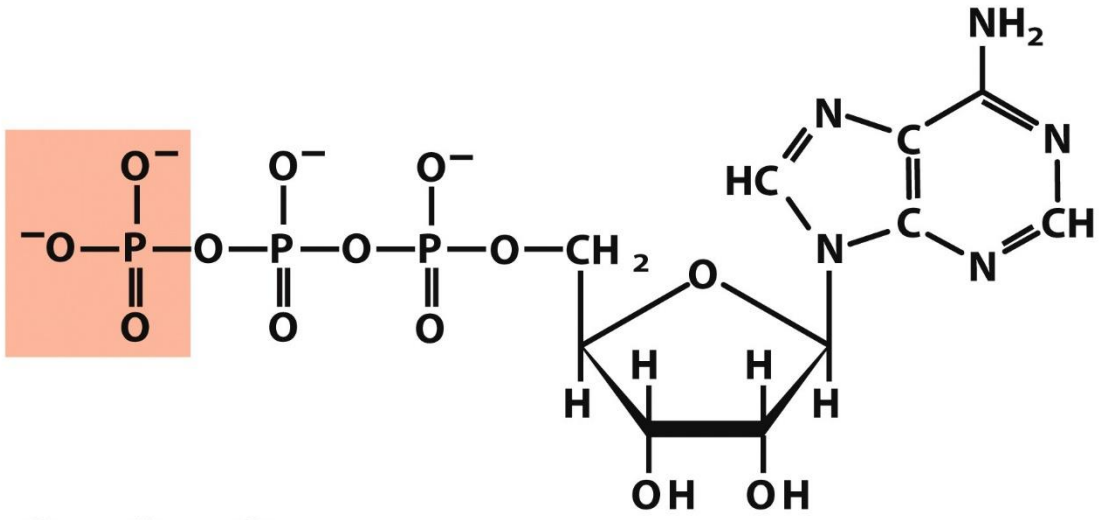
Purines and pyrimidines, nucleosides, nucleotides: Structure, function and metabolism



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Nucleotides have a variety of roles in cellular metabolism

- They are activated precursors of **nucleic acids**.

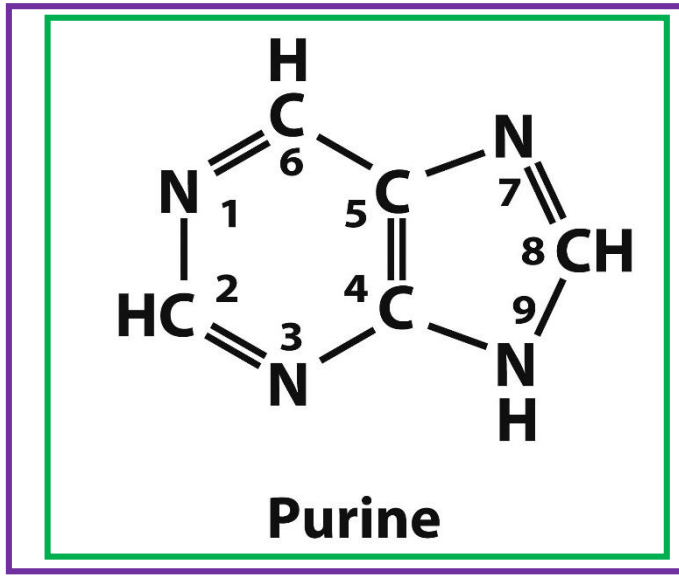
- ATP is the universal **currency of energy**; GTP also serves as an energy source for a more select group of biological processes.

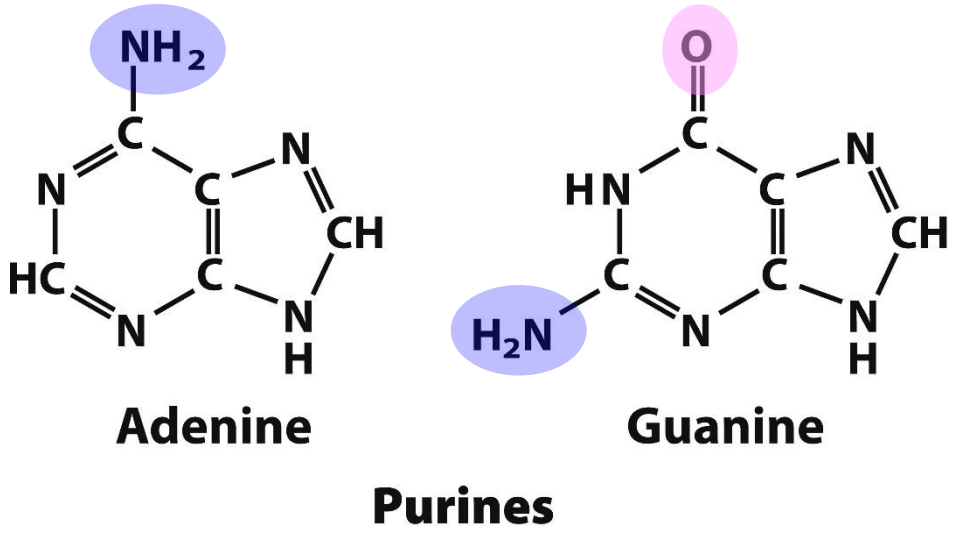
- Nucleotide derivatives (such as UDP-glucose) participate in **biosynthetic processes** (such as the formation of glycogen).

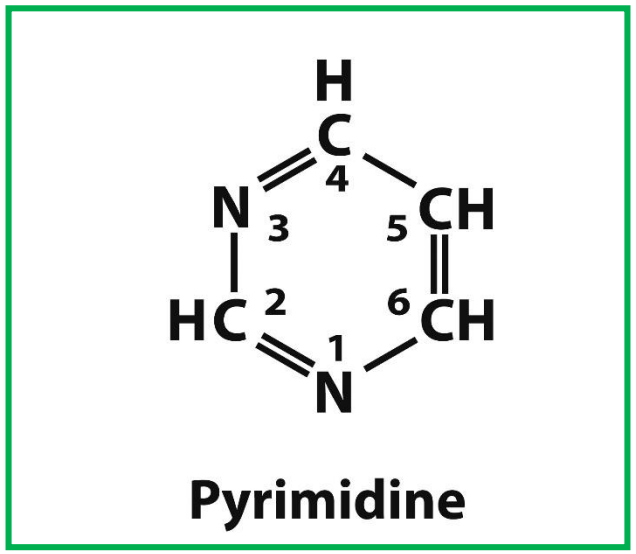
- They are essential components of **signal-transduction pathways** (cyclic nucleotides such as cAMP and cGMP are second messengers that transmit signals both within and between cells).

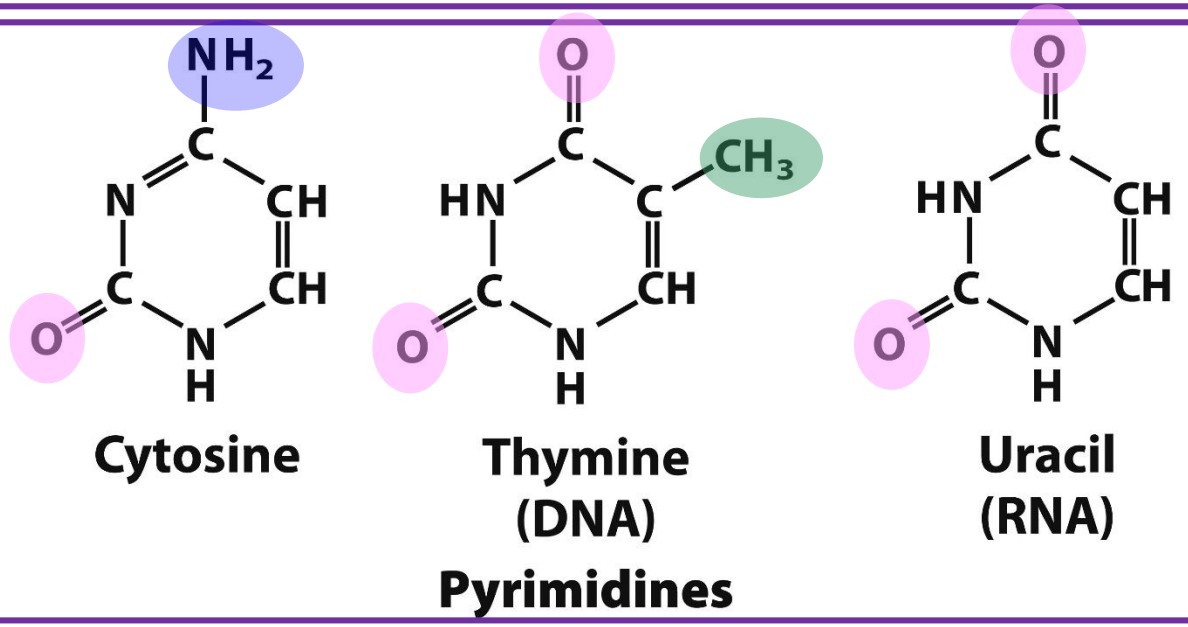
- They are components of the **cofactors** NAD, FAD, *S*-adenosylmethionine and coenzyme A.

Major purine and pyrimidine bases



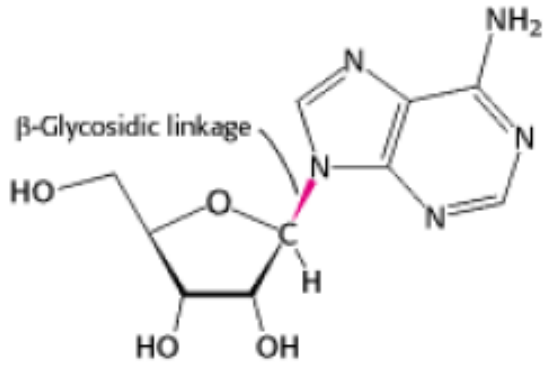


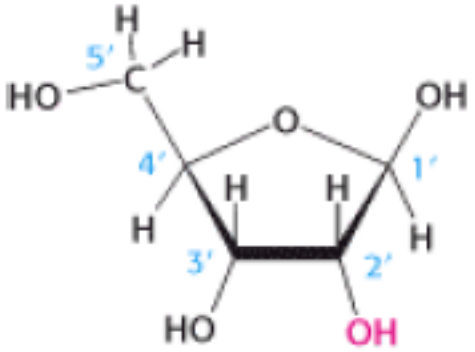




NUCLEOSIDES

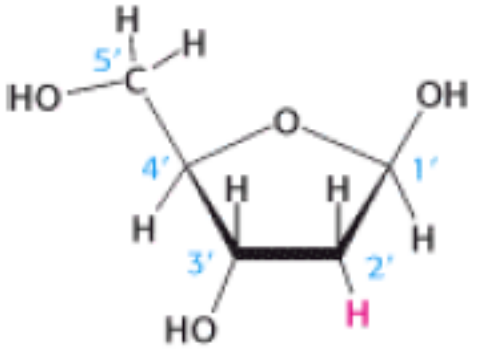
purine or pyrimidine base + sugar





ribose

in ribonucleic acid (RNA)



deoxyribose

in deoxyribonucleic acid (DNA)

NUCLEOTIDES

phosphate esters of nucleosides (base + sugar + phosphate)

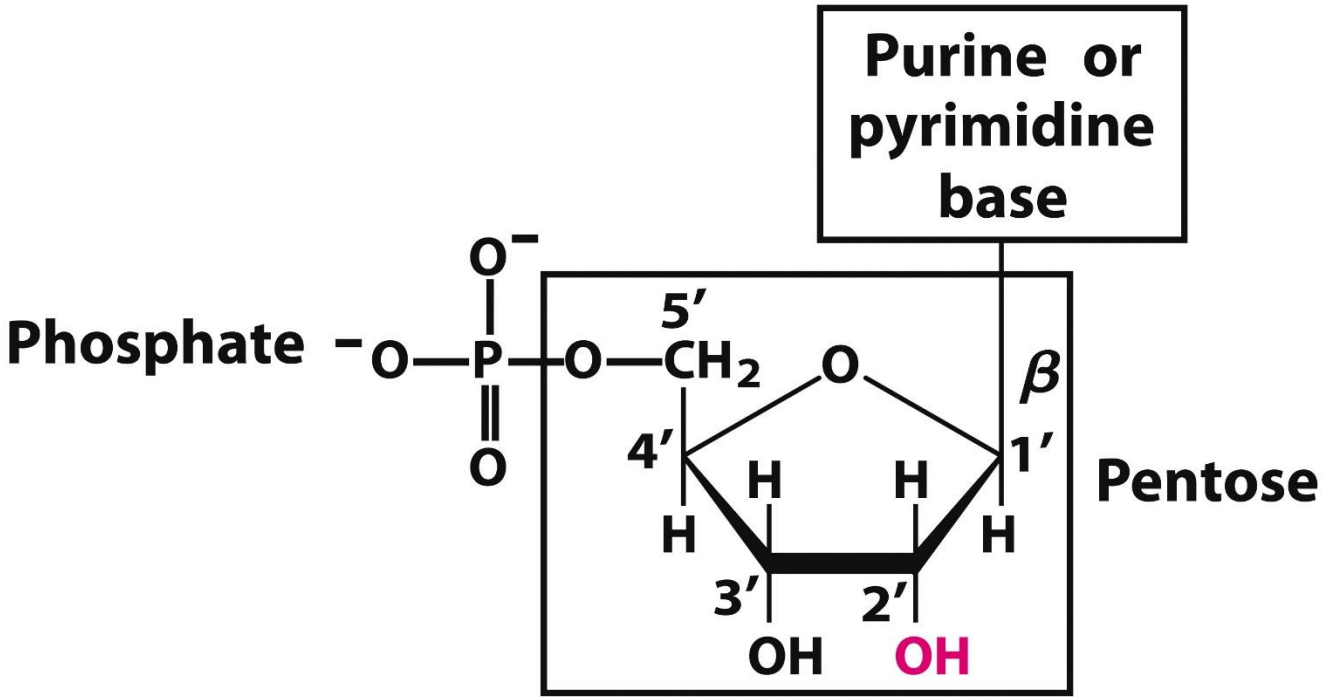
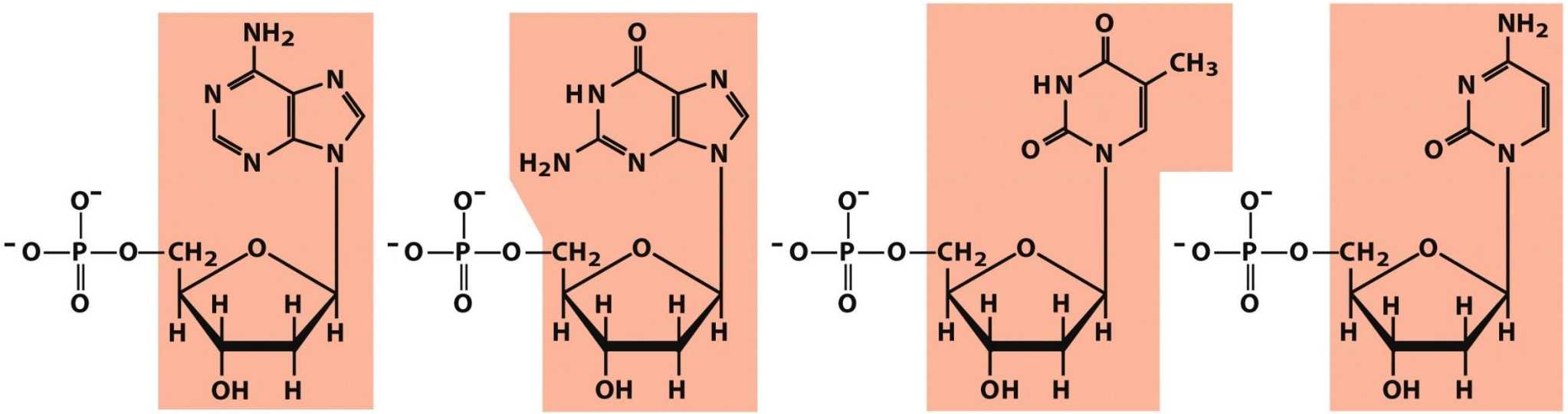


TABLE 8–1 Nucleotide and Nucleic Acid Nomenclature

Base	Nucleoside	Nucleotide	Nucleic acid
Purines			
Adenine	Adenosine	Adenylate	RNA
	Deoxyadenosine	Deoxyadenylate	DNA
Guanine	Guanosine	Guanylate	RNA
	Deoxyguanosine	Deoxyguanylate	DNA
Pyrimidines			
Cytosine	Cytidine	Cytidylate	RNA
	Deoxycytidine	Deoxycytidylate	DNA
Thymine	Thymidine or deoxythymidine	Thymidylate or deoxythymidylate	DNA
Uracil	Uridine	Uridylate	RNA

Note: "Nucleoside" and "nucleotide" are generic terms that include both ribo- and deoxyribo- forms. Also, ribonucleosides and ribonucleotides are here designated simply as nucleosides and nucleotides (e.g., riboadenosine as adenosine), and deoxyribonucleosides and deoxyribonucleotides as deoxynucleosides and deoxynucleotides (e.g., deoxyriboadenosine as deoxyadenosine). Both forms of naming are acceptable, but the shortened names are more commonly used. Thymine is an exception; "ribothymidine" is used to describe its unusual occurrence in RNA.

