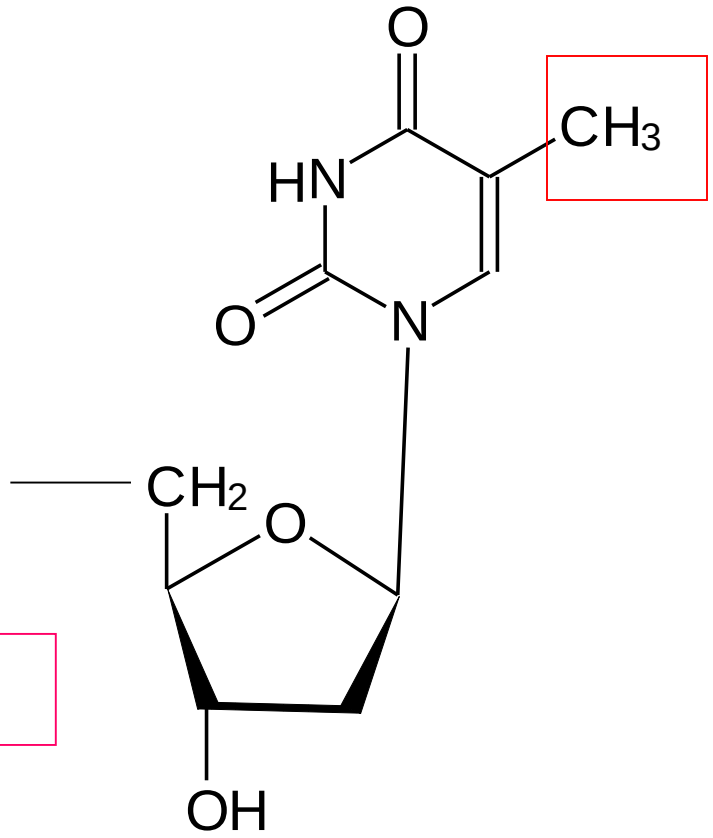
TEST

Biosyntesis of UTP and CTP



dTMP (methylation)

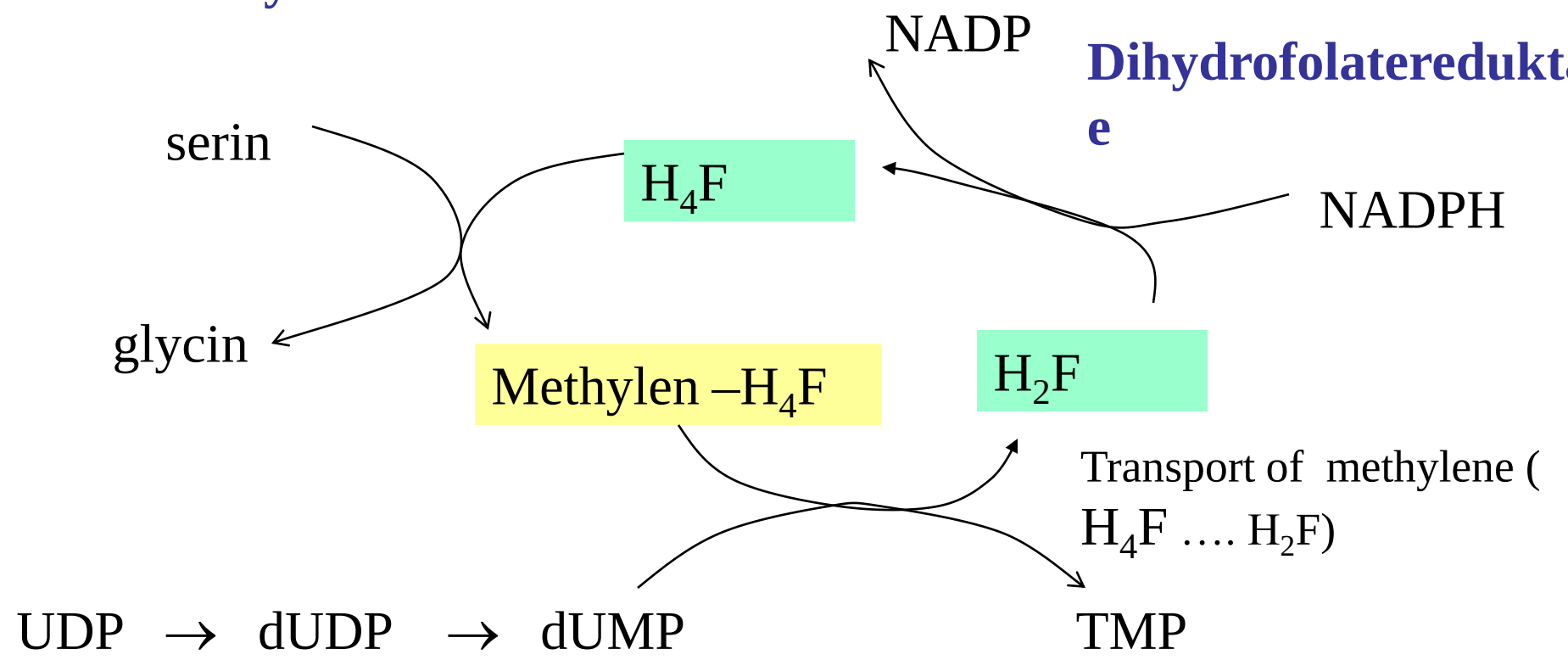
Deoxythymidintri-P



Methylation- H_4F

Methylen group in H_4F is reduced to methyl dUMP

Synthesis of TMP

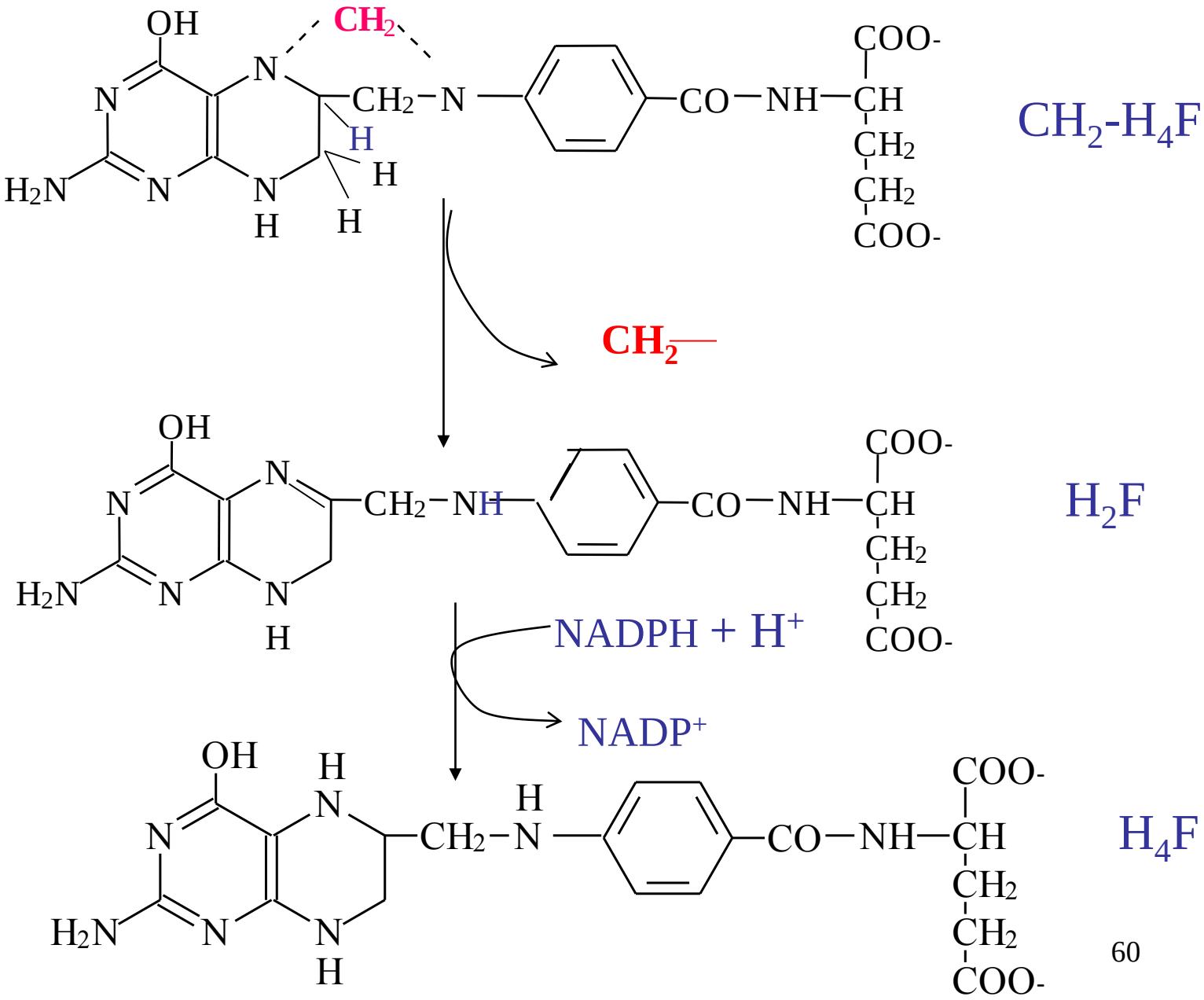


TEST

Thymidylatesynthase

(enzym dependent on folate)

Anticancer drugs


CH2
CH2-H4F
CH2
H2F
NADPH + H+
NADP+
H4F

Dihydrofolate reductase - an objective antitumor therapy.

Dihydrofolate reductase was the first enzyme for which focused antitumor therapy.

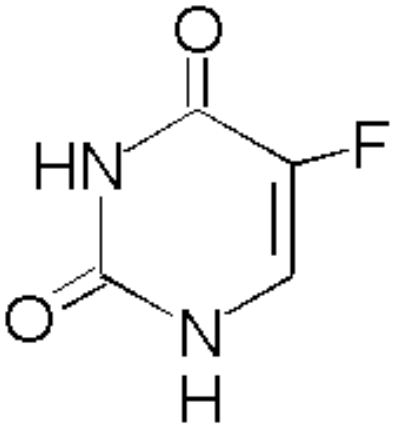
The first-used inhibitor was aminopterin.

It binds to the enzyme 1000 times tighter than folate, acts as a competitive inhibitor.
Currently used methotrexate and similar derivatives.

All drugs which affect the synthesis of purines and pyrimidines, deplete rapidly dividing cells - but not only cancer cells but also cells in the bone marrow and GI tract cells such as hair follicles.

