Nucleotide biosynthesis

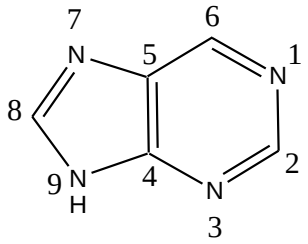
- Purine and Pyrimidine biosynthesis
- Horton ch 18

Cellular functions of Nucleotides

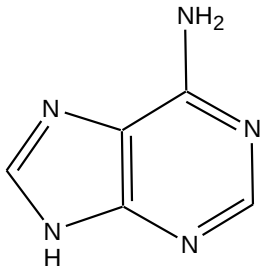
- **Activated precursors of nucleic acids (DNA, RNA)**
- **Universal carriers of chemical energy (e.g., ATP, GTP)**
- **Building blocks of co-factors (e.g., NAD, CoA etc.)**
- **Substrates for covalent enzyme modification (e.g., phosphorylation)**
- **Second messengers in cellular communication (e.g., cAMP)**

DNA and RNA bases

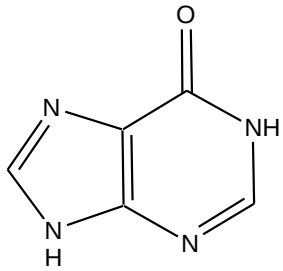
Basic structure



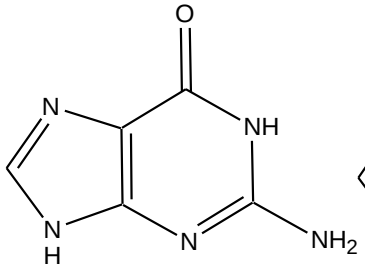
Purine



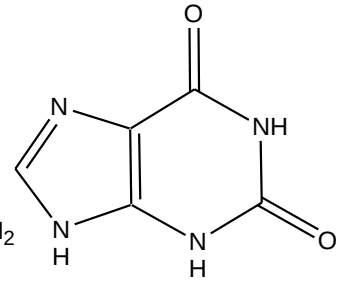
Adenine (A)



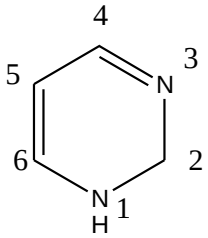
Hypoxanthine



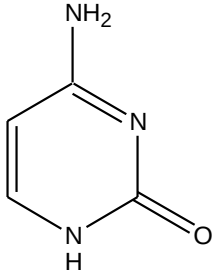
Guanine (G)



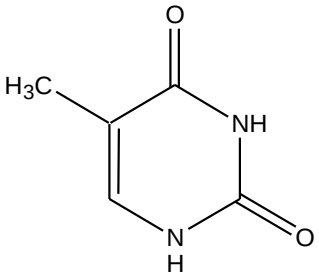
Xanthine



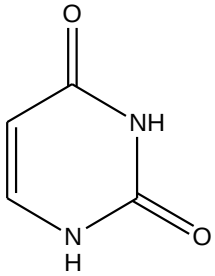
Pyrimidine



Cytosine (C)



Thymine (T)



Uracil (U)

DNA

RNA

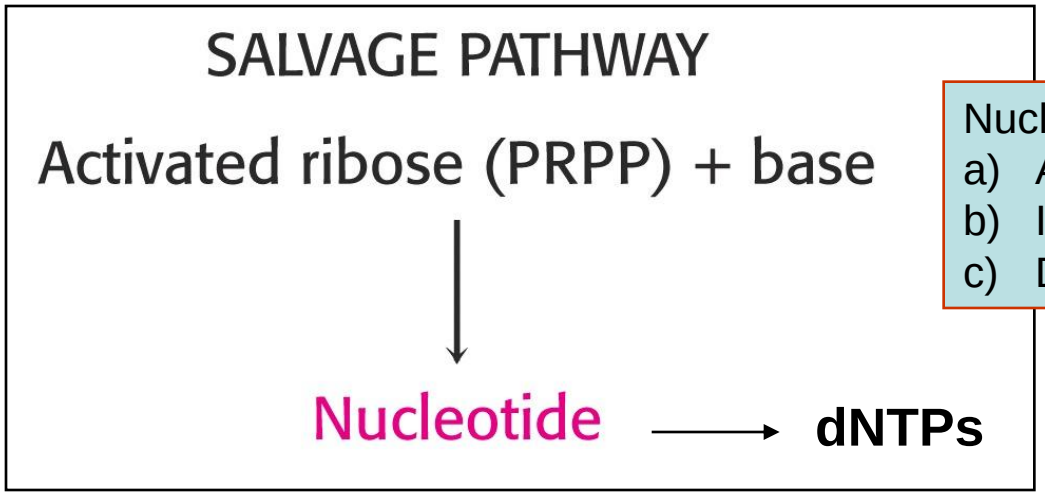
Base + Pentose + Phosphate

TABLE 25.1 Nomenclature of bases, nucleosides, and nucleotides

RNA		
Base	Ribonucleoside	Ribonucleotide (5′-monophosphate)
Adenine (A)	Adenosine	Adenylate (AMP)
Guanine (G)	Guanosine	Guanylate (GMP)
Uracil (U)	Uridine	Uridylate (UMP)
Cytosine (C)	Cytidine	Cytidylate (CMP)
DNA		
Base	Deoxyribonucleoside	Deoxyribonucleotide (5′-monophosphate)
Adenine (A)	Deoxyadenosine	Deoxyadenylate (dAMP)
Guanine (G)	Deoxyguanosine	Deoxyguanylate (dGMP)
Thymine (T)	Thymidine	Thymidylate (TMP)
Cytosine (C)	Deoxycytidine	Deoxycytidylate (dCMP)