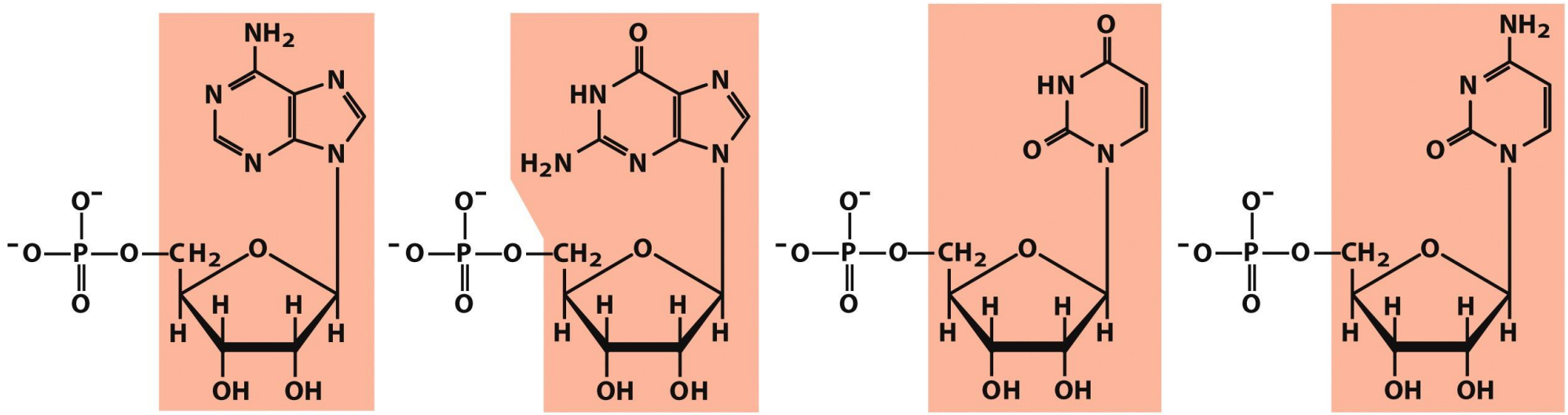
Major deoxyribonucleotides



	Deoxyadenylate (deoxyadenosine 5′-monophosphate)	Deoxyguanylate (deoxyguanosine 5′-monophosphate)	Deoxythymidylate (deoxythymidine 5′-monophosphate)	Deoxycytidylate (deoxycytidine 5′-monophosphate)
Nucleotide:				
Symbols:	A, dA, dAMP	G, dG, dGMP	T, dT, dTMP	C, dC, dCMP
Nucleoside:	Deoxyadenosine	Deoxyguanosine	Deoxythymidine	Deoxycytidine

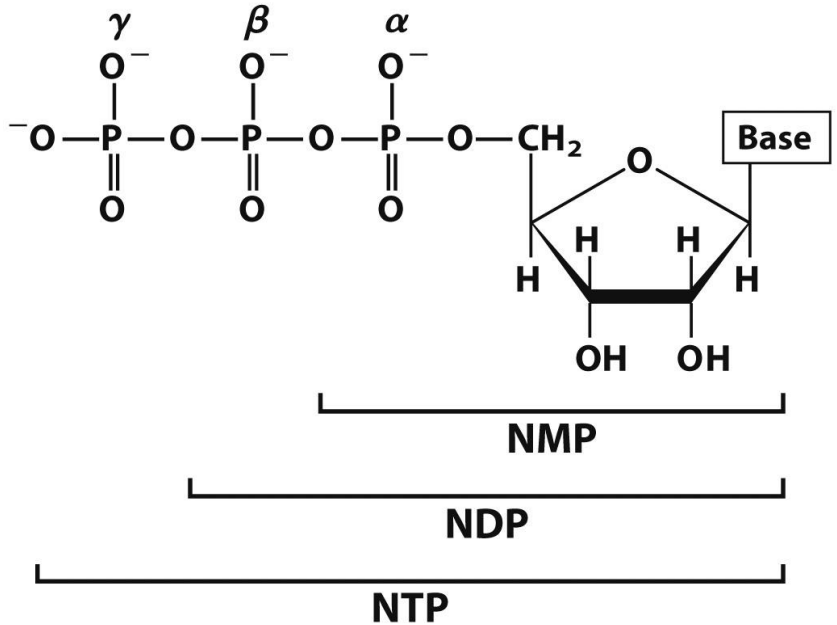
Deoxyribonucleotides

Major ribonucleotides



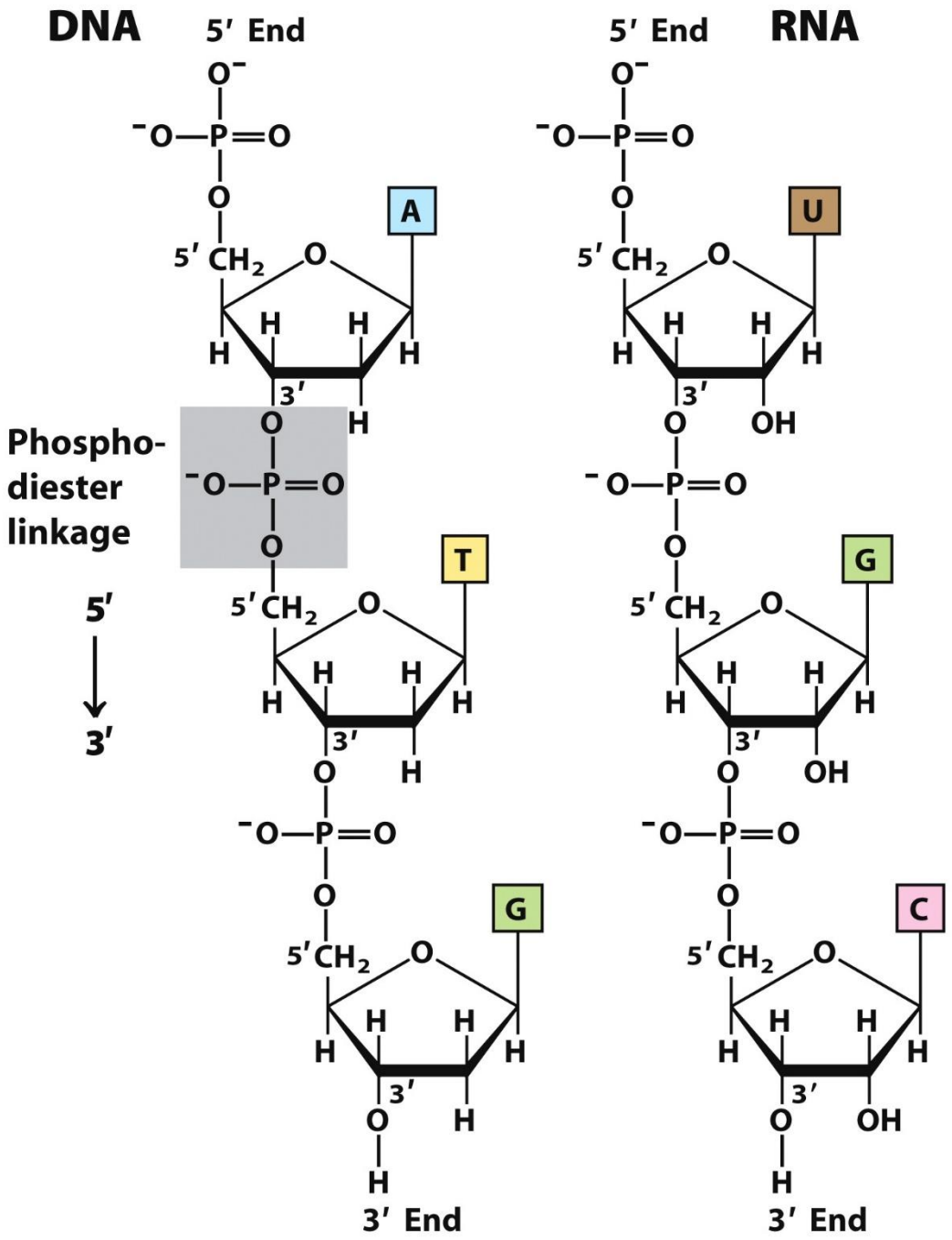
Nucleotide:	Adenylate (adenosine 5′-monophosphate)	Guanylate (guanosine 5′-monophosphate)	Uridylate (uridine 5′-monophosphate)	Cytidylate (cytidine 5′-monophosphate)
Symbols:	A, AMP	G, GMP	U, UMP	C, CMP
Nucleoside:	Adenosine	Guanosine	Uridine	Cytidine

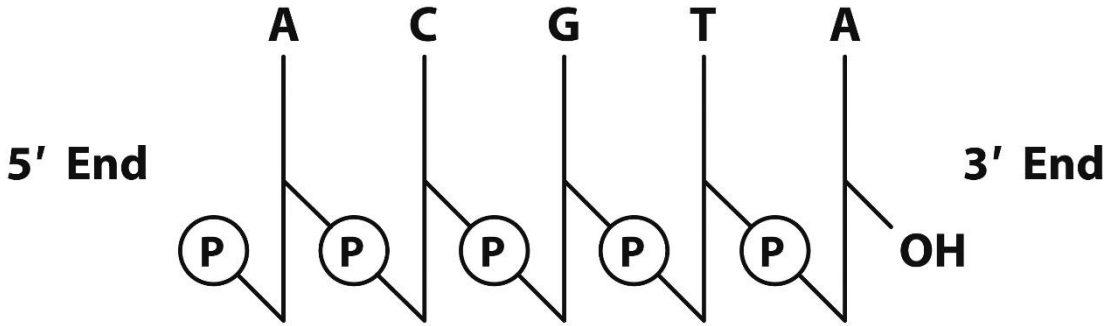
Ribonucleotides



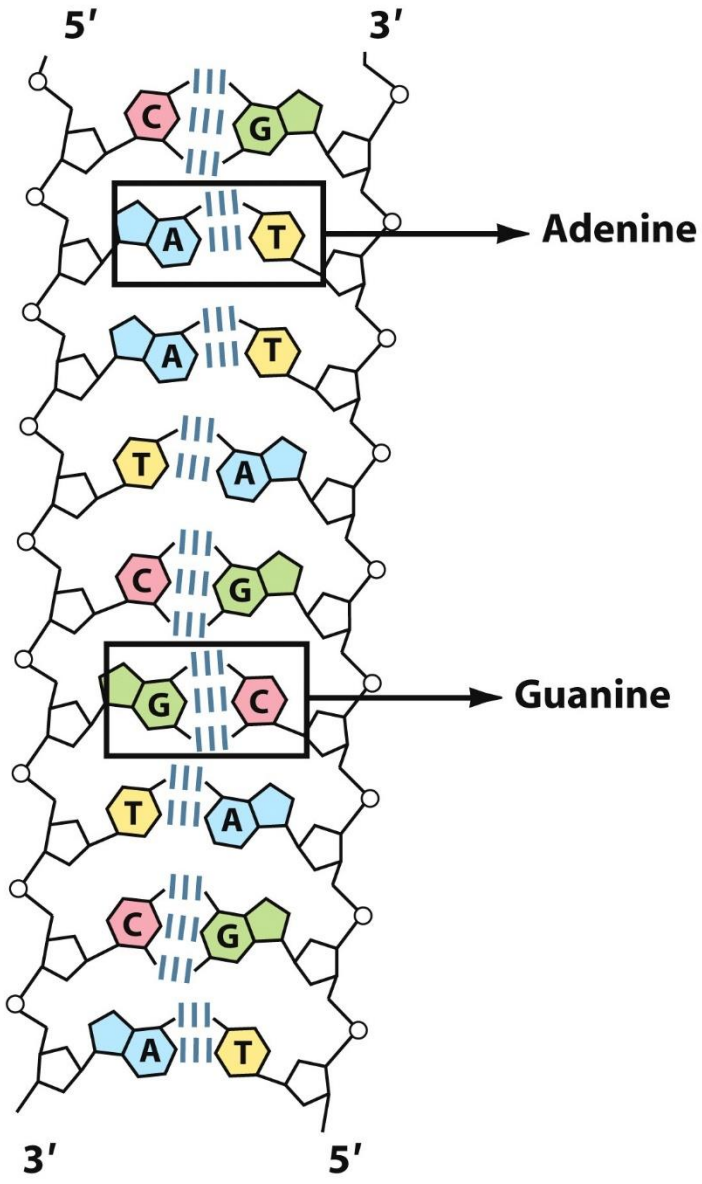
Abbreviations of ribonucleoside 5′-phosphates			
Base	**Mono-**	**Di-**	**Tri-**
Adenine	AMP	ADP	ATP
Guanine	GMP	GDP	GTP
Cytosine	CMP	CDP	CTP
Uracil	UMP	UDP	UTP

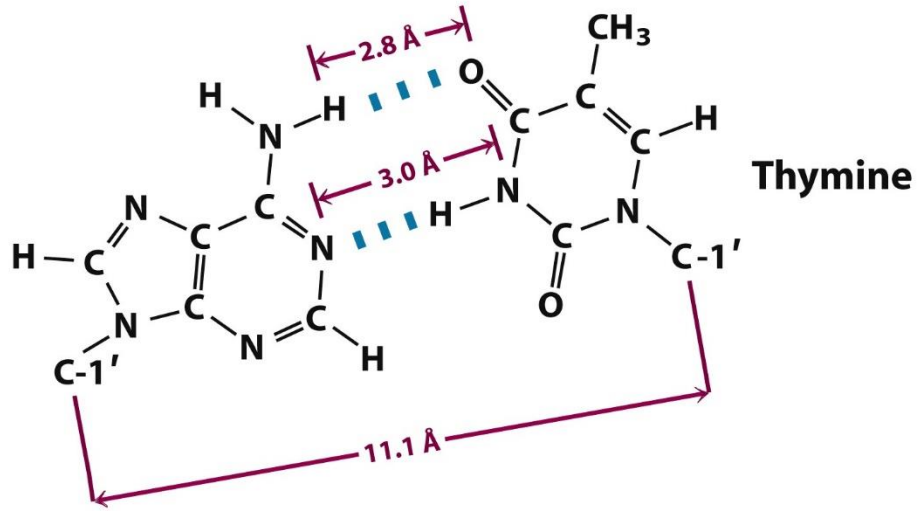
Abbreviations of deoxyribonucleoside 5′-phosphates			
Base	**Mono-**	**Di-**	**Tri-**
Adenine	dAMP	dADP	dATP
Guanine	dGMP	dGDP	dGTP
Cytosine	dCMP	dCDP	dCTP
Thymine	dTMP	dTDP	dTTP