TEST

thymidylate synthase



5-fluorouracil

The administration of fluorouracil

↓

organism conversion to
5-fluorodeoxyuridine monophosphate

↓

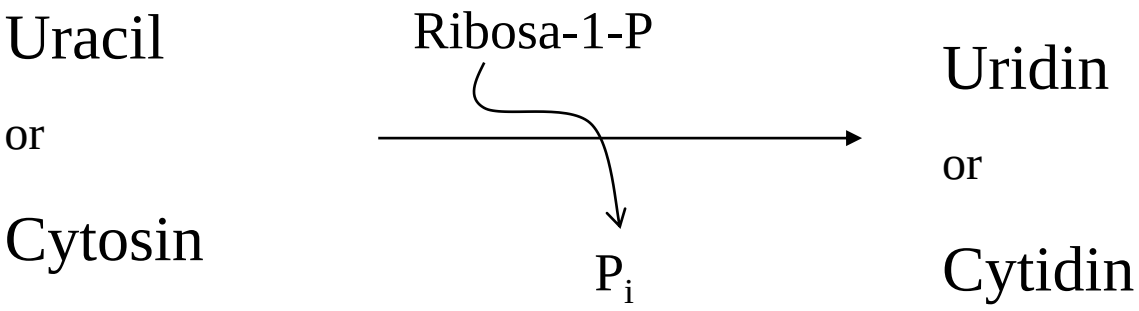
Competitive inhibition
thymidylatesynthasy

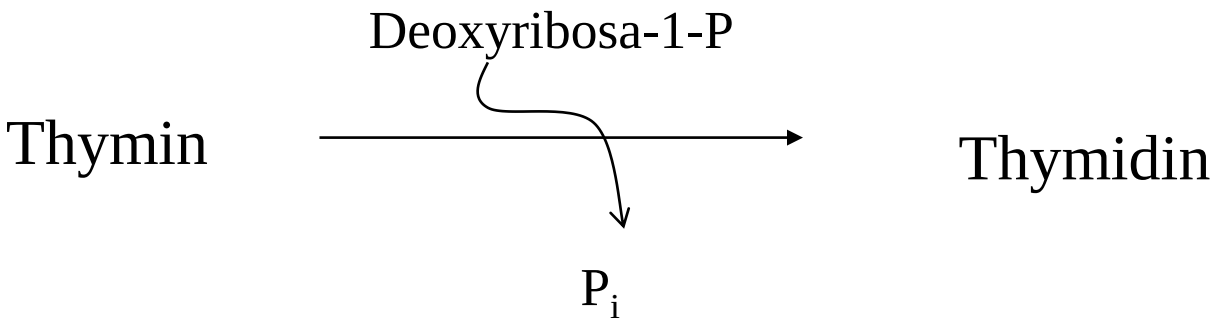
Thymidylate
synthase because it is blocked by a competitive inhibitor, which in effect prevents dTMP, resulting in a slowdown (disabling) of cell division.

The cytostatic effect of a drug

2. Synthesis of pyrimidins by *salvage pathway*

1. nukleosides





2. Kinase - phosphorylation

- thymidin + ATP → TMP + ADP
- cytidin + ATP → CMP + ADP
- deoxycytidin + ATP → dCMP + ADP
- uridin + ATP → UMP + ADP

Salvage pathway – extrahepatal tissues

Regulation of biosyntesis of pyrimidins

❑ **Allosteric:**

- KarbamoylPsynthetase:
  inhibition by UTP, purins nucleotides,

  aktivation by PRPP

❑ dependence on cell cycle

KarbamoylP-synthetase in S phase is more sensitive to activation by PRPP

Degradation of pyrimidins nucleotides

TEST

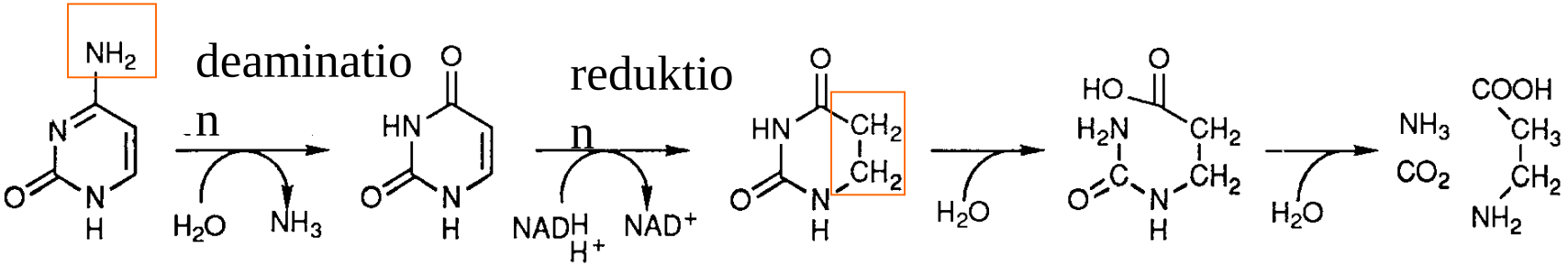
Pyrimidins – to the simple compounds – urine
pyrimidine base, we are able in our body break down into simpler components
STEPS:

a) Release of P
b) Release of sugar
c) Degradation of pyimidin base

End products of cleavege of pyrimidines:

NH_3, CO_2, β-alanin, (β -aminoisobutyrate)

Soluble metabolist – excretion by urine



β-alanin

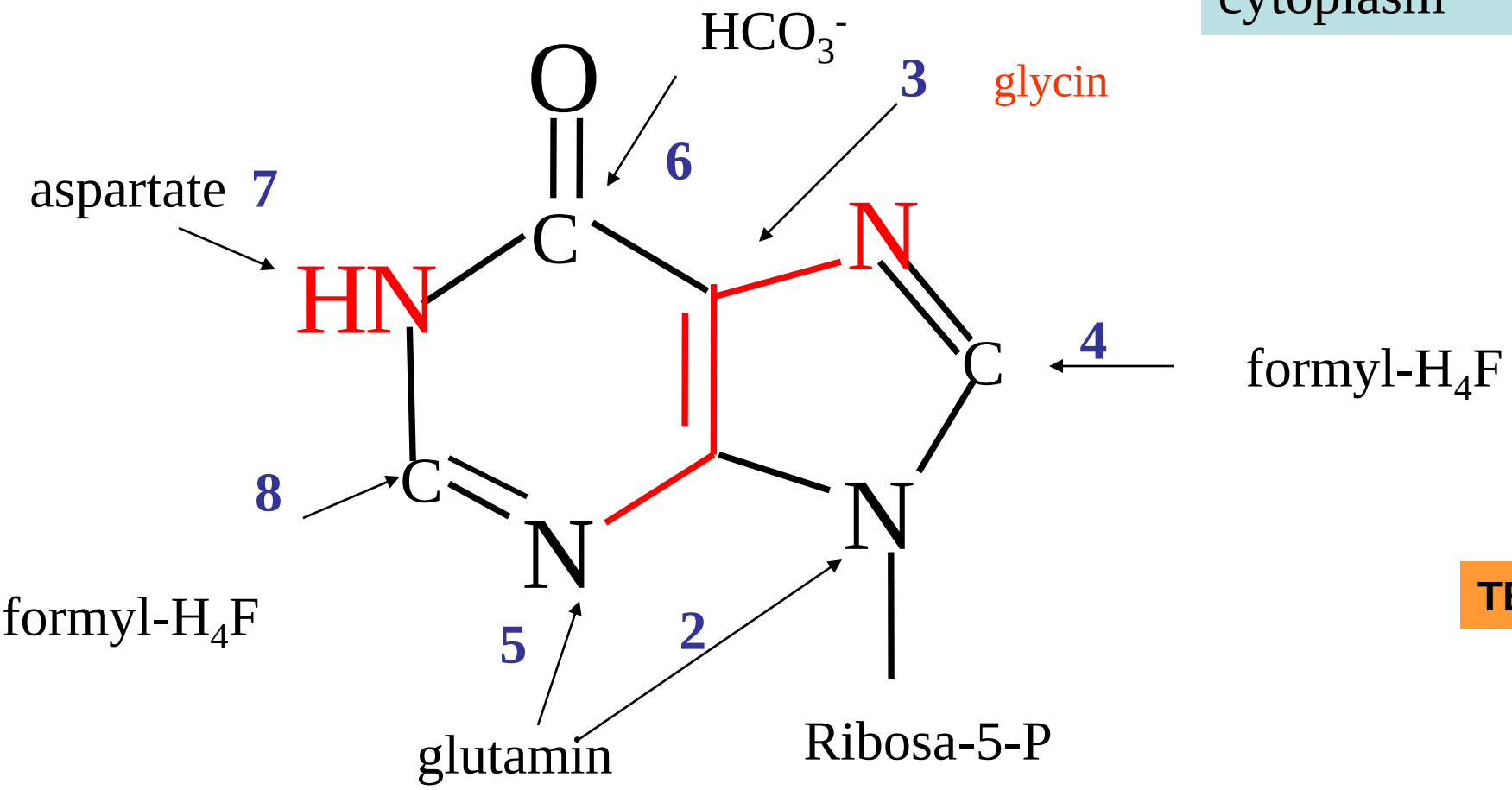
β-aminoisobutyrate

Biosyntesis of purins

(multienzym complex)

liver

cytoplasm

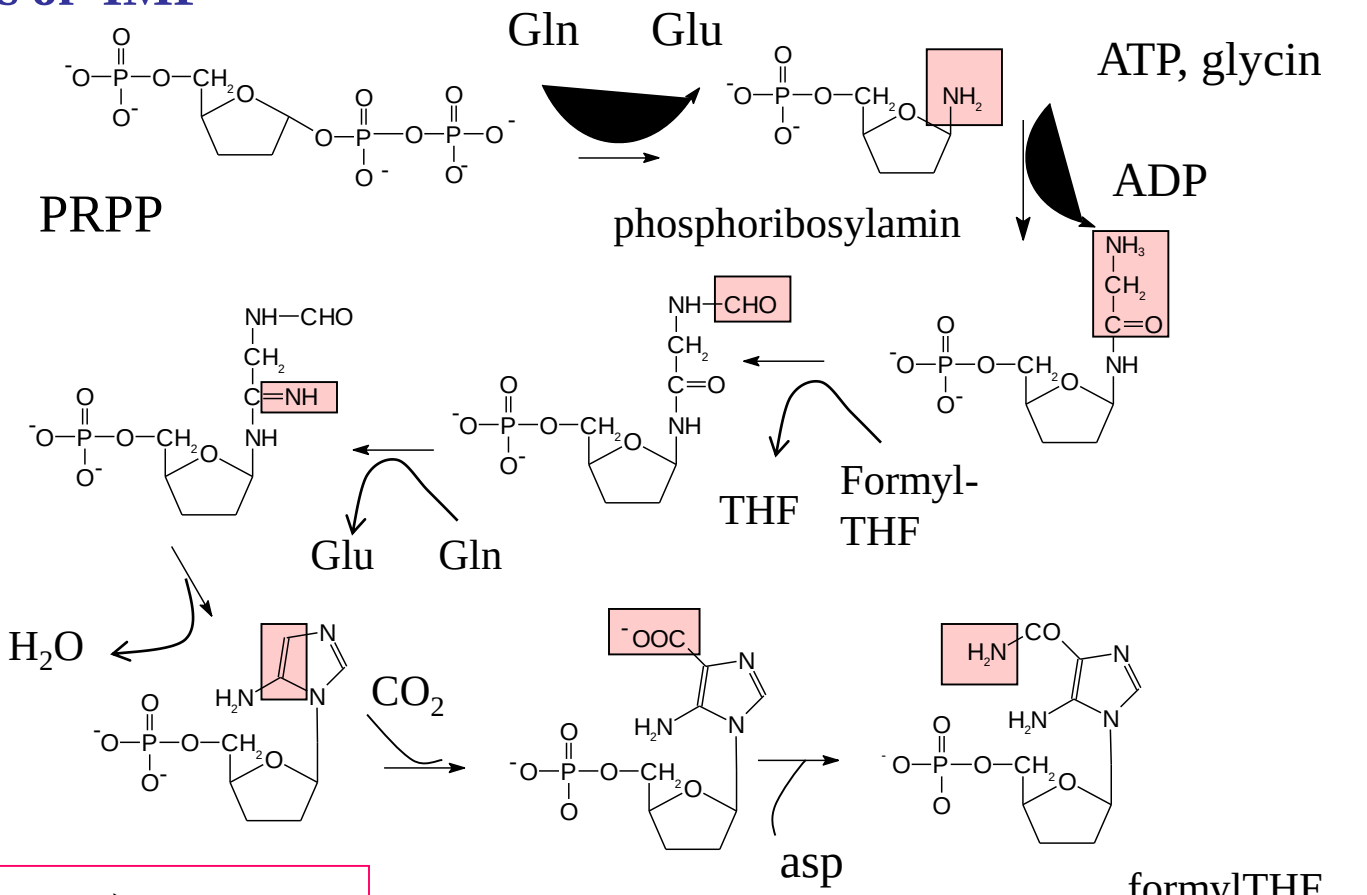


TEST

Inosin-5-P (IMP)