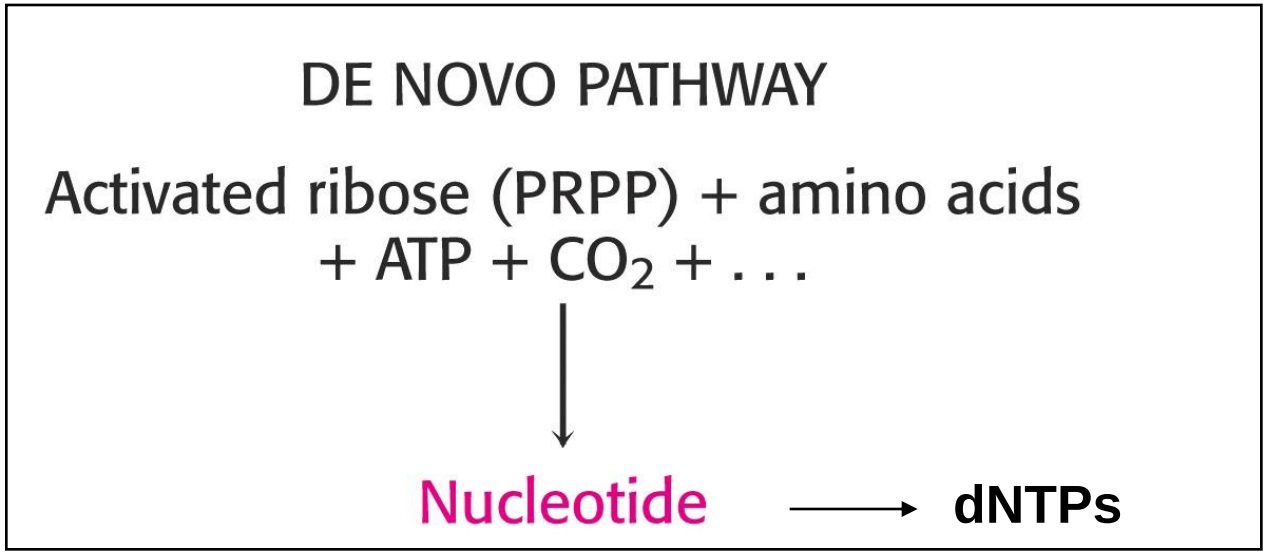
Hypoxanthine	Inosine	Inosinate (IMP)
Xanthine	Xanthosine	Xanthylate (XMP)

Two major routes for nucleotide biosynthesis

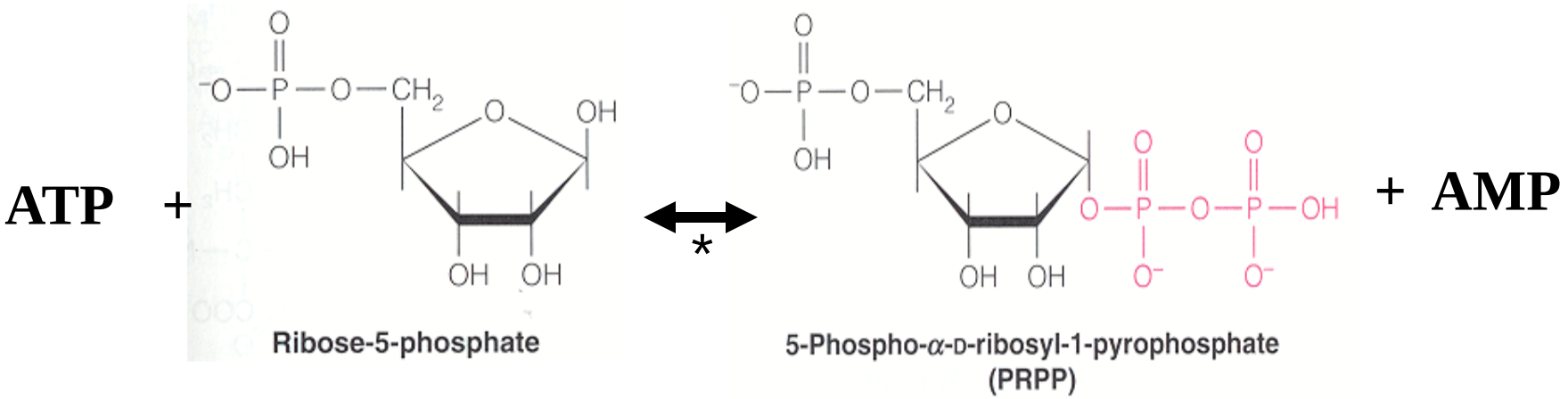


Nucleotides obtained:

a) Apoptosis of cells
b) Intracellular – DNA/RNA in lysosomes
c) Diet



PRPP is a Central Metabolite in Nucleotide Metabolism



In both the de novo synthesis pathway and salvage pathway, PRPP is the key point for nucleotide metabolism.

***Ribose phosphate pyrophosphokinase**
Or PRPP Synthetase

Salvage Pathway (overview):

Eg. AMP

1. Nucleic acids are broken down (or degraded) to monophosphate nucleosides by nuclease activities