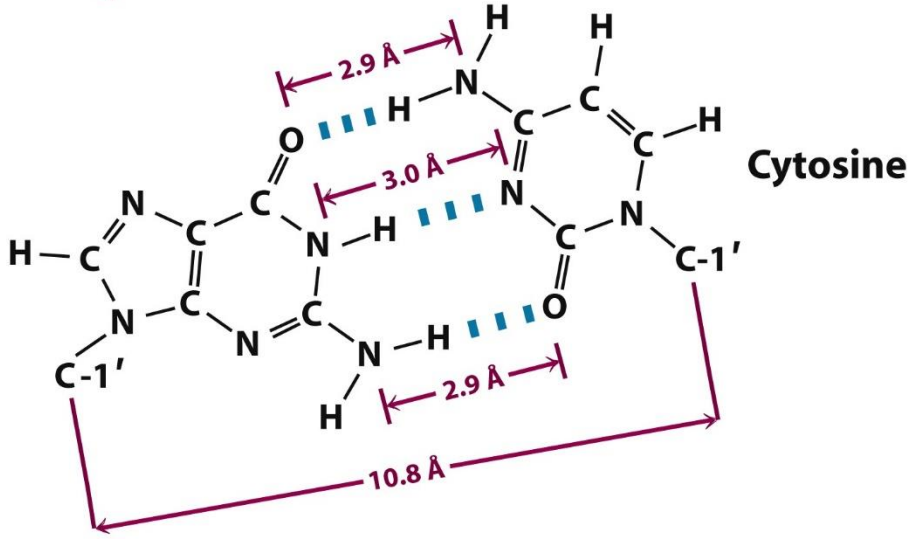




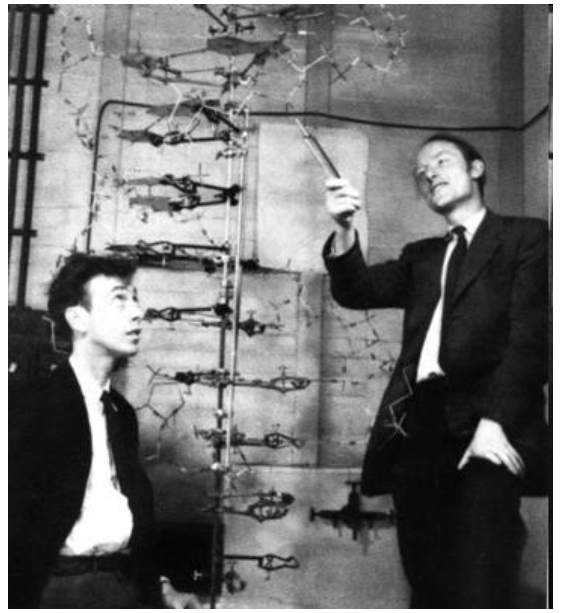
The successive nucleotides of both DNA and RNA are covalently linked by phosphodiester bonds

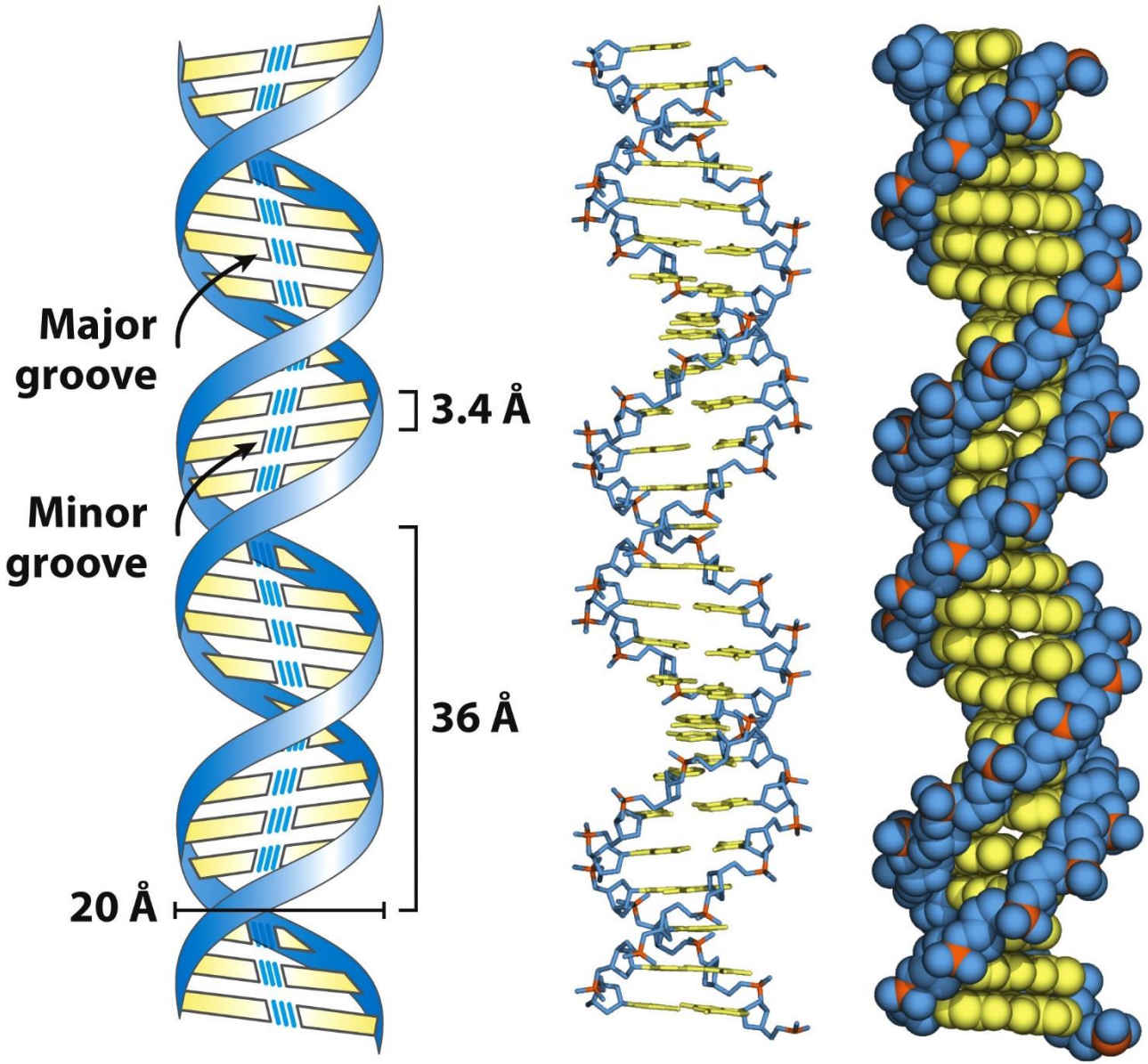




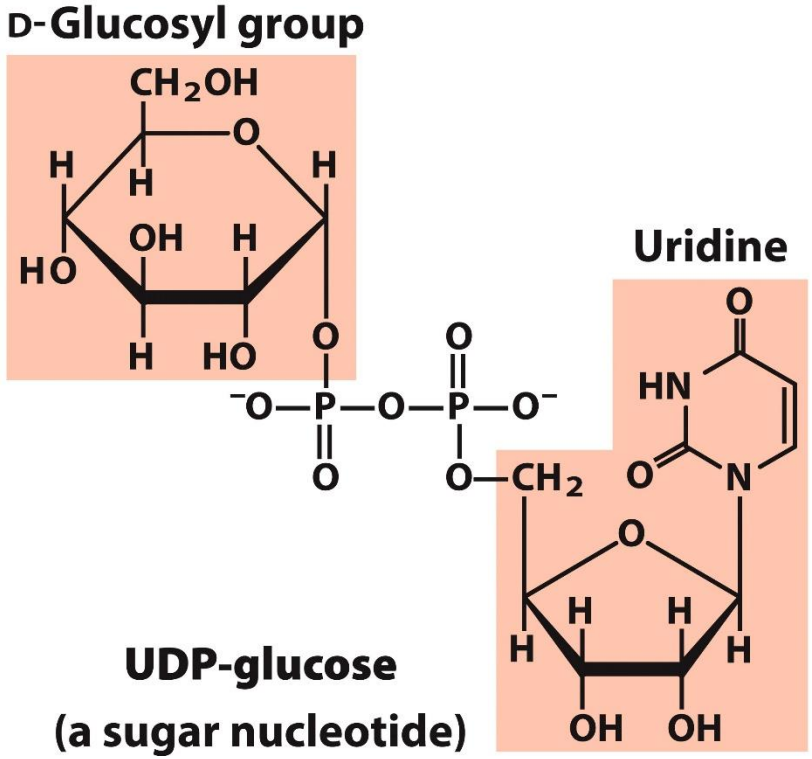


the double helix structure of the DNA molecule was proposed by Watson and Crick in 1953.

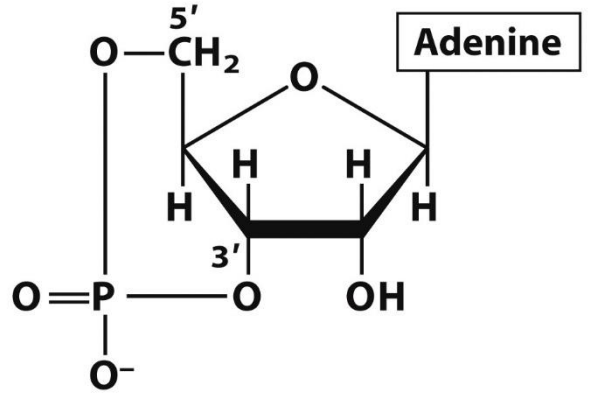



Major groove
Minor groove
3.4 Å
36 Å
20 Å

UDP-glucose

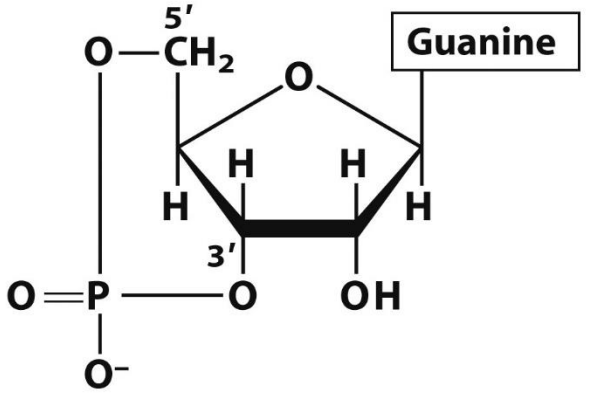


cyclic AMP (cAMP)



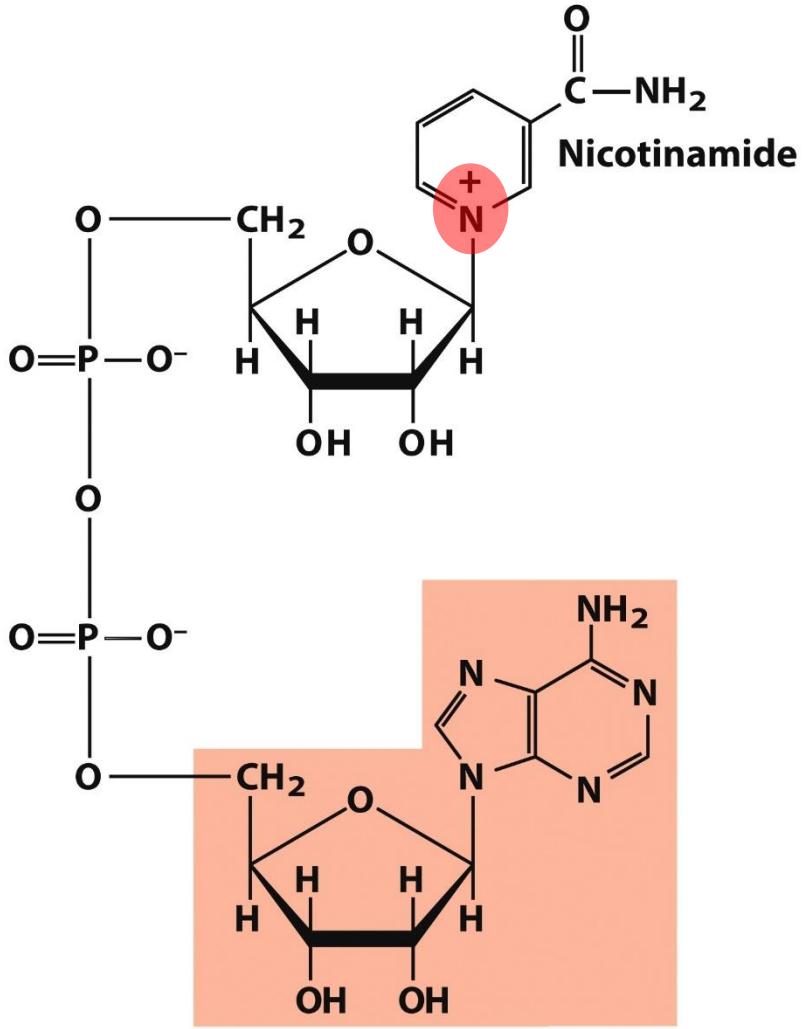
Adenosine 3′,5′-cyclic monophosphate (cyclic AMP; cAMP)

cyclic GMP (cGMP)



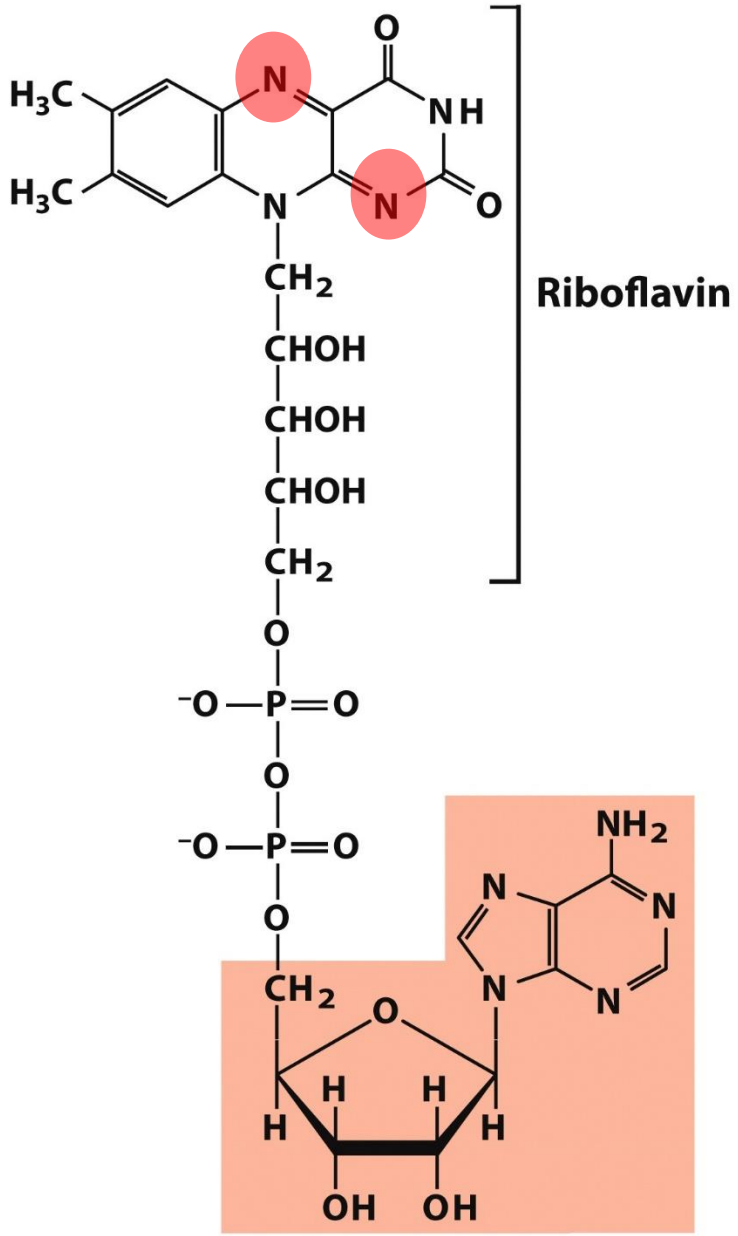
Guanosine 3′,5′-cyclic monophosphate (cyclic GMP; cGMP)