Biosyntesis of IMP

TEST

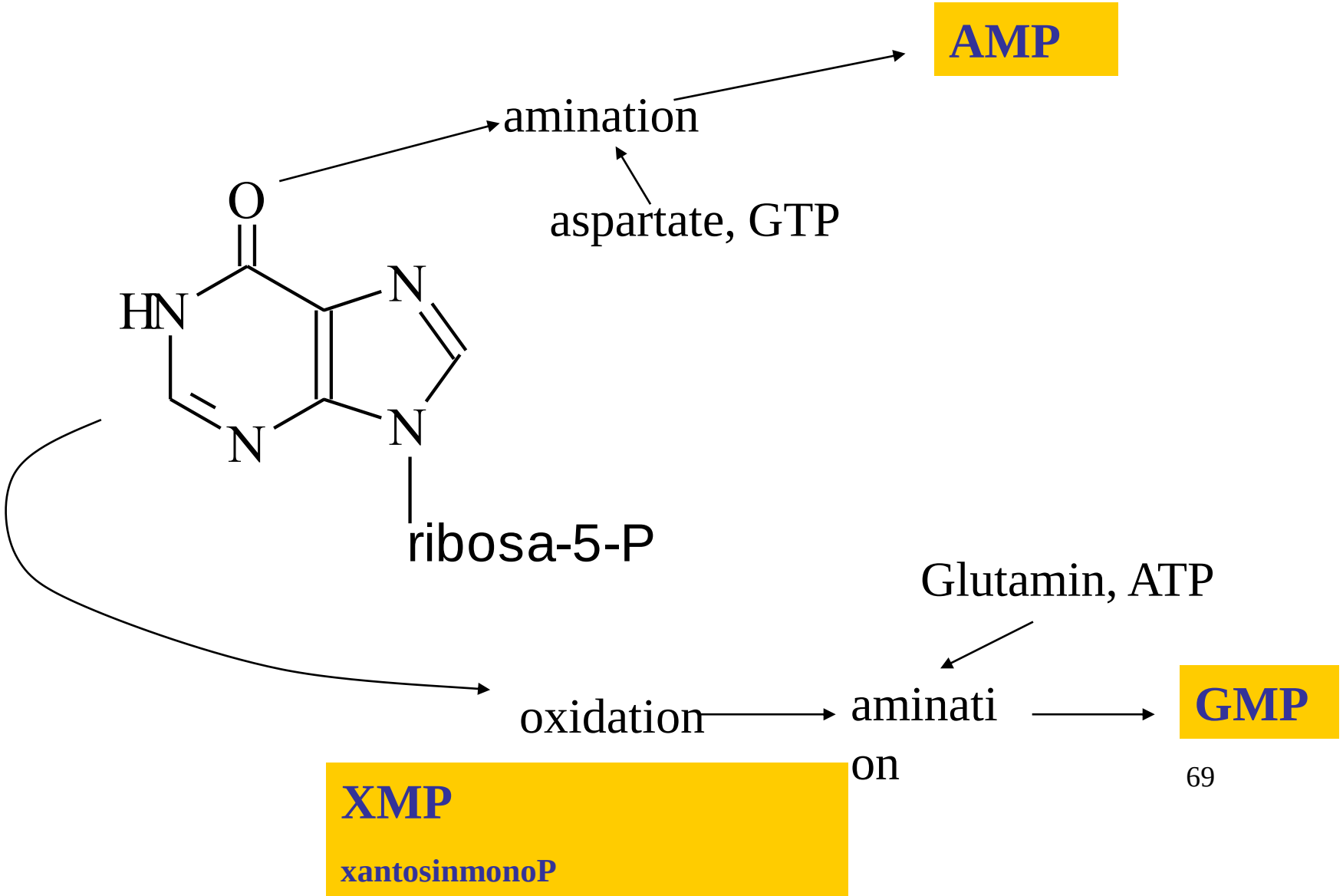


Inosin-5-P (IMP)

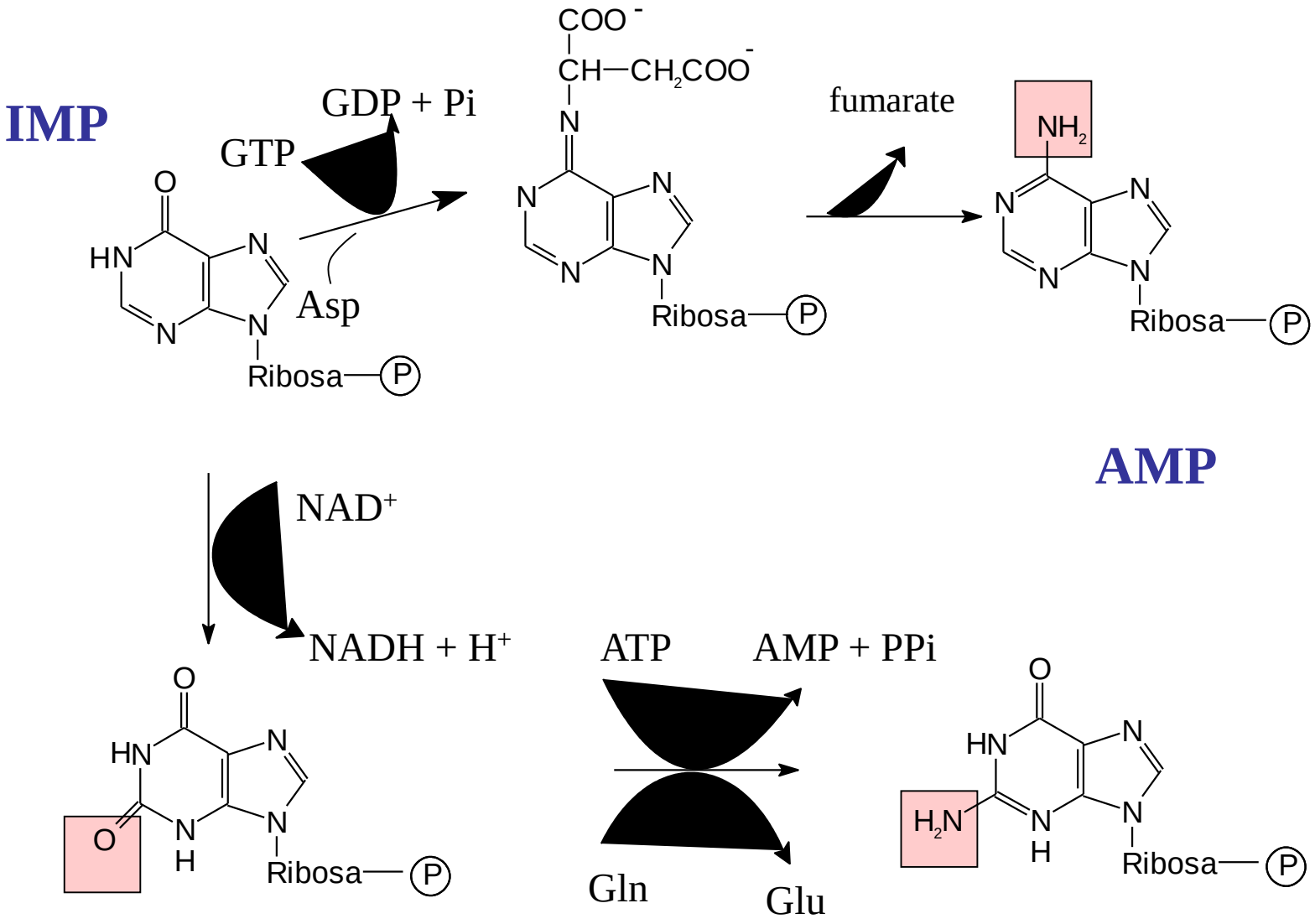
inosinmonophosphate

Inosin-5-P (IMP)

Initial substance for synthesis of other basis

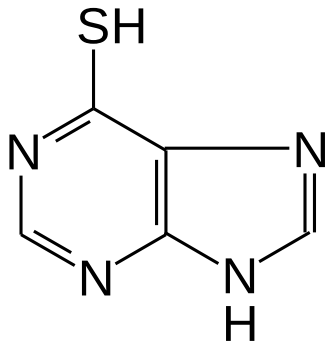


Syntesis of AMP a GM



Inhibitors of syntesis of purins (cytostatics)

- inhibitors dihydrofolate reductase
- analogy glutamin (azaserin)
- 6-merkaptopurin- inhibition of change IMP to AMP and GMP