NAD+



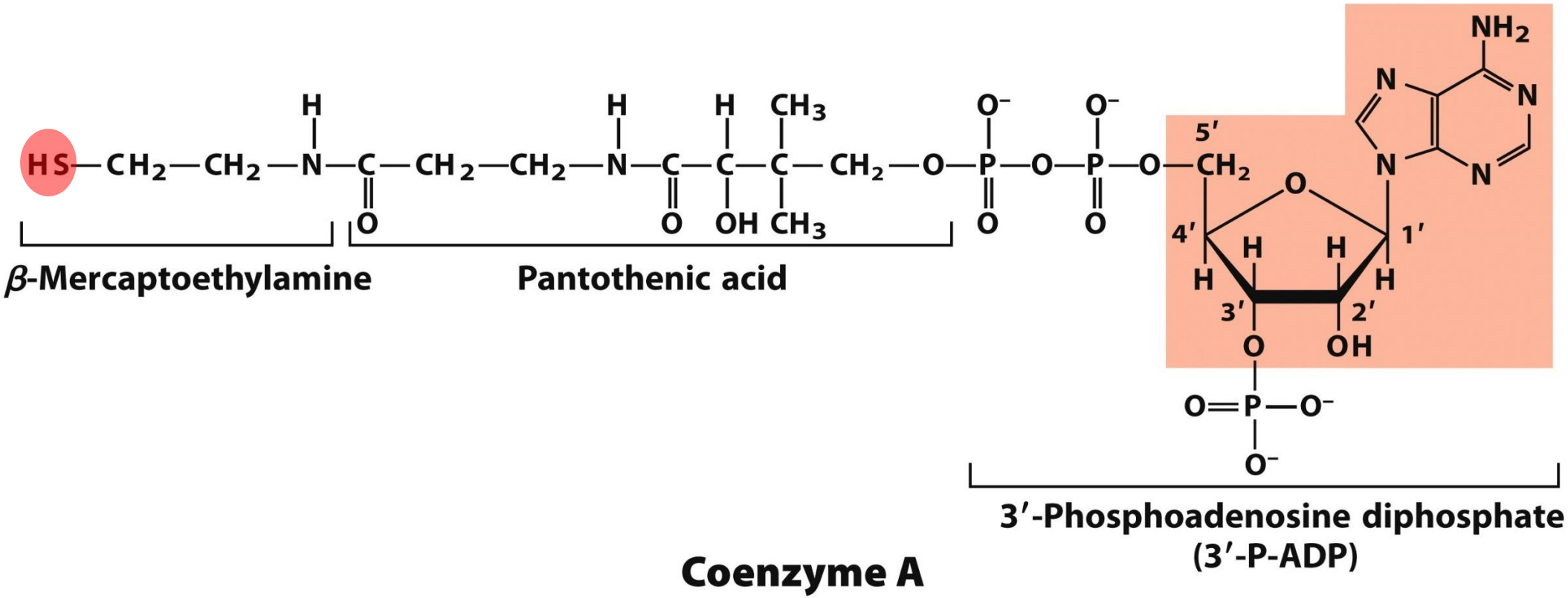
Nicotinamide adenine dinucleotide (NAD^+)

FAD



Flavin adenine dinucleotide (FAD)

CoA


β-Mercaptoethylamine
Pantothenic acid
3′-Phosphoadenosine diphosphate
(3′-P-ADP)
Coenzyme A

NUCLEOTIDE BIOSYNTHESIS

DE NOVO pathway

activated ribose (PRPP)
+ amino acids
+ ATP + CO_2

SALVAGE pathway

activated ribose (PRPP) + base

DE NOVO NUCLEOTIDE BIOSYNTHESIS

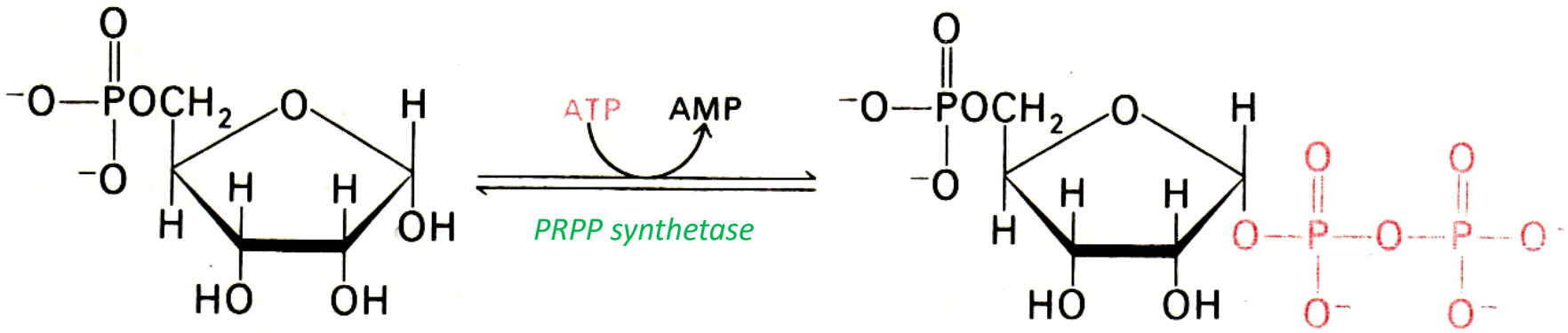
- *De novo* pathways for purine and pyrimidine biosynthesis appear to be nearly identical in all living organisms.

- **Purine ring** is synthesized from amino acids glycine, glutamine and aspartate; N^{10}-formyltetrahydrofolate; CO_2.

- **Pyrimidine ring** is synthesized from carbamoyl phosphate (bicarbonate + NH_3) and aspartate

- The enzymes involved in *de novo* pathway are mostly present as large, multienzyme complexes in the cell.

PRPP is the donor of ribose-phosphate in nucleotides

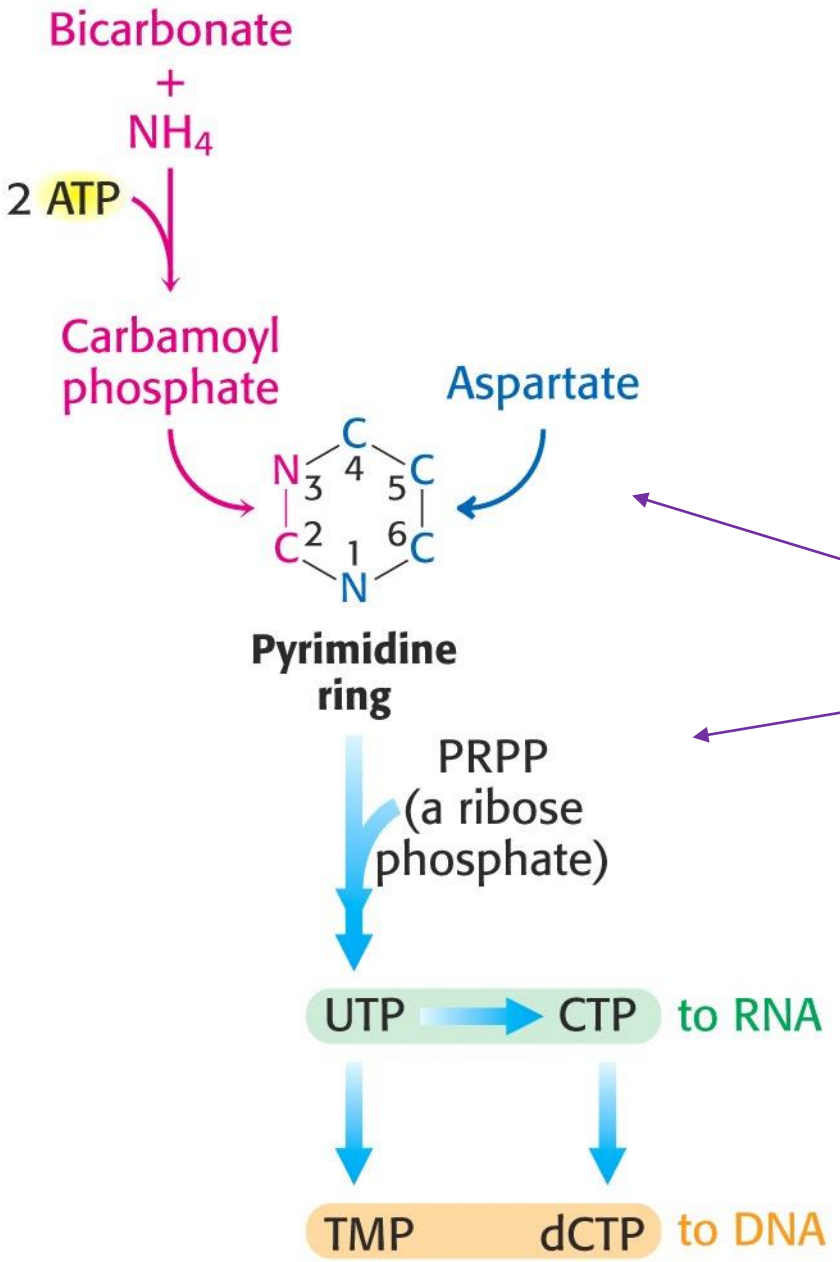


ribose-5-phosphate

5-phosphoribosyl-1-pyrophosphate (PRPP)

ATP + ribose-5-phosphate ⇌ phosphoribosyl-pirophosphate (PRPP)

Pyrimidine synthesis *de novo*



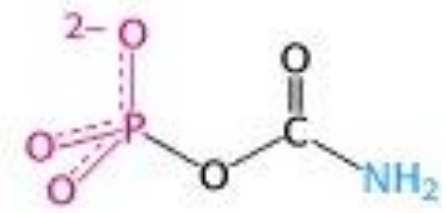
- The biosynthesis of pyrimidines is a simpler process than that of purines.

the pyrimidine ring is assembled first

and then it is attached to a ribose phosphate

- the first step in *de novo* pyrimidine biosynthesis is the synthesis of **carbamoyl phosphate**

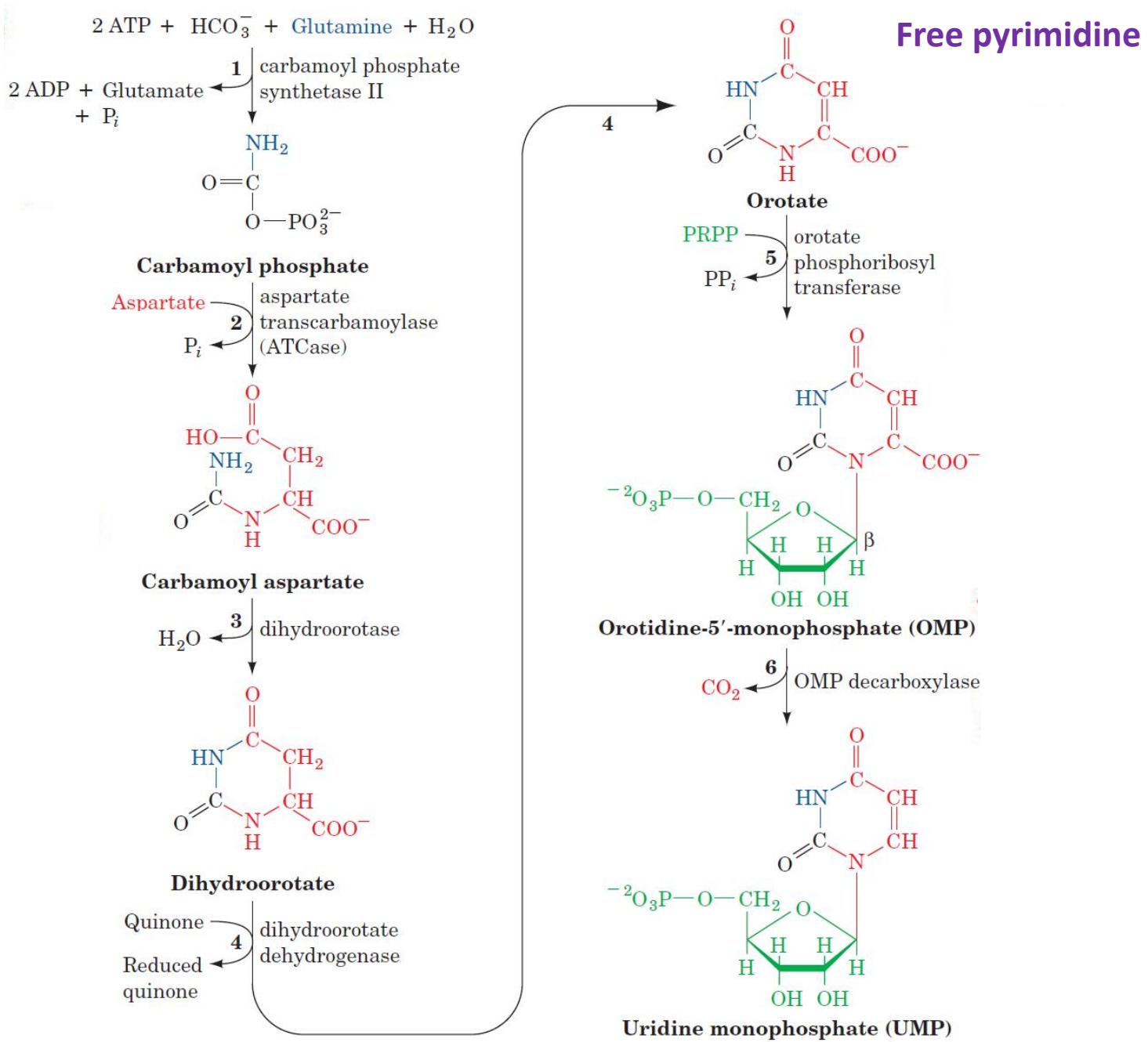
glutamine + 2 ATP + HCO_3^- ⟶ carbamoyl-phosphate + 2 ADP + P_i + glutamate



Reminder: differences between carbamoyl phosphate synthetase I and II (CPS I and CPS II)

	CPS I	CPS II
Intracellular localisation	Mitochondria	Cytosol
Tissue localisation	Liver cells	Cells of most tissues
Function	**Urea biosynthesis**	**Pyrimidine biosynthesis**
Activation	*N*-acetylglutamate	Phosphoribosyl-pyrophosphate (PRPP)
Source of nitrogen	Ammonia	Amide nitrogen of glutamine

Free pyrimidine



Nucleoside monophosphates, diphosphates and triphosphates are interconvertible

NUCLEOSIDE MONOPHOSPHATES are phosphorylated by **nucleoside-monophosphate-kinases** – enzymes specific for a particular base, but nonspecific for a sugar

Adenylate-kinase: AMP + **ATP** ↔ ADP + ADP

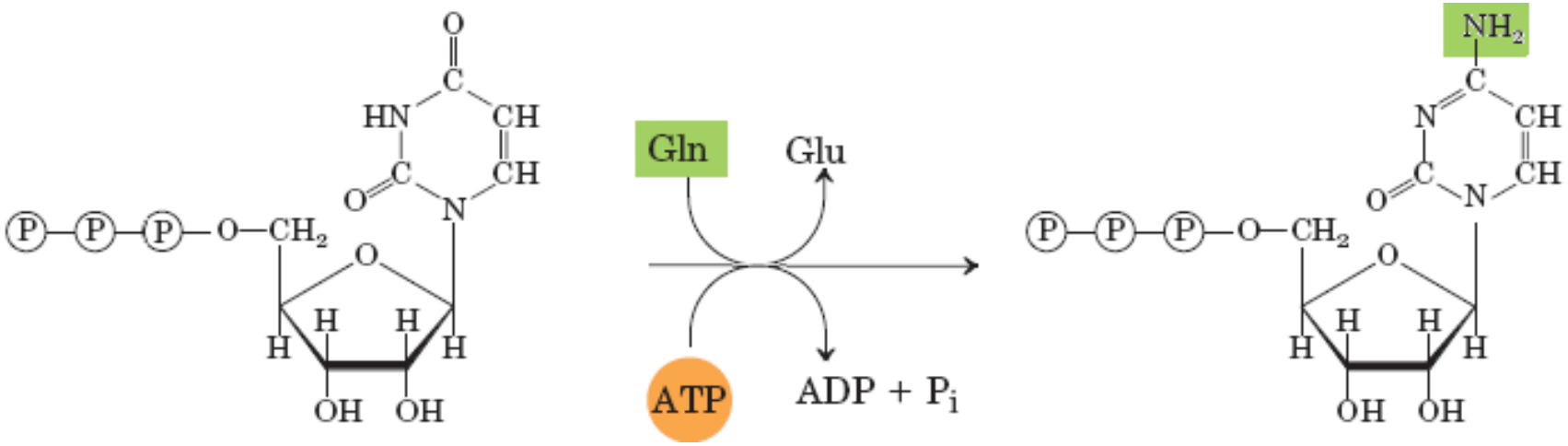
UMP-kinase: UMP + **ATP** ↔ UDP + ADP

NUCLEOSIDE DIPHOSPHATES AND TRIPHOSPHATES are interconverted by **nucleoside-diphosphate-kinases**, enzymes with broad specificity (**X** and **Y** can be any ribonucleoside or deoxyribonucleoside):

XDP + **Y**TP ↔ **X**TP + **Y**DP

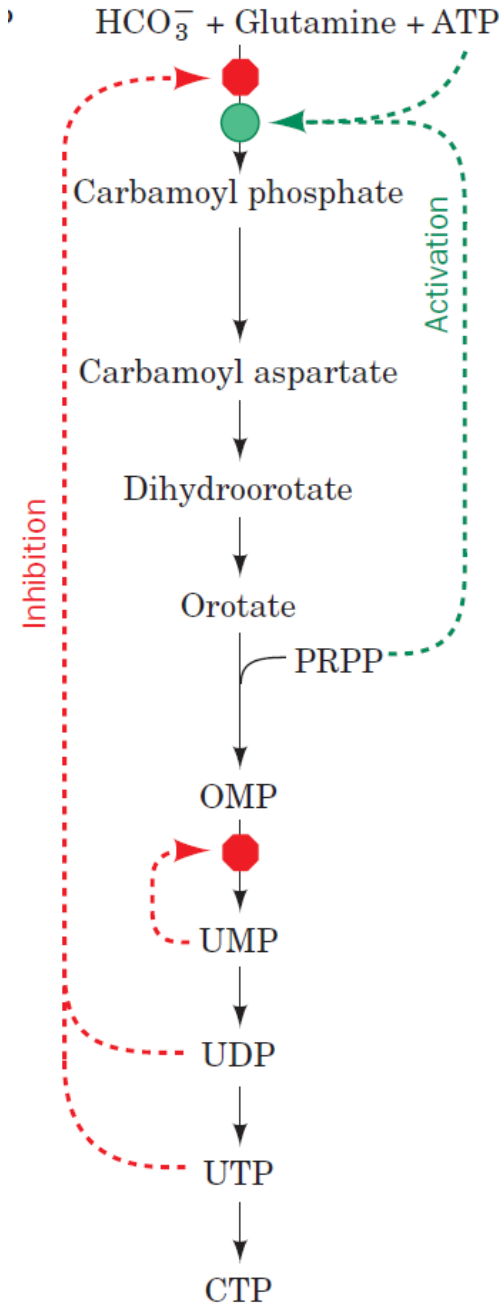
e.g. **U**DP + **A**TP ↔ **U**TP + **A**DP

Cytidine-triphosphate (CTP) is formed by amination of UTP

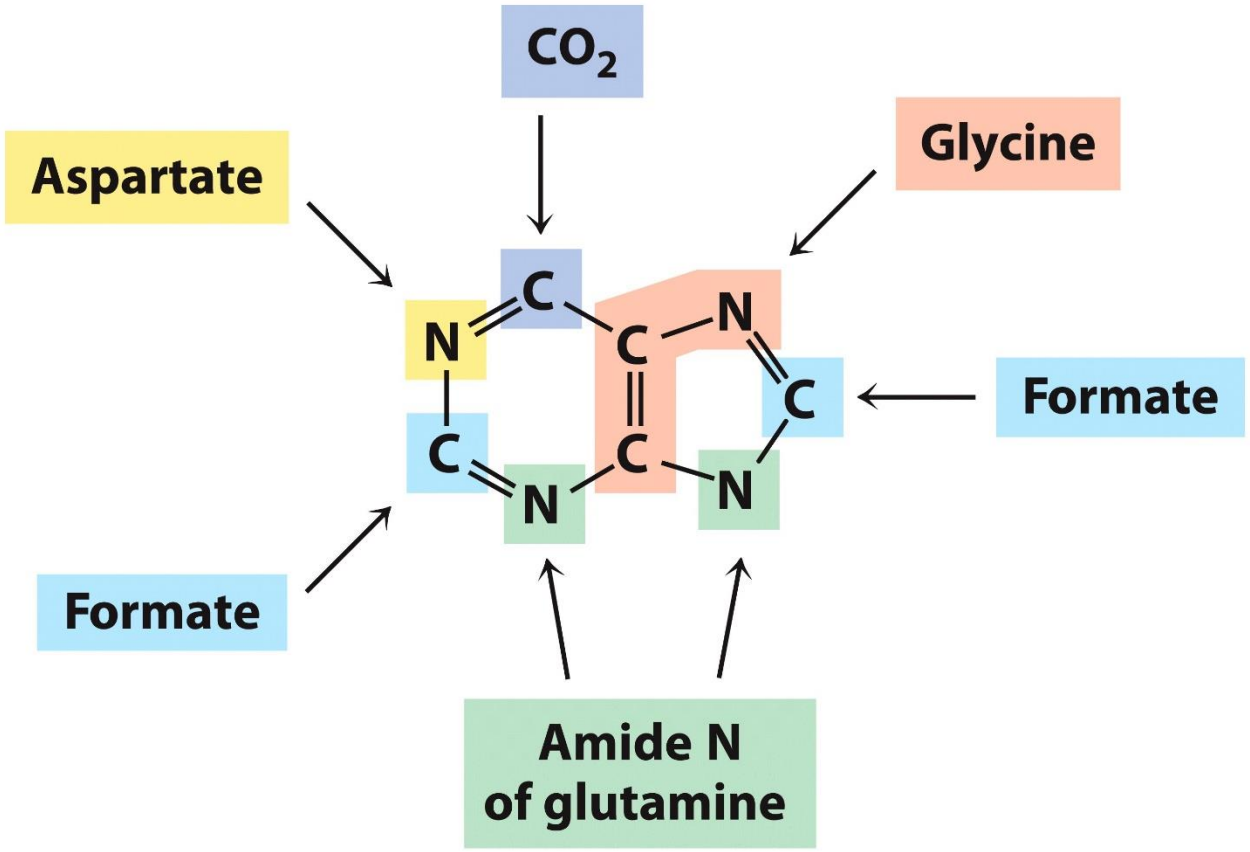


Regulation of pyrimidine biosynthesis

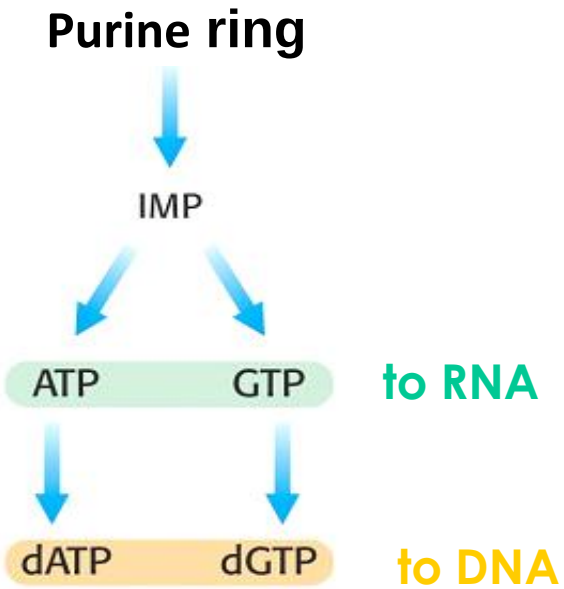
Regulation by feedback inhibition

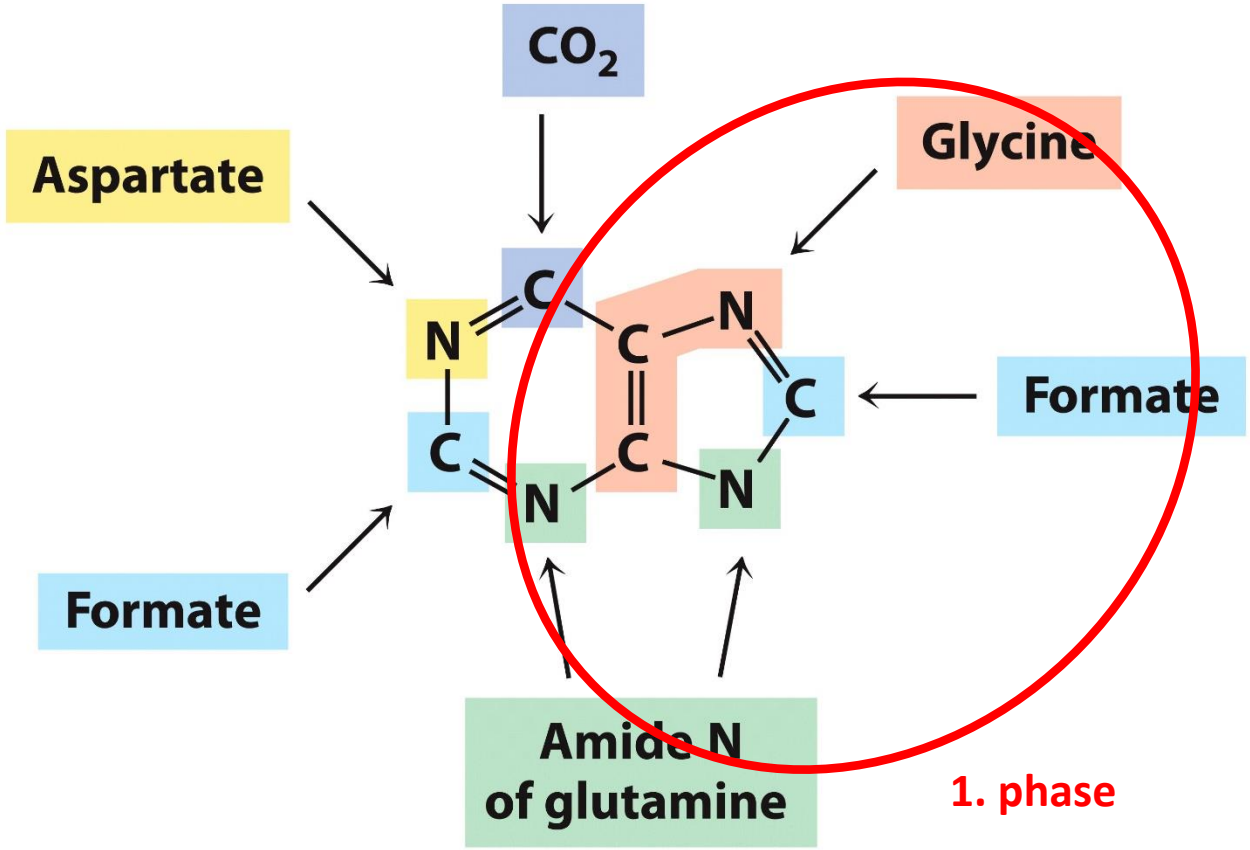


Purine synthesis *de novo*



- As opposed to pyrimidine synthesis, **here PRPP provides the foundation** on which the bases are constructed step by step.



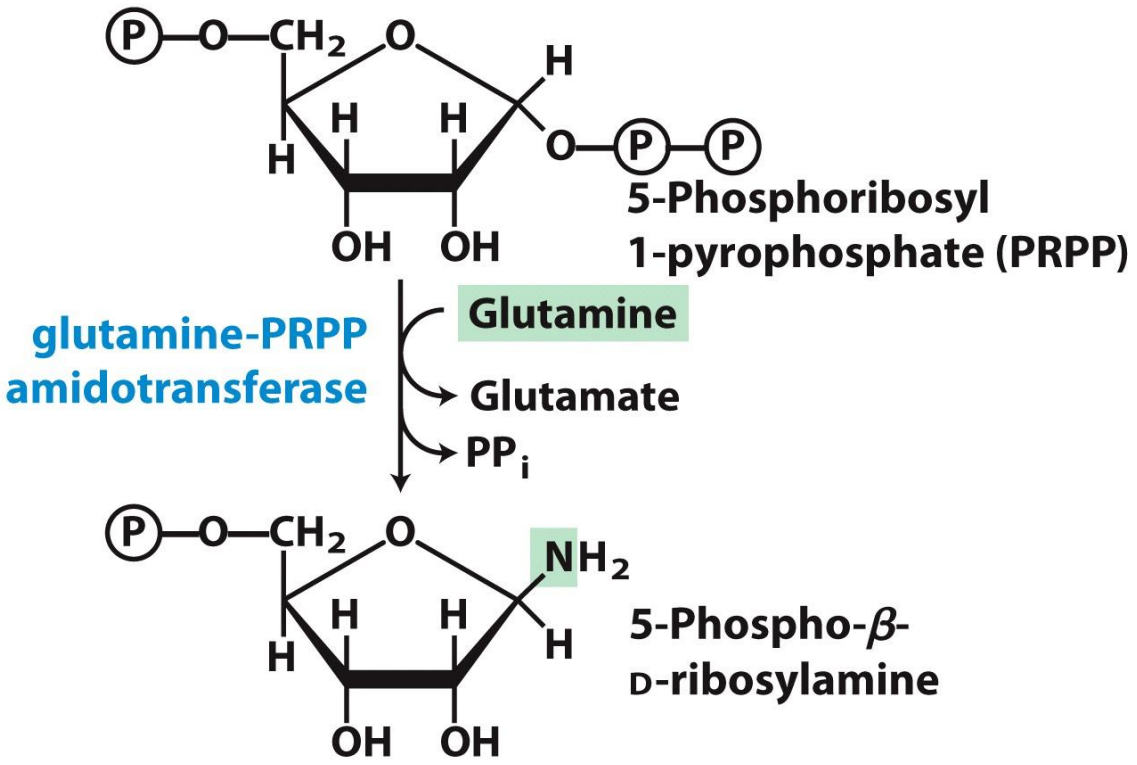


1. **phase of the synthesis** – intermediar product *5-aminoimidazole-ribonucleotide* – complete 5 atom ring of the purine skeleton