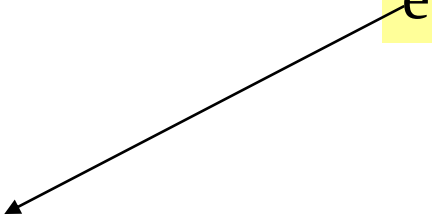


merkaptopurin

Syntesis of purins by salvage pathway

Extrahepatal tissue

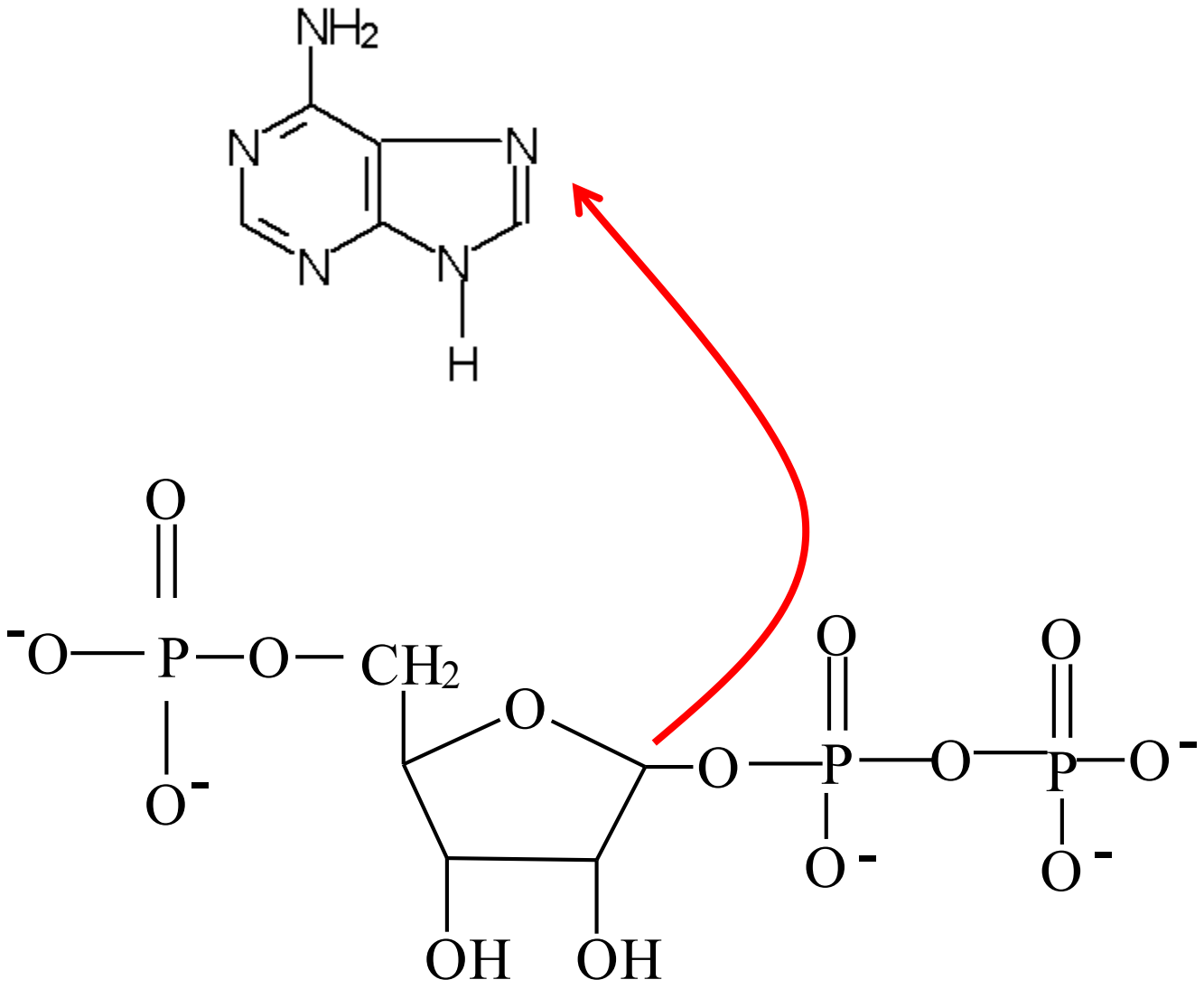
phosphoribosyltransferase



Purin + PRPP → purinnukleotidmonoP + PP

Recyclation of purins **phosphoribosyltransferase**

phosphoribosyltransferase

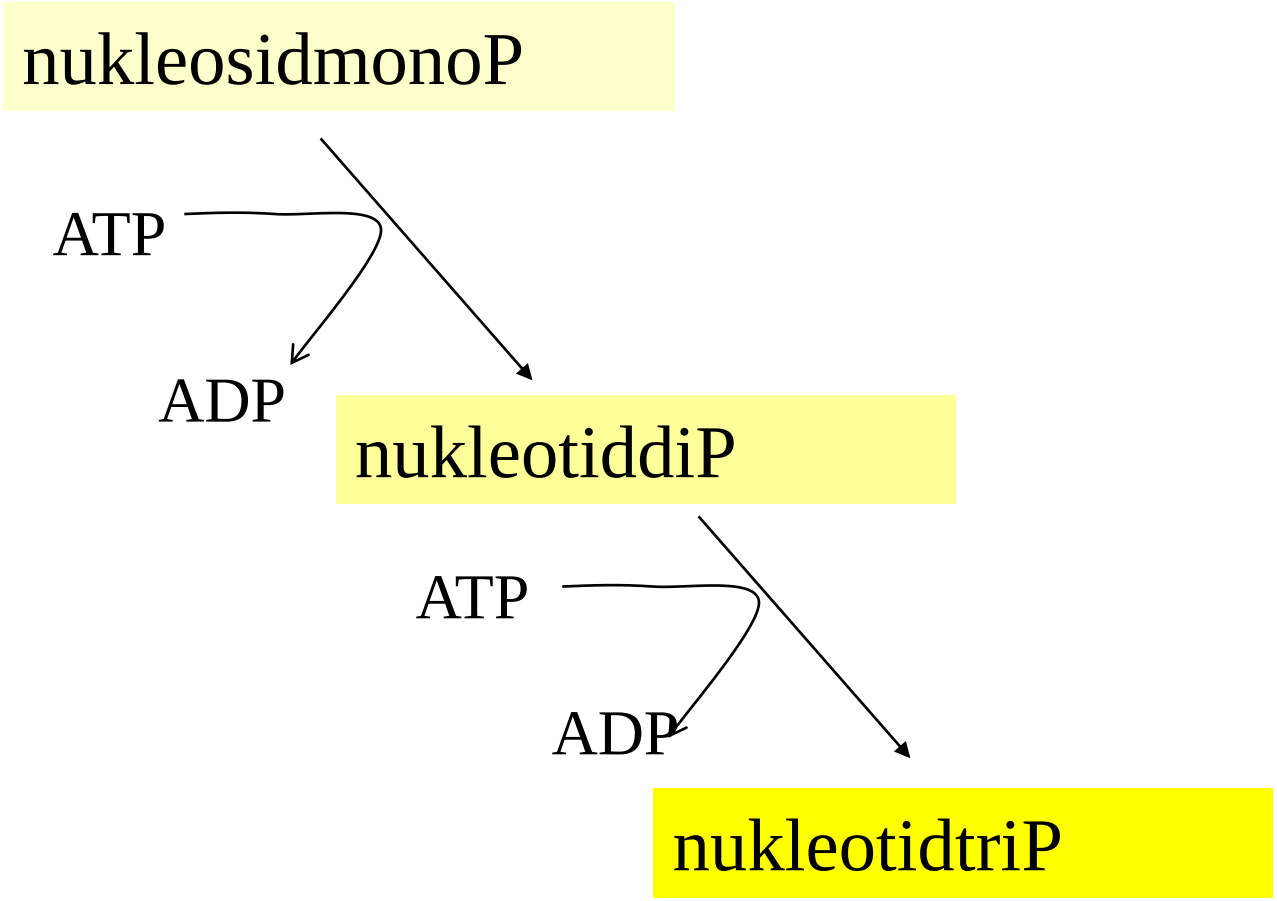


AMP adeninphosphoribosyltransferase

Phosphoribosyltransferase deficiency causes Lesch-Nyhan syndrome

hereditary disease
overproduction purine bases
accumulation of uric acid - DNA
mental retardation, self-mutilation

Syntesis of nukleotiddiP and triP



Regulation of biosyntesis of purins

1.

- concentration of PRPP

Speed of use

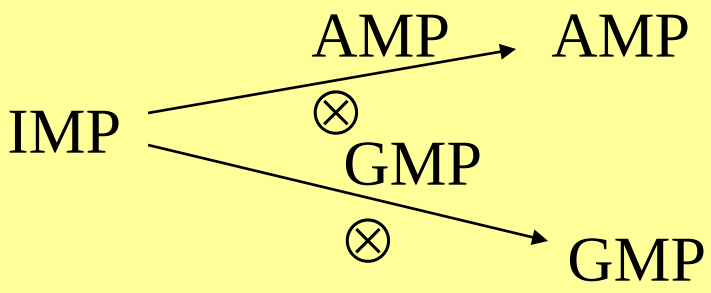
Speed of synthesis

Speed of degradation

2.

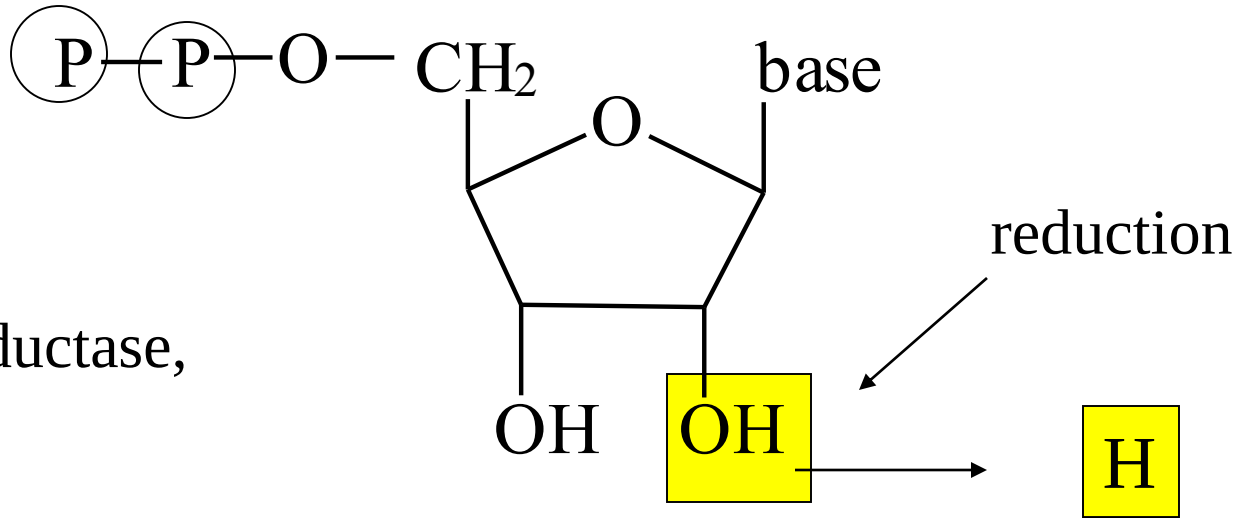
- inhibice PRPP-glutamylamidotransferase by AMP and GMP (end products)

3.