2. **phase of the synthesis** – the assembly of the 6 atom ring

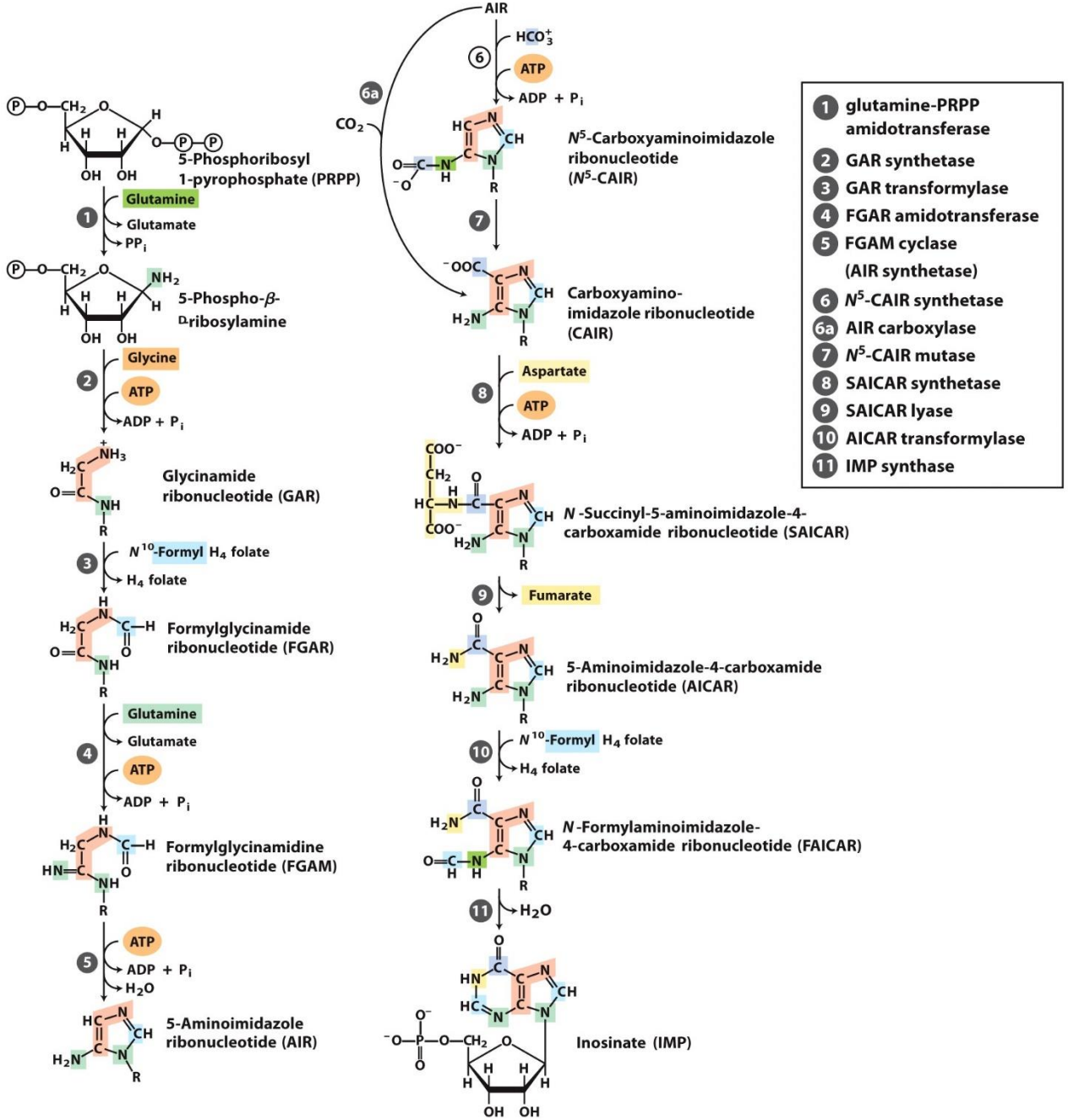
Purine ring is assembled gradually on ribose-phosphate

- the first committed step of the pathway is the formation of **5-phosphoribosyl-1-amine** from **PRPP** and **glutamine.**

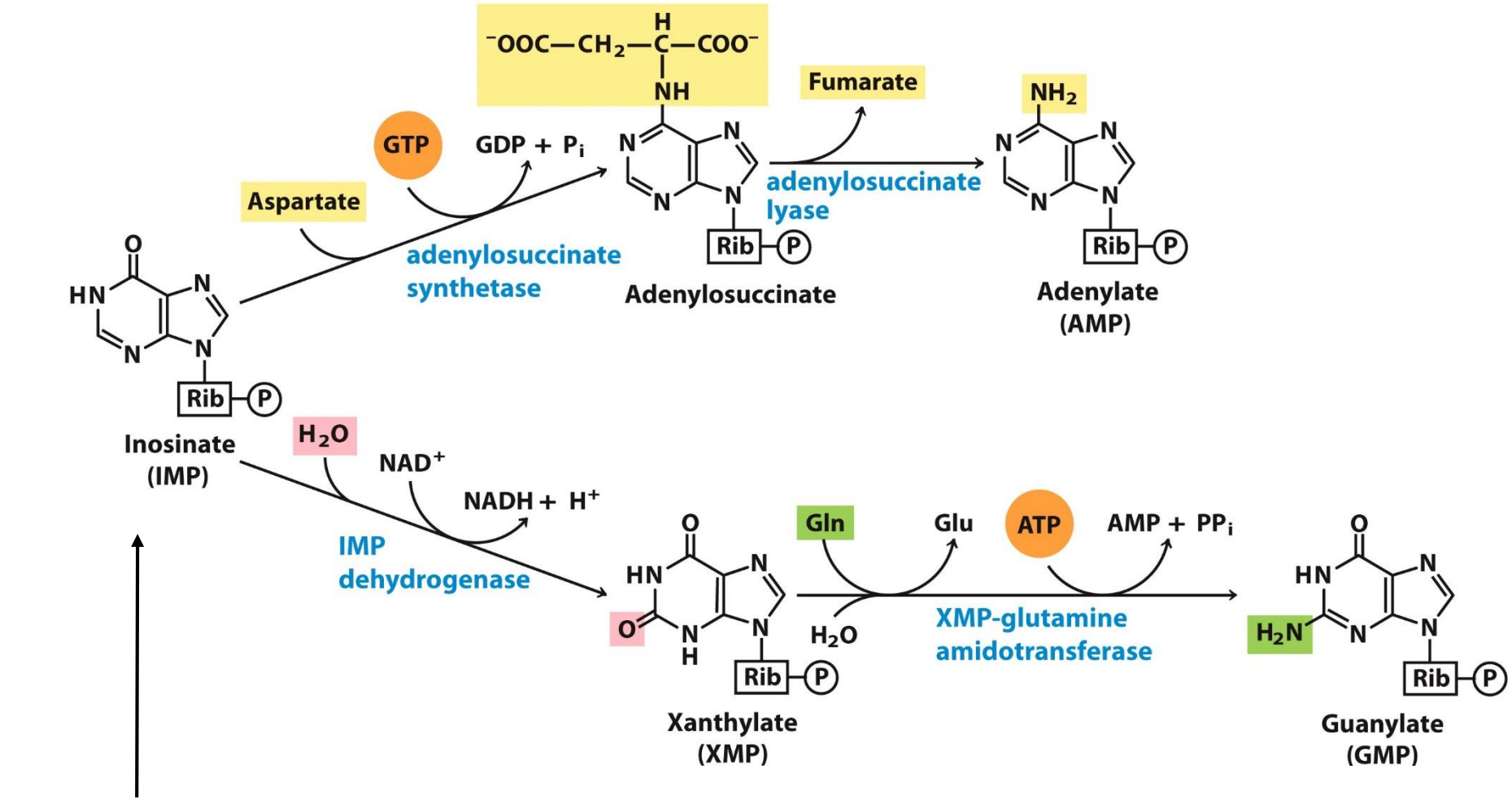


the reaction is driven by the hydrolysis of pyrophosphate

Phase 1 Phase 2



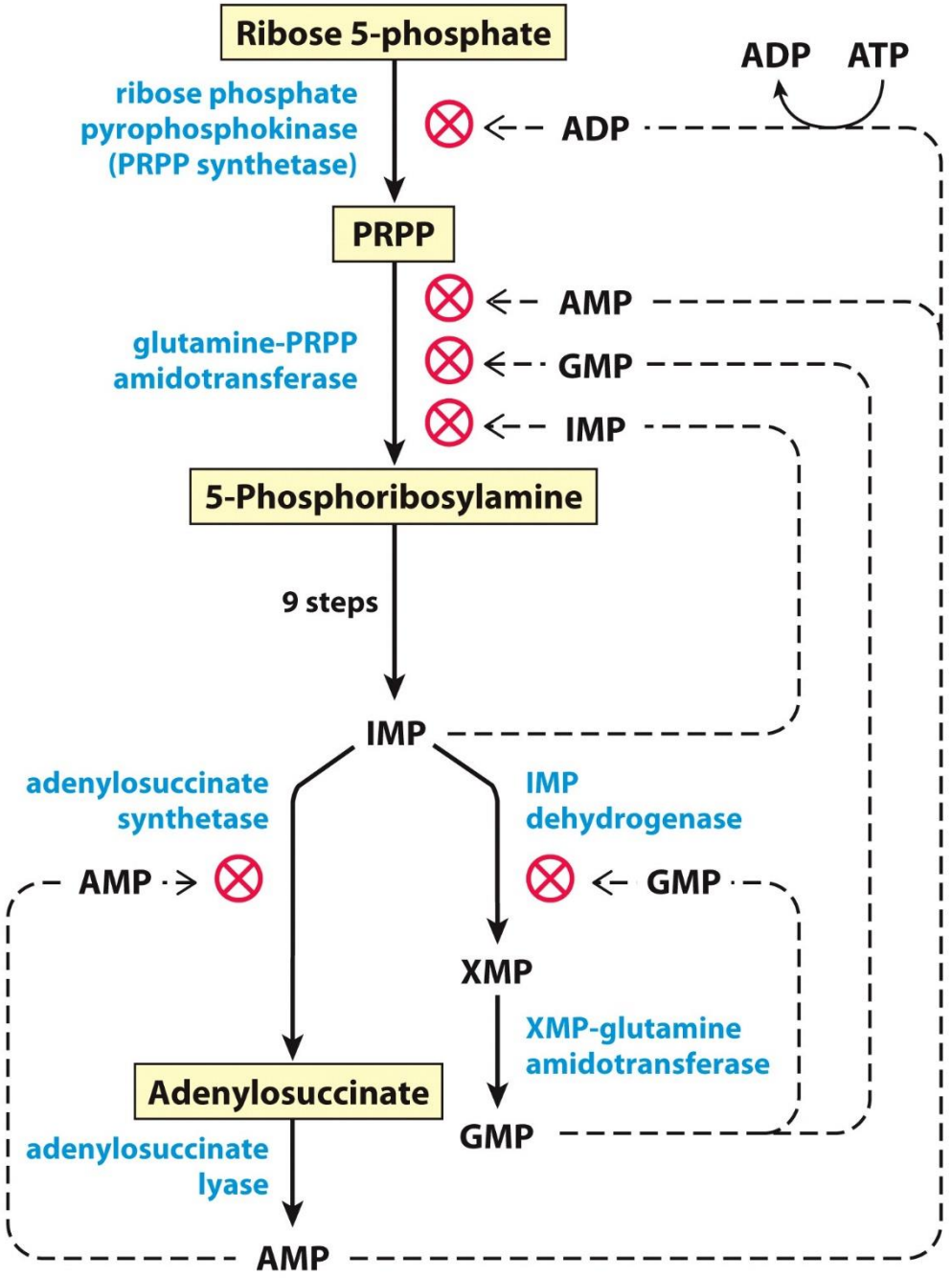
AMP and GMP are formed from IMP



the first intermediate with a complete purine ring is inosinate (IMP)

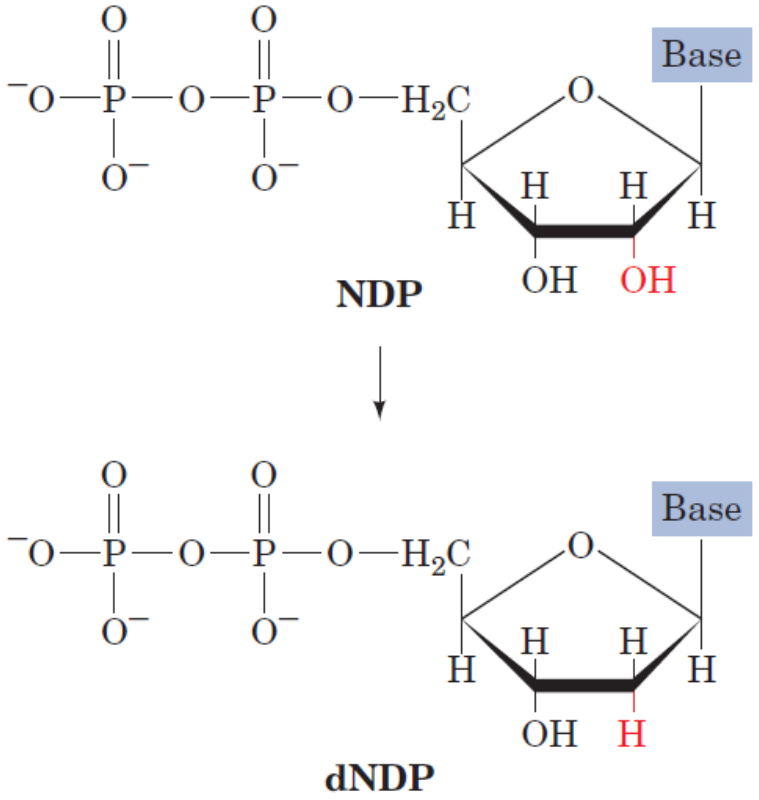
Regulation of purine biosynthesis

Regulation by feedback inhibition

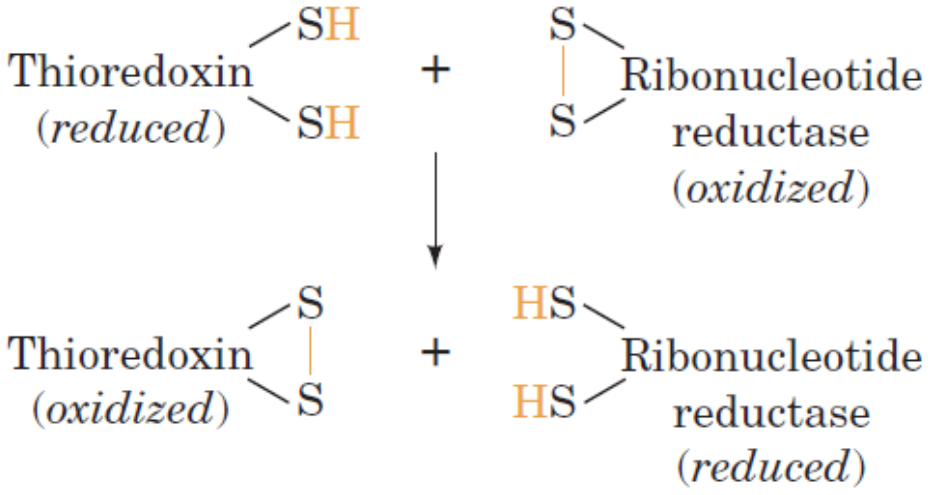


Ribonucleotides are the precursors of **deoxyribonucleotides**

- Deoxyribonucleotides are derived from the corresponding **ribonucleotides by** direct reduction at the 2'-carbon atom of the D-ribose; the reaction is catalyzed by **ribonucleotide reductase.**