2-deoxyribonucleotides

NucleotiddiP → 2-deoxynucleotiddiP



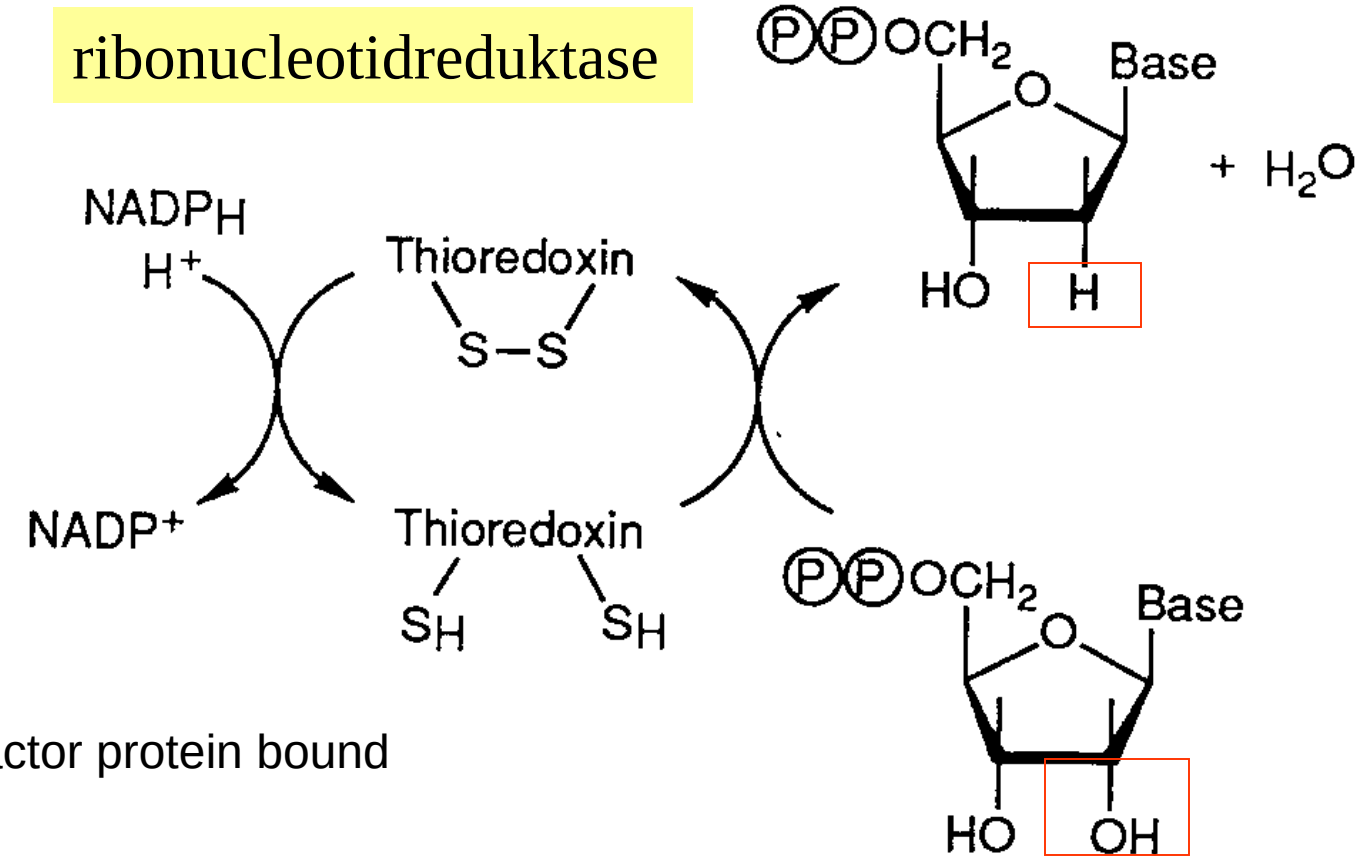
thioredoxin,
thioredoxinreductase,
NADPH

Thioredoxinreductase - Se

deoxygenati
on

Nukleotiddiphosphate → deoxynucleotiddiphosphate

ribonucleotidreduktase



cofactor protein bound

Degradation of purines

liver

AMP,GMP,

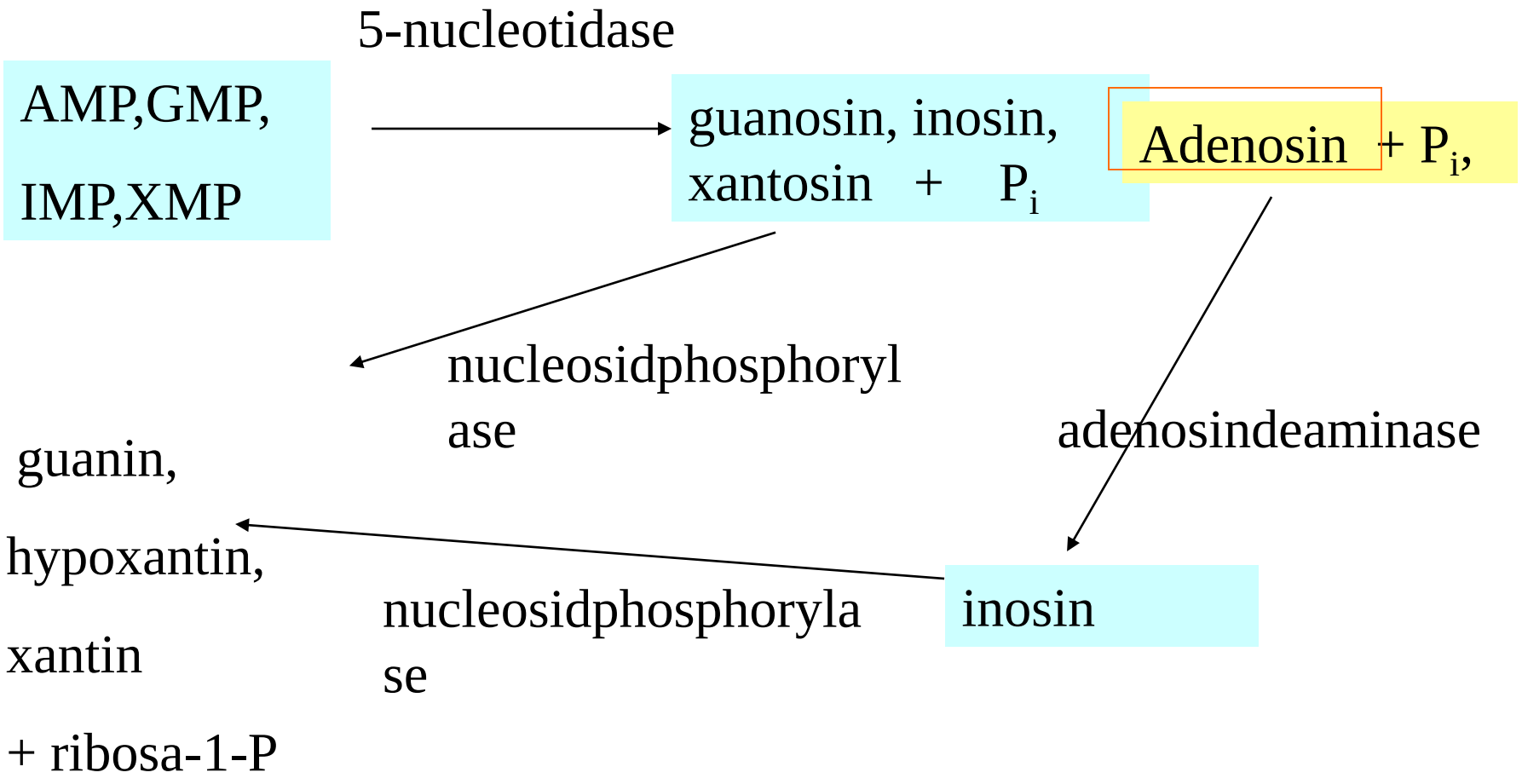
IMP,XMP

5-nukleotidase

Cleavage of P

guanosin, inosin,
xantosin + P_i

Adenosin + P_i,


5-nucleotidase
AMP,GMP,
IMP,XMP
guanosin, inosin,
xantosin + P_i
Adenosin + P_i,
nucleosidphosphoryl
ase
adenosindeaminase
guanin,
hypoxantin,
xantin
+ ribosa-1-P
nucleosidphosphoryla
se
inosin

adenosine deaminase deficiency

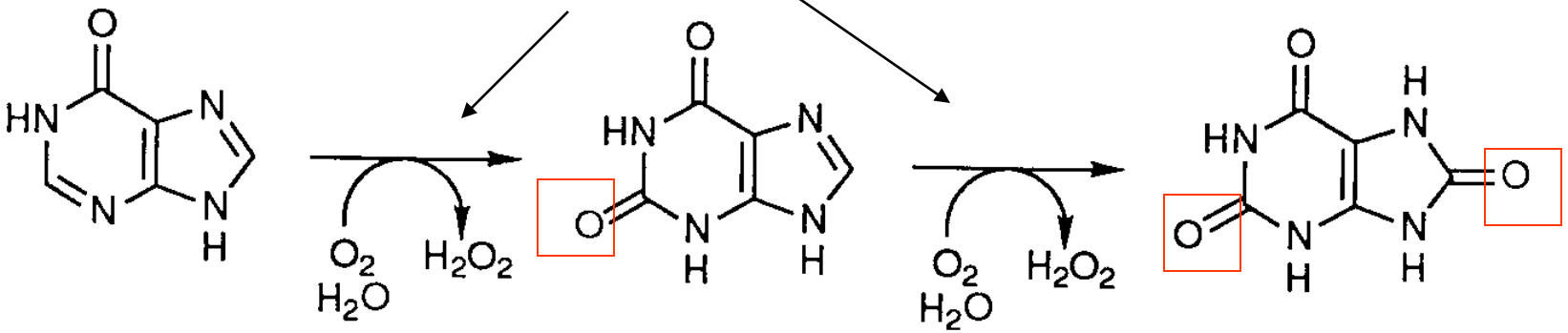
Enzyme deficiency leads to the accumulation of toxic deoxyadenosine, which affects immunocompetent cells
One of the causes of severe combined immunodeficiency (severe combined immunodeficiency disease-SCID).

Degradation of purins

TEST

Inhibition by allopurinolem

xantinoxidase



hypoxantin

xantin

Uric acids

guanase

guanin

end metabolit primate, ….. (400-600 mg /den)

TEST

Defects in metabolism of purins

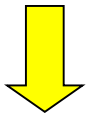
gout

- increasing of production and decreasing of excretion of uric acid
- defect in salwa pathway

⟶ (deficit hypoxantin-guaninphosphoribosyltransferase) (HGPRT)

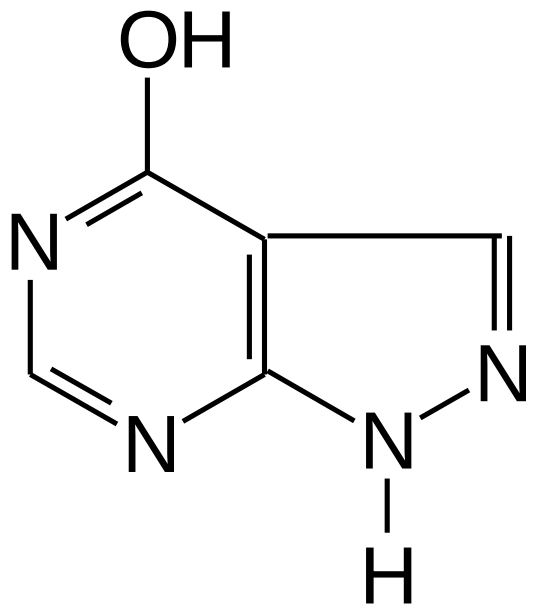
hypoxantin + PRPP ⟶⊗ IMP + PP

- decrease of clearance in kidney



Keeping of crystals of UA in tissue

Allopurinol – competitive inhibitor xantinoxidasy



Gout: allopurinol inhibits the oxidation of hypoxanthine to xanthine

hypoxanthine is more soluble and more readily excreted

hypouricemia

xantinoxidasy deficit (excretion of hypoxanthine and xanthine)

protein synthesis

Synthesis of proteins - translations

Where: in cells containing nuclear DNA

Where cell: ribosomes (free or bound to the ER, mitochondria)

prokaryotes: transcription, editing, transcript and translation are spatially separated

eukaryotes: translation in progress to mature mRNA is transported to the cytoplasm

Molecules which are necessary for protein synthesis?

Amino acids

A number of enzymes

protein factors

ATP and GTP

The inorganic ions (Mg2 +, K +)

Effects of antibiotics on protein synthesis of prokaryotes

antibiotic effect

Streptomycin binds to the 30S ribosomal subunit, inhibits the formation of initiation complex errors in reading the mRNA.

Tetracycline binds to the 30S ribosomal subunit and inhibits the binding of aminoacyl-tRNA to A
Chloramphenicol binds to the 50S ribosomal subunit and inhibits peptidyltransferasu
Erythromycin binds to the 50S ribosomal subunit and inhibits translocation

Puromycin Populated A-site of the ribosome, causing premature termination

TEST

Protein folding (folding)

The nascent polypeptide chain is transported through the ribosome

Gradually getting out of a "protected" area of the ribosome and set its spatial folding

Folding (folding) is mediated by specific proteins - chaperones (heat shock proteins)

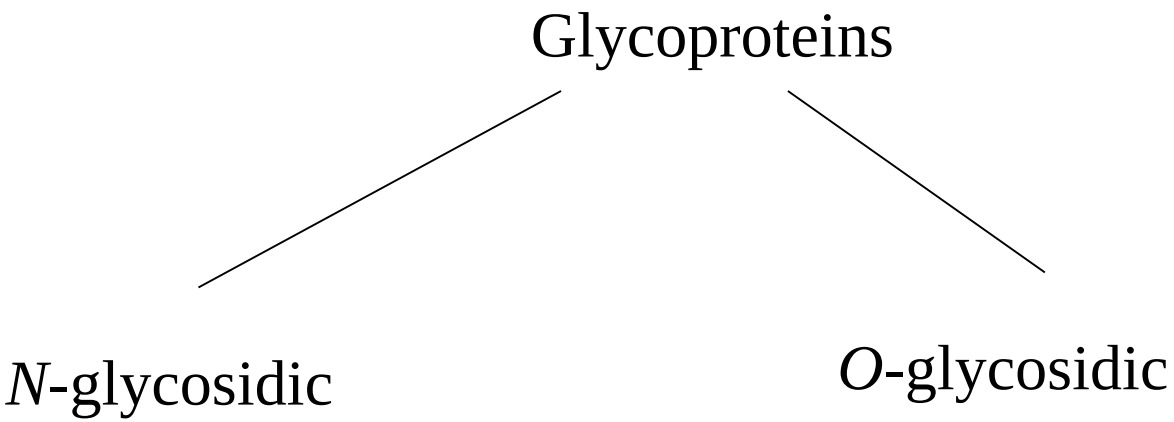
Faults in composing - Alzheimer's disease, BSE, cystic fibrosis ad.

TEST

Post-translational modification of proteins

-Removing methionine residue
-Changing the length of the molecule (cleavage of the polypeptide chain)
-glycosylation
-acetylation
-carboxylation
-methylation
-prenylation
- hydroxylation
-Sulfation ad.

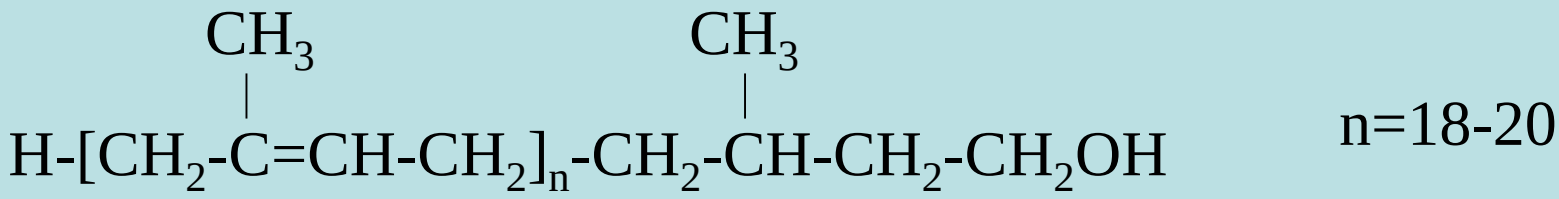
Glykosylation of proteins



They differ in carbohydrate synthesis method

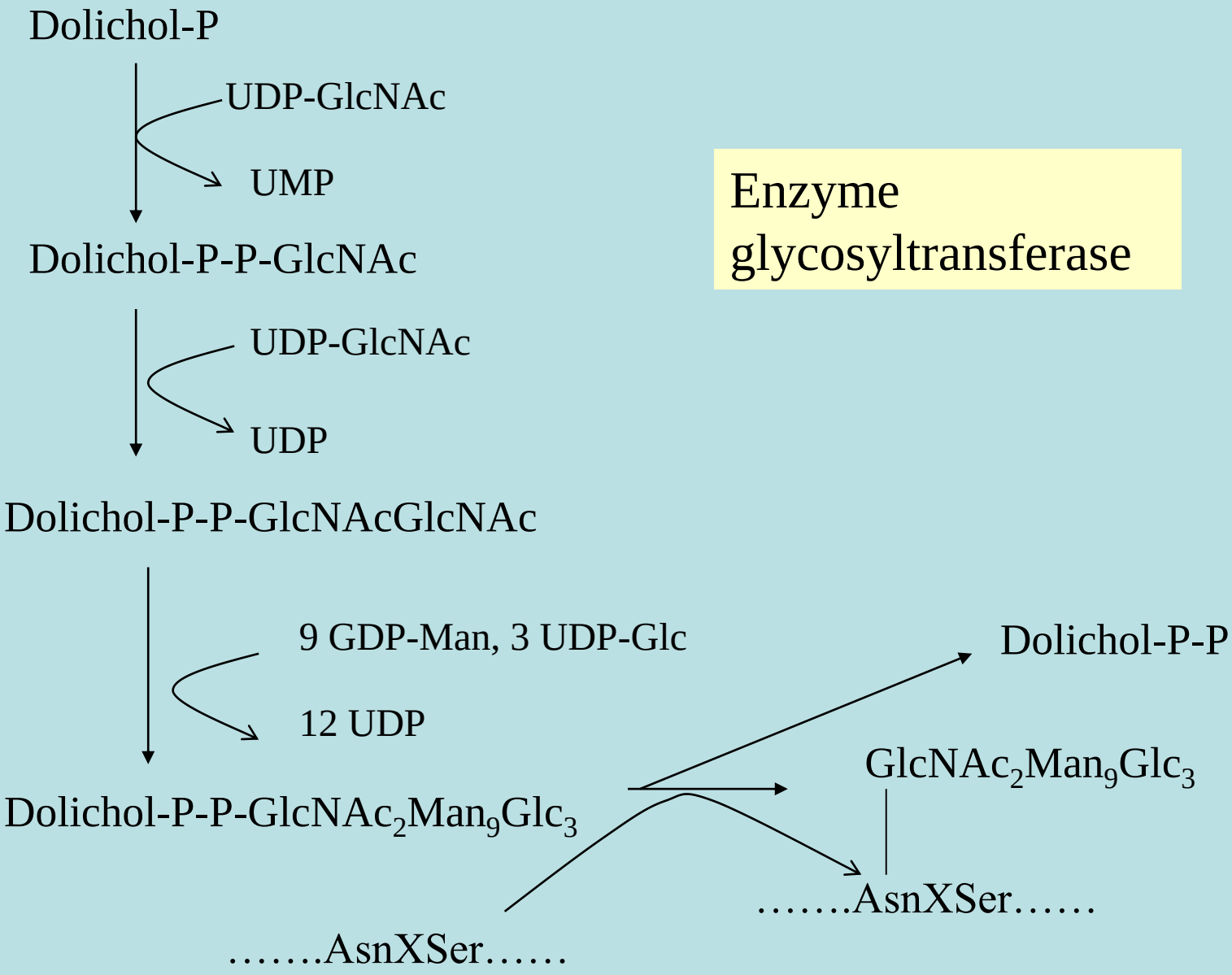
Synthesis of N-glycoproteins

Synthesis of sugar components takes place outside the protein
The base is polyisoprene dolichol (see synthesis of cholesterol)



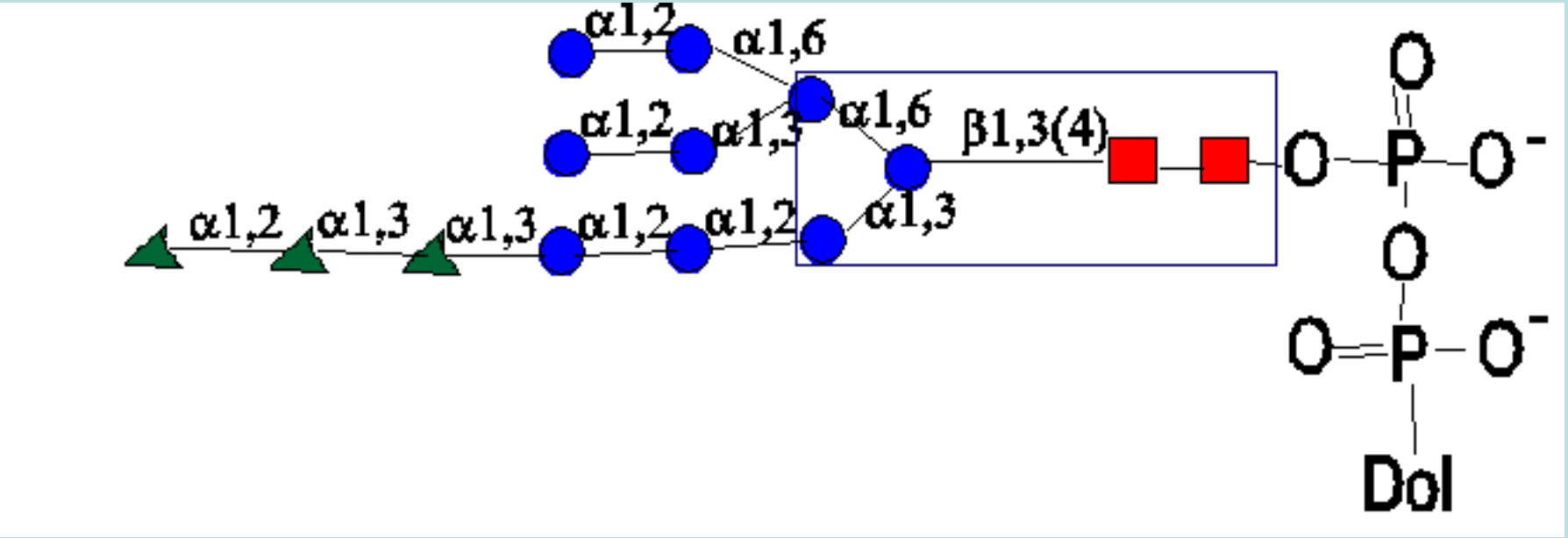
Dolichol diphosphate as it is bound in the ER membrane, the terminal phosphate is gradually adjoin the activated monosaccharides.
Ready oligosaccharide is transferred to a protein is bound via asparagine N-glycosidic linkage. In plasma protein binding will take place finish oligosaccharide component.