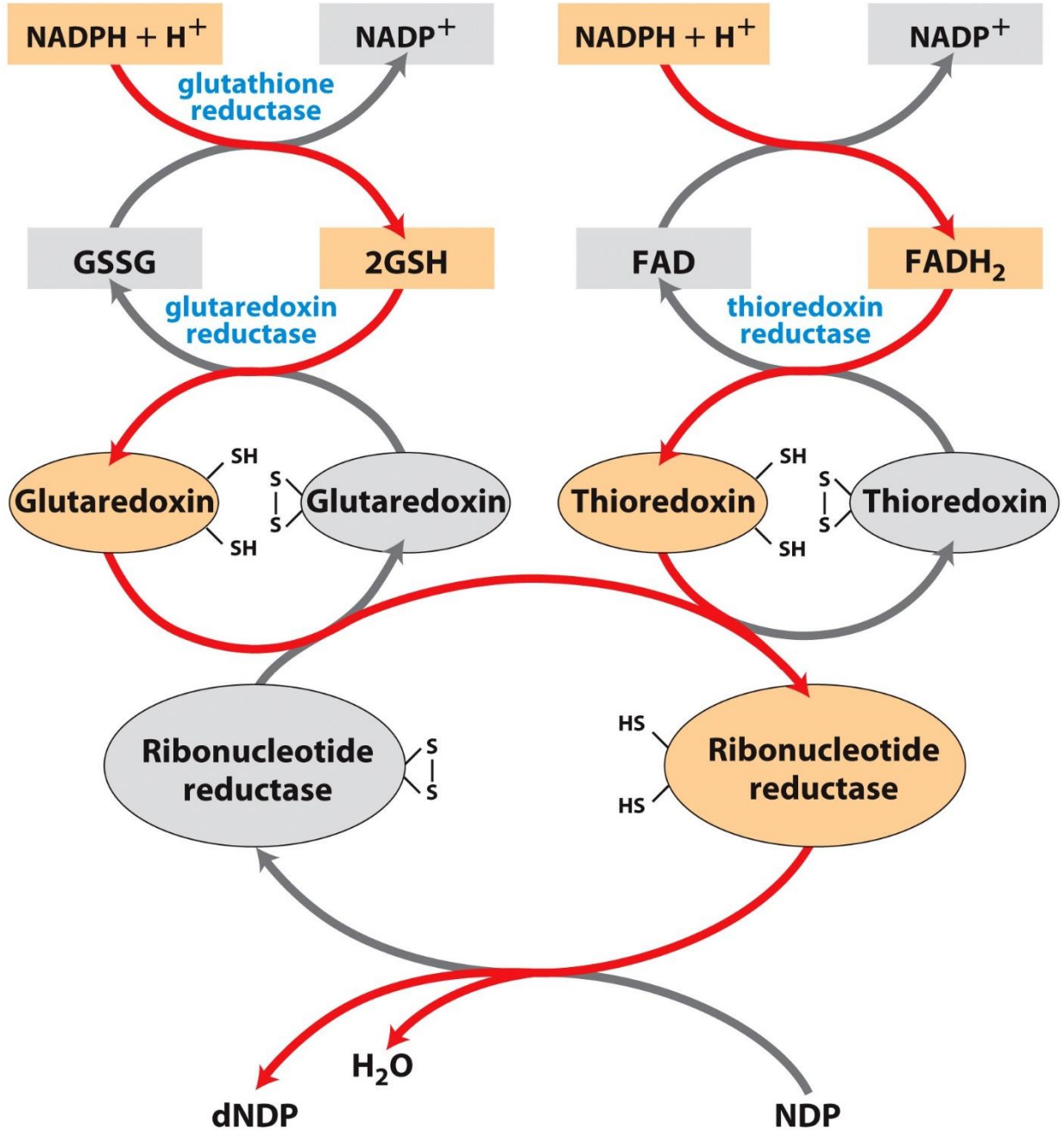


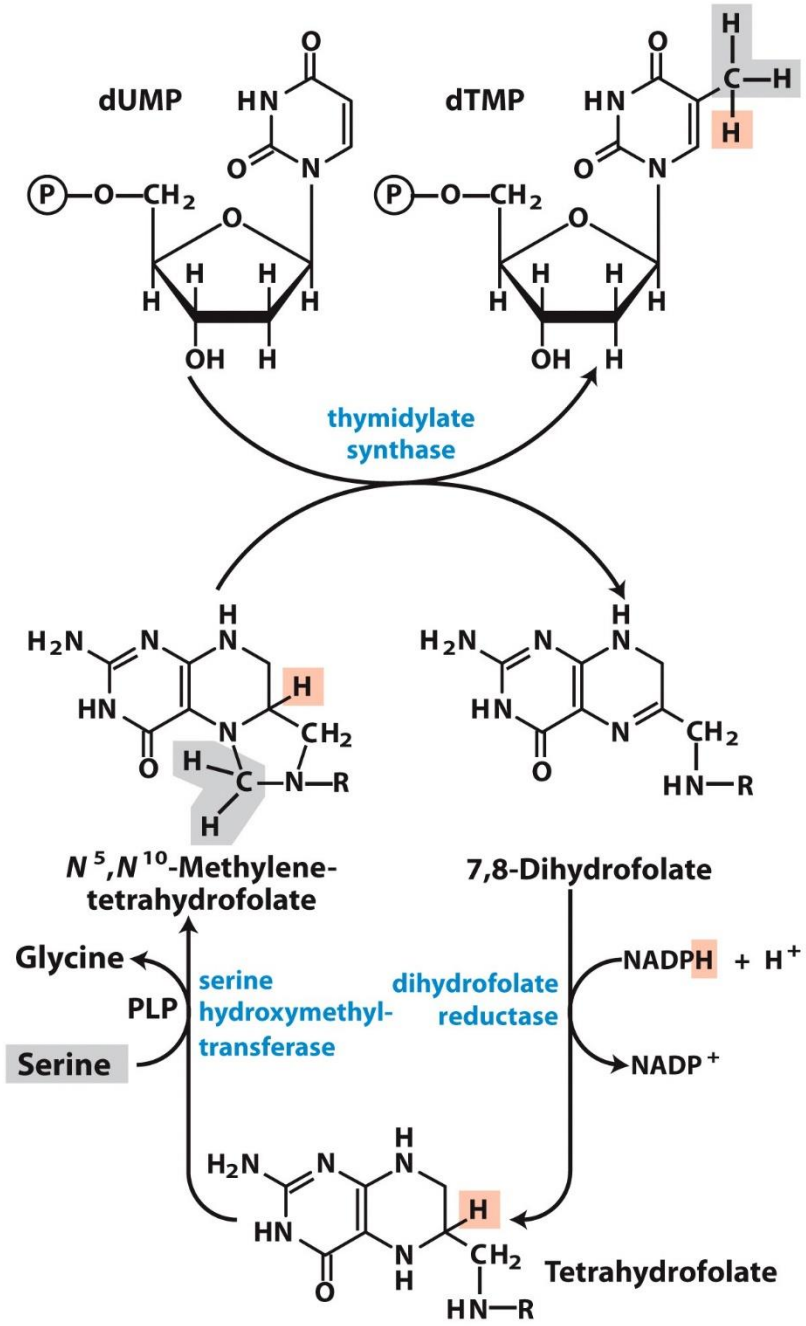
Thioredoxin and glutaredoxin are ribonucleotide reductase's physiological reducing agents



- The reduction of ribose requires a **pair of hydrogen atoms.**

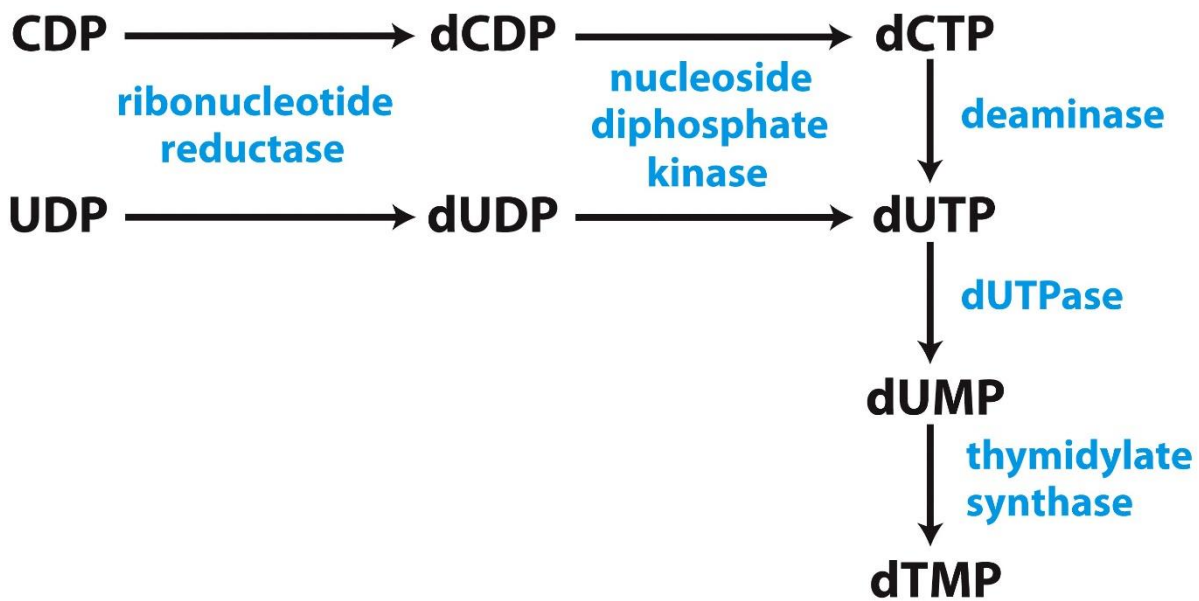
- Those hydrogen atoms are ultimately donated by **NADPH** via an intermediate hydrogen-carrying proteins: **thioredoxin** and **glutaredoxin.**


NADPH + H+
NADP+
glutathione reductase
GSSG
2GSH
glutaredoxin reductase
Glutaredoxin
SH
S
S
SH
Glutaredoxin
NADPH + H+
NADP+
FAD
FADH2
thioredoxin reductase
Thioredoxin
SH
S
S
SH
Thioredoxin
Ribonucleotide reductase
S
S
HS
HS
Ribonucleotide reductase
H2O
dNDP
NDP

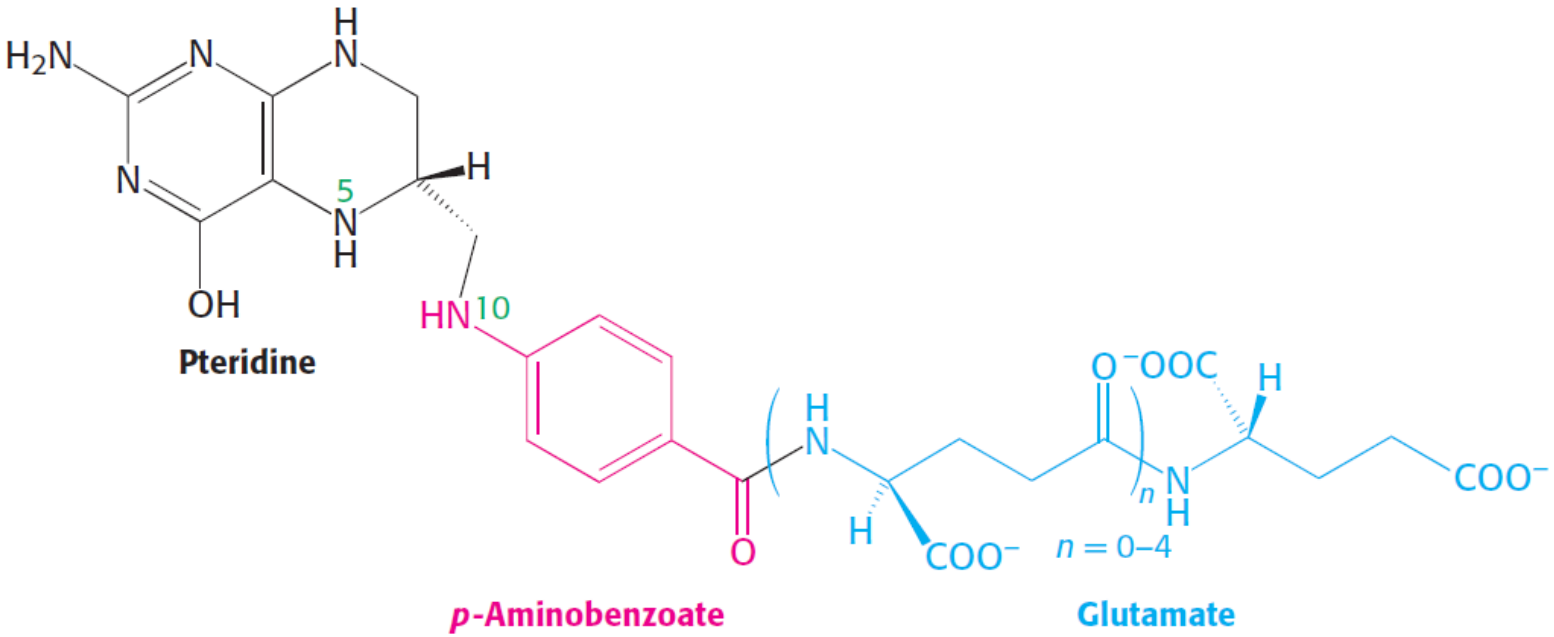


Deoxythymidylate is derived from deoxyuridylate

- A one-carbon unit at the hydroxymethyl ($-CH_2OH$) oxidation level is transferred from ***N^5,N^{10}*-methylenetetrahydrofolate** to dUMP, then reduced to a methyl group.
- The reduction occurs at the expense of oxidation of **tetrahydrofolate to dihydrofolate.**

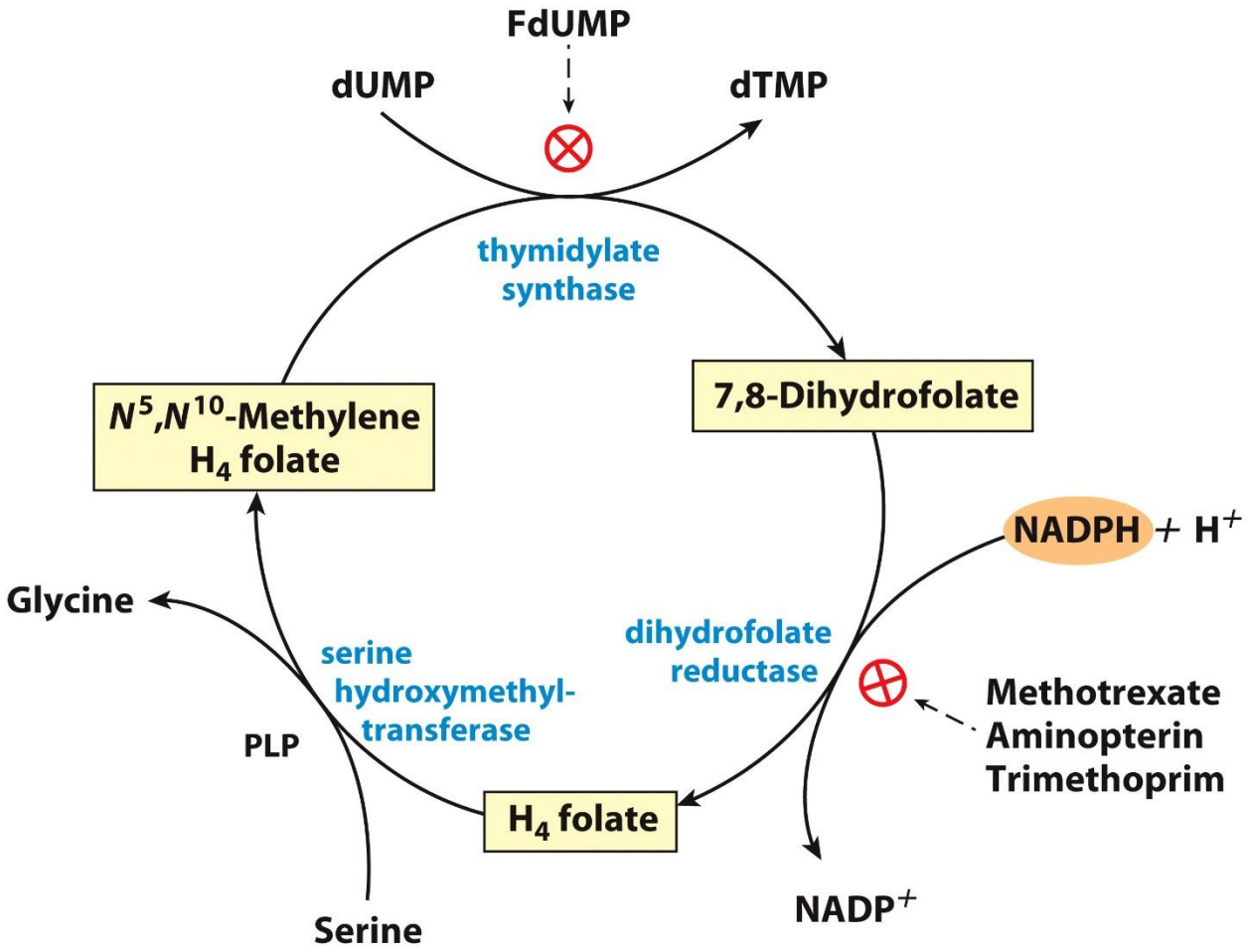

CDP
dCDP
dCTP
ribonucleotide reductase
nucleoside diphosphate kinase
deaminase
UDP
dUDP
dUTP
dUTPase
dUMP
thymidylate synthase
dTMP