Glykosylation of dolichol



Enzyme glycosyltransferase

The oligosaccharide precursor bound dolichol

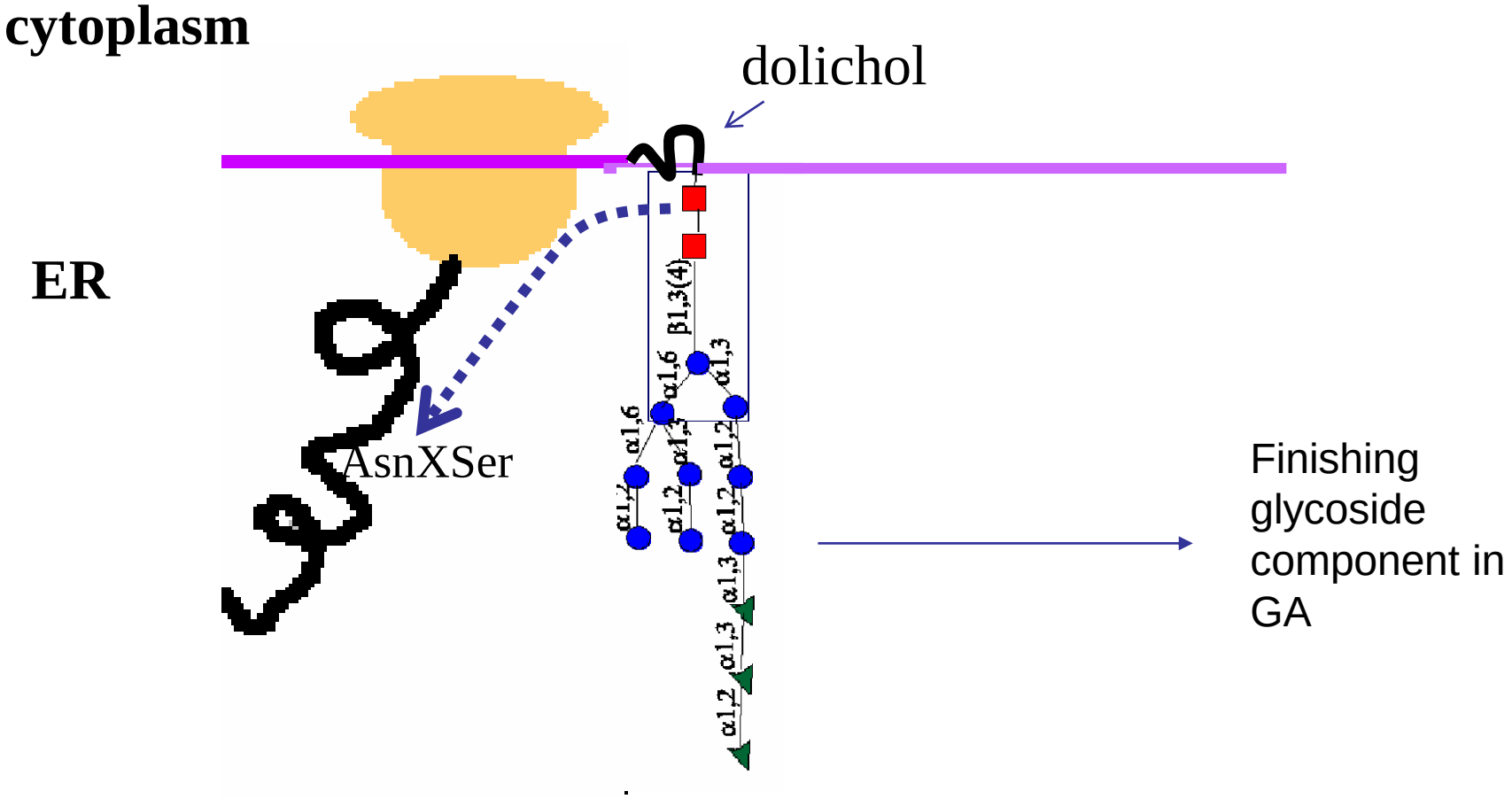


glucose

mannose

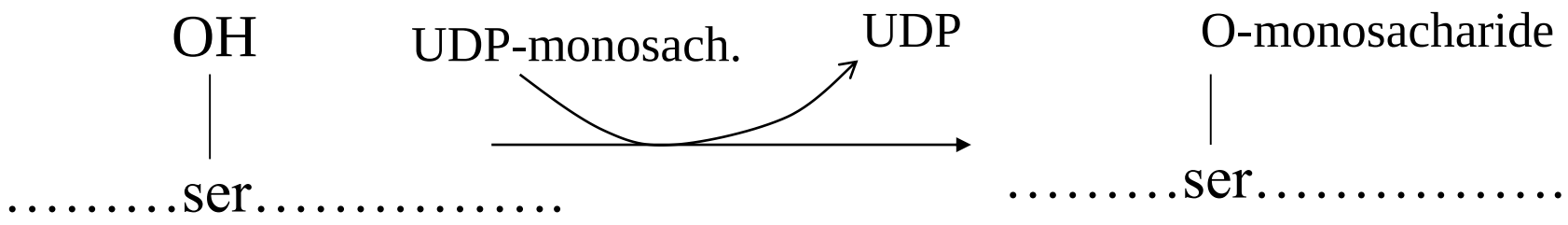
N-acetylglukosamin

Cotranslation glycosylation



Synthesis of O-glycoproteins

Takes place in the ER and the Golgi



Activated monosaccharides are sequentially attached
O-glycosidic linkage

TEST

Transport of proteins to subcellular and extracellular spaces (targeting)

Protein synthesis on free ribosomes

↓

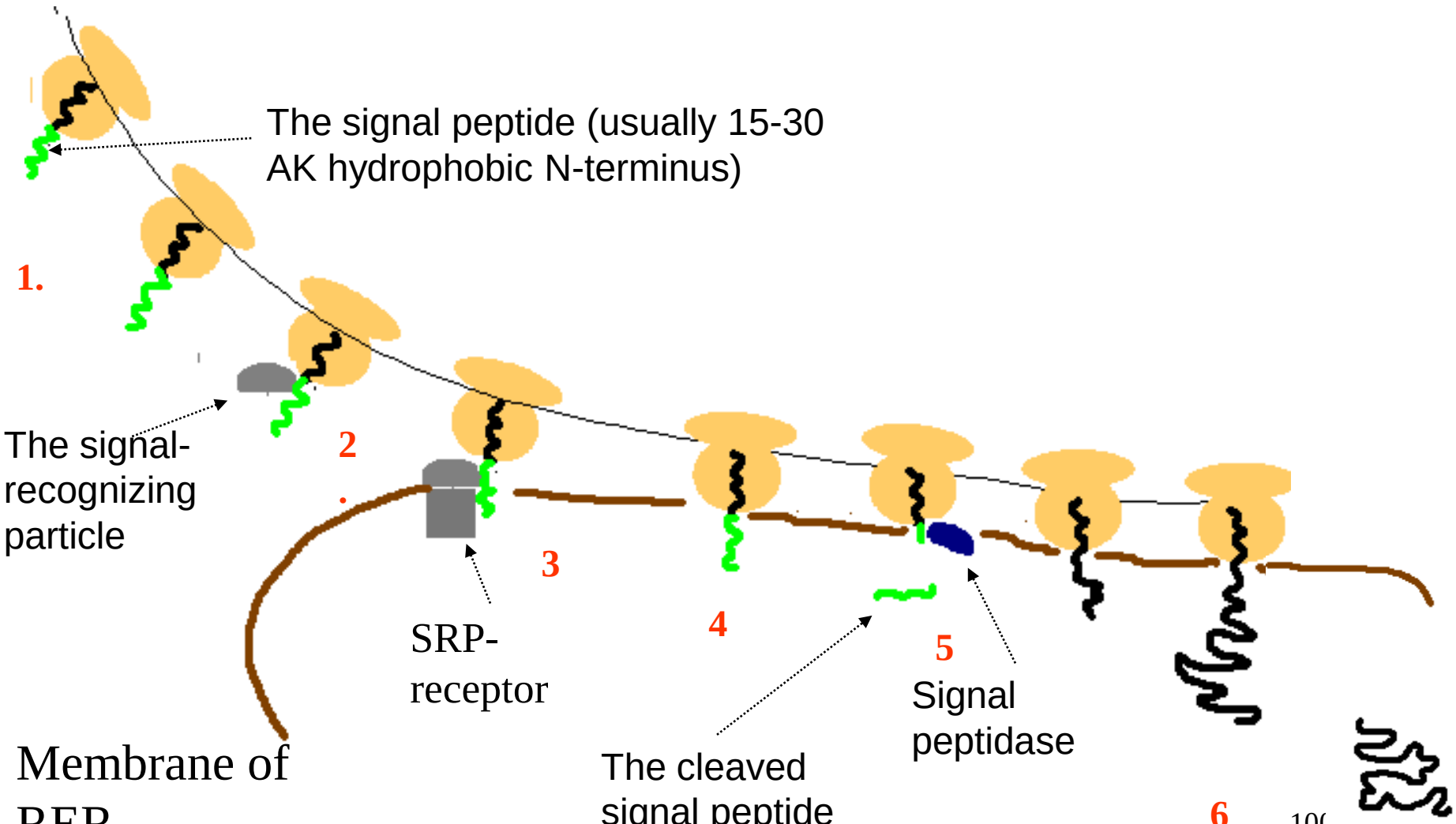
Proteins remain in the cytoplasm and are transported into the organelles (nucleus, mitochondria). AK contain a sequence which directs the transport

Protein synthesis on the RER

↓

Transport into lysosomes, ER, Golgi apparatus or the membranes, secretion from a cell

Transport of proteins synthesized on the RER



TEST

Transport of proteins synthesized on the RER

1. The translation begins in the cytosol

2. Once the signal peptide leaves the ribosome, it binds to the signal-recognizing particle (signal recognition particle-SRP). At the same time binds the ribosome and inhibits further synthesis

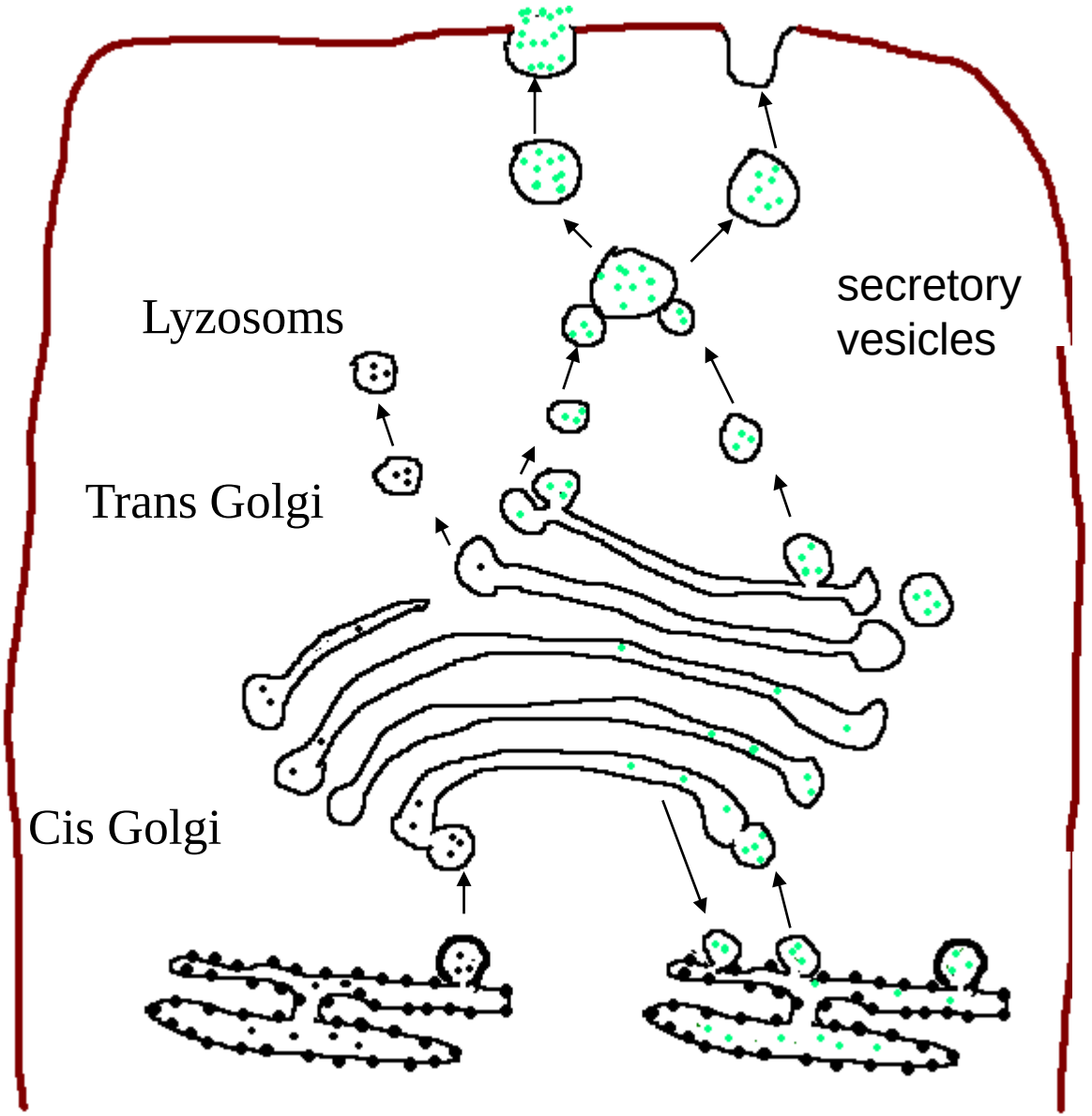
3. SRP particle binds to SRP receptor in the RER membrane and attaches to the ribosome RER

4. SRP is released and continues synthesis

5. Once the signal peptide penetrates the RER, signal peptidase deletes it

6. The synthesis of nascent protein and continues complete protein is released into the RER

Transport of proteins synthesized on the RER



Transport of proteins synthesized on the RER-cont.

1. Proteins synthesized on the RER are transported in the form of vesicles of the cis-Golgi

2. Here is a sorting center - structural features determine where the protein routing (sorting)

3. Some remain in the Golgi apparatus, while others are returned to the RER

4. Another wander in the form vesicles in the trans Golgi delivery

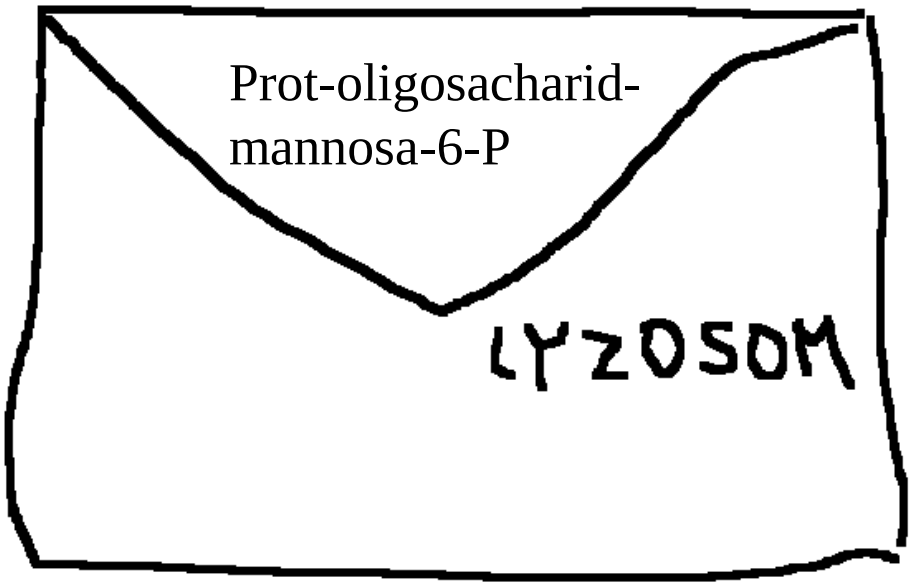
5. Here are separating lysosomes and secretory vesicles

6. The contents of secretory vesicles is released extracellularly

7. Hydrophobic proteins embedded in the membranes of vesicles become membrane proteins

Principles of intracellular sorting (sorting)

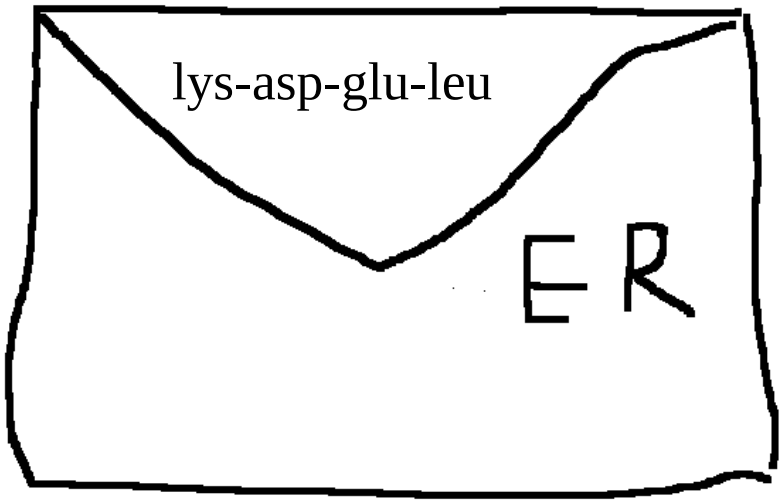
Example 1:
Proteins destined for lysosomes are labeled N-linked oligosaccharide terminated with mannose-6-P



"Address" is recognized by specific membrane receptors in the Golgi, the protein is incorporated into a coated vesicle klathrinem

Principles of intracellular sorting

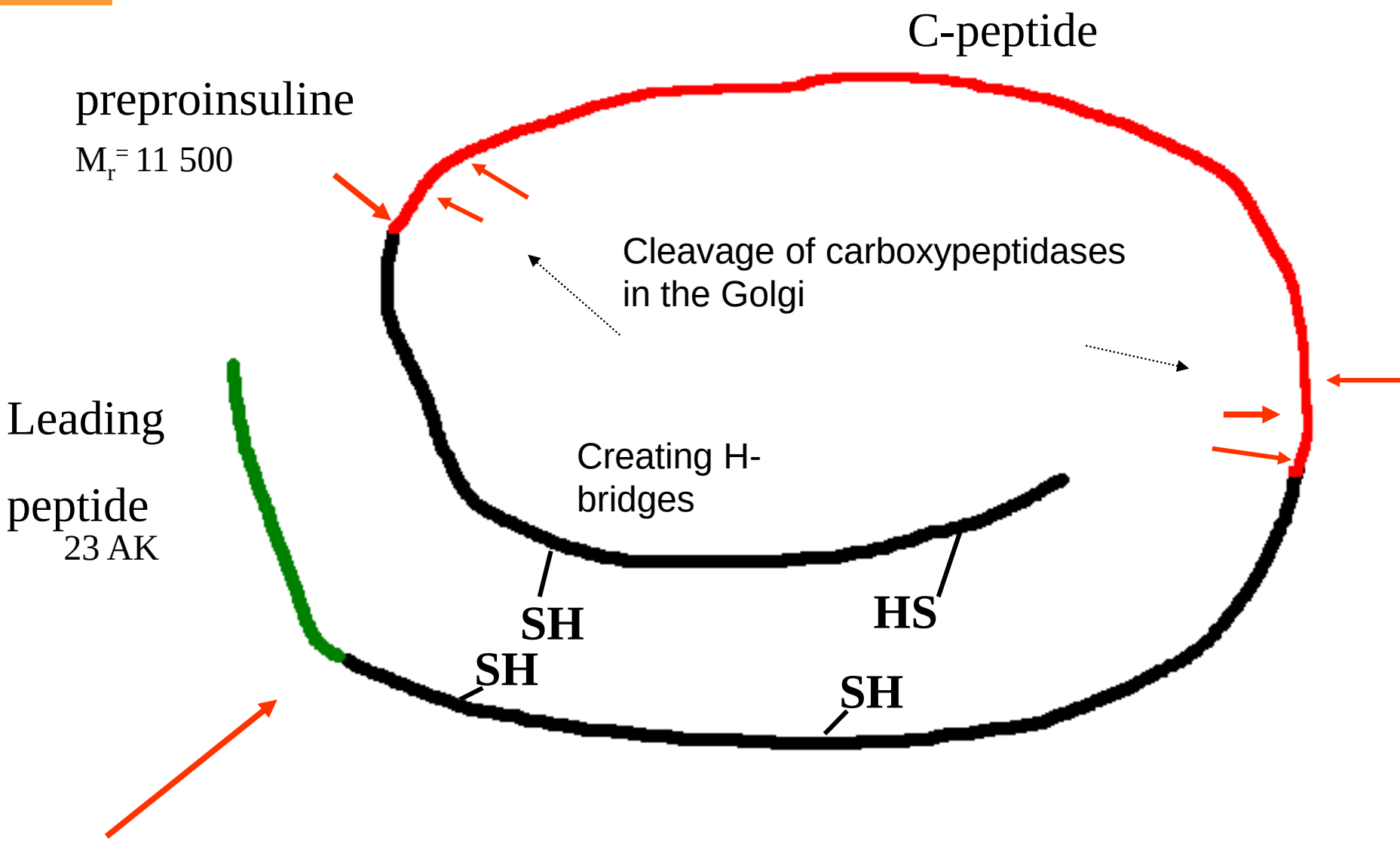
Example 2:
Proteins destined for the ER to the carboxyl terminus of the sequence Lys-Asp-Glu-Leu



The proteins are transported from the Golgi back to the ER

Example posttranslational modifications: the synthesis of insulin

TEST



1 Cleavage of the peptide lead ER