Tetrahydrofolate is the donor of C_1-groups in different biosynthetic processes



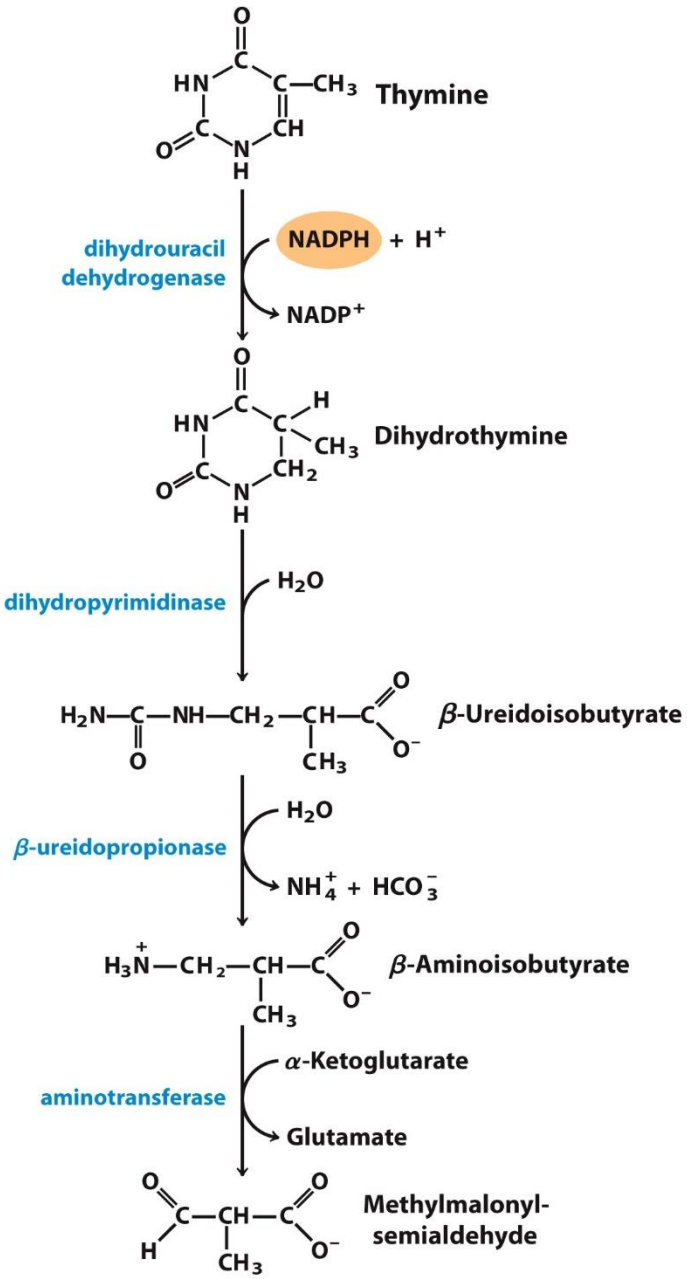
- it is involved in the synthesis of Met, Gly, thymine, purines

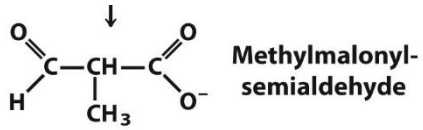
Anticancer drugs block the synthesis of thymidylate by inhibiting thymidylate-synthase and dihydrofolate-reductase



- Thymidylate synthase and dihydrofolate reductase are choice targets in cancer chemotherapy because the generation of large quantities of precursors for DNA synthesis is required for rapidly dividing cancer cells.
- Fluorodeoxyuridylate (F-dUMP) is an analog of dUMP and it irreversibly inhibits thymidylate synthase → suicide inhibition.
- Aminopterin and methotrexate are potent competitive inhibitors of dihydrofolate reductase.

Catabolism of pyrimidines





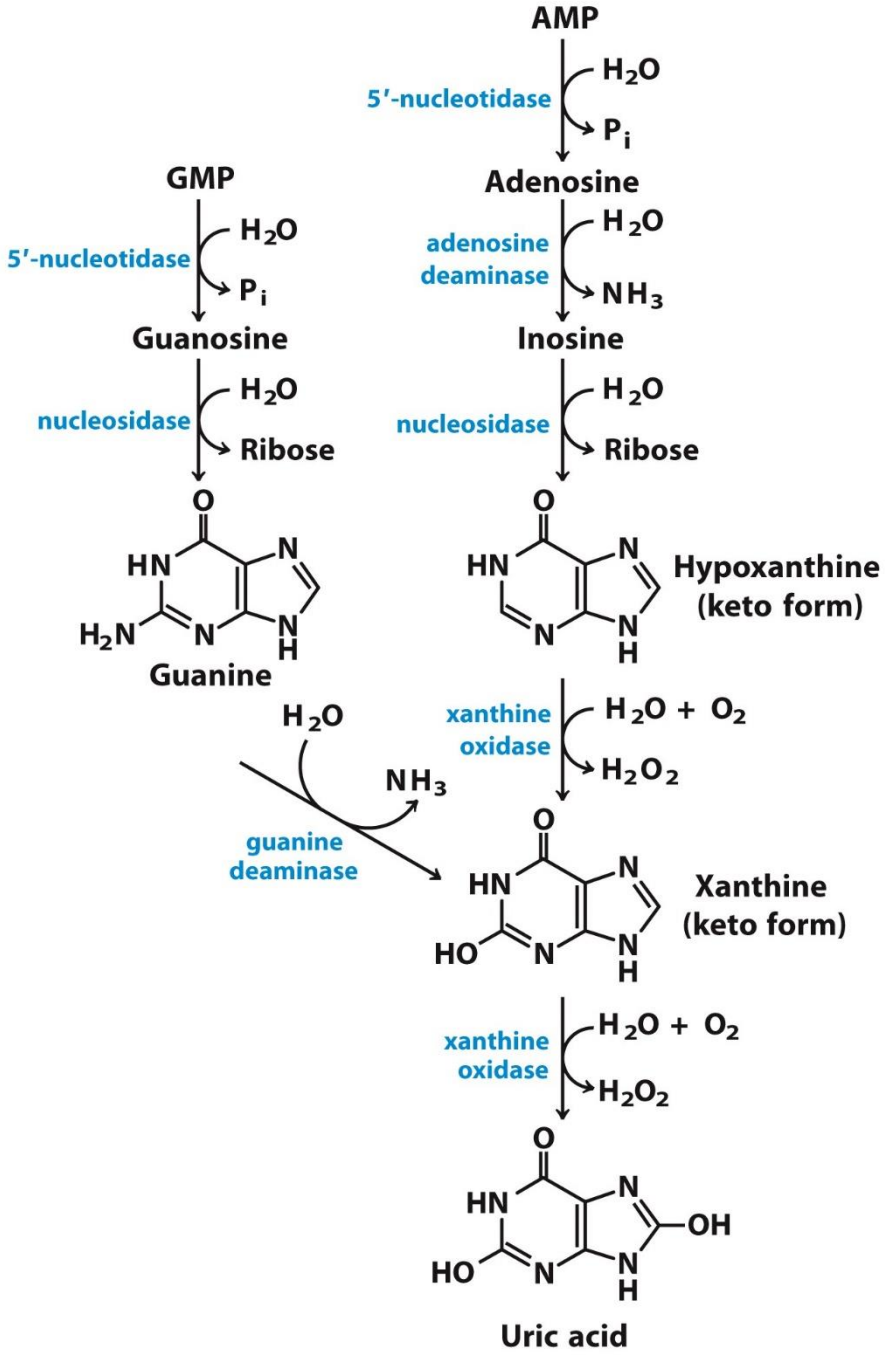
Methylmalonyl CoA

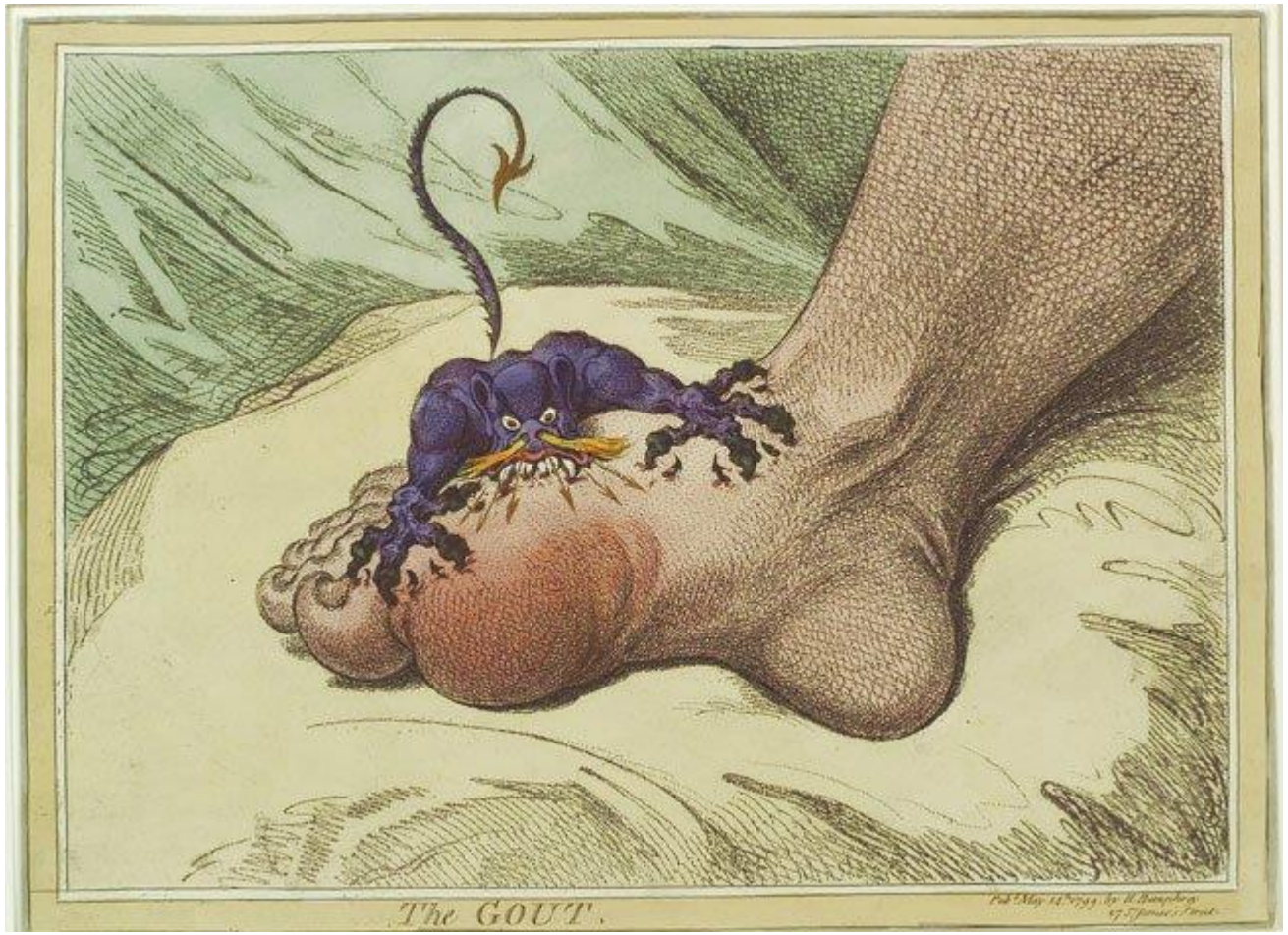
Succinyl CoA

entry into citric acid cycle

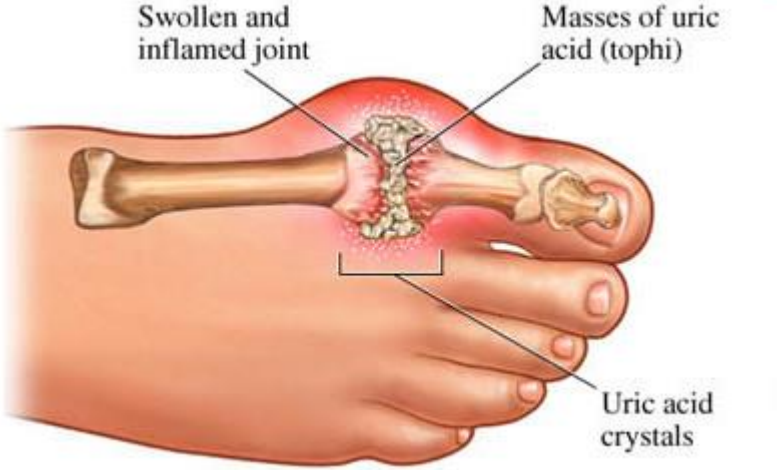
Catabolism of purines

- Purine bases are converted to **urate** for excretion.
- Long-term elevated concentration of uric acid (hyperuricemia) → gout.
- It's precise cause is not known, but it often involves an underexcretion of urate.
- The joints become inflamed, painful, and arthritic, owing to the abnormal deposition of sodium urate crystals; the kidneys are also affected, as excess uric acid is deposited in the kidney tubules.

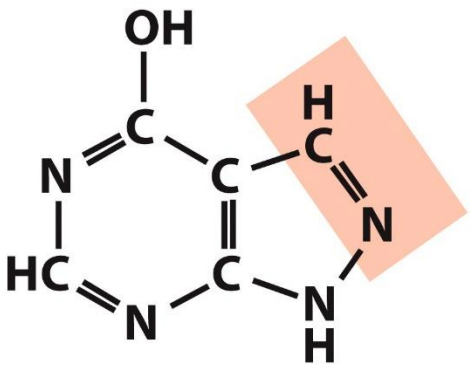




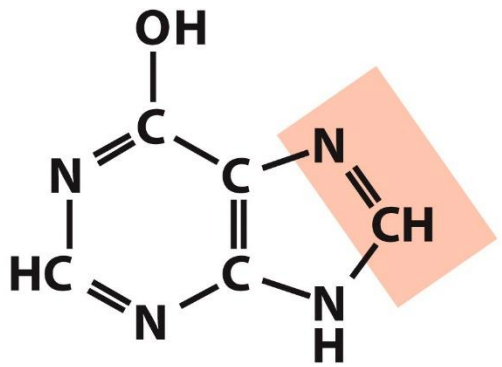
"The Gout" by James Gillray (1799)



Micrograph of sodium urate crystals

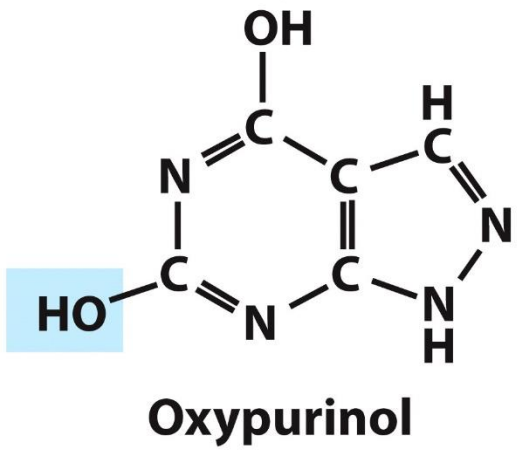


Allopurinol



Hypoxanthine
(enol form)

xanthine
oxidase



Oxypurinol

- Major alleviation of the symptoms is provided by the drug allopurinol, which inhibits xanthine oxidase, the enzyme that catalyzes the conversion of purines to uric acid.

- The mechanism is “suicide inhibiton” – allopurinol is a substrate of xanthine oxidase, which converts allopurinol to oxypurinol (alloxanthine) - oxypurinol inactivates the reduced form of the enzyme by remaining tightly bound in its active site.

- **When xanthine oxidase is inhibited, the excreted products of purine metabolism are xanthine and hypoxanthine**, which are more watersoluble than uric acid and less likely to form crystalline deposits.

Literature used to prepare the presentation

1. D.L. Nelson and M.M. Cox: **Lehninger Principles of Biochemistry**, 6 th edition, W.H. Freeman and Company, USA, 2013.

2. J.M. Berg, J.L. Tymoczko and L. Stryer: **Biochemistry**, 7 th edition, W.H. Freeman and Company, USA, 2010.

3. D. Voet i J.G. Voet: **Biochemistry**, 4th edition, John Wiley & Sons Inc., USA, 2010.

Review questions/questions you should know the answers to:

1. Explain the difference between nucleotides and nucleosides.
2. Name the major purine and pyrimidine bases.
3. What are the two main pathways nucleotides can be synthesized by?
4. Orotate is an intermediar in which metabolic pathway?
5. Explain the major differences in purine and pyrimidine *de novo* biosynthetic pathway.
6. Purine ring is assembled *de novo* from: _____, _____, _____, _____ and _____.
7. Pyrimidine ring is assembled *de novo* from: _____ and _____.
8. Give an example of a disease caused by an elevated concentration of uric acid (hyperuricemia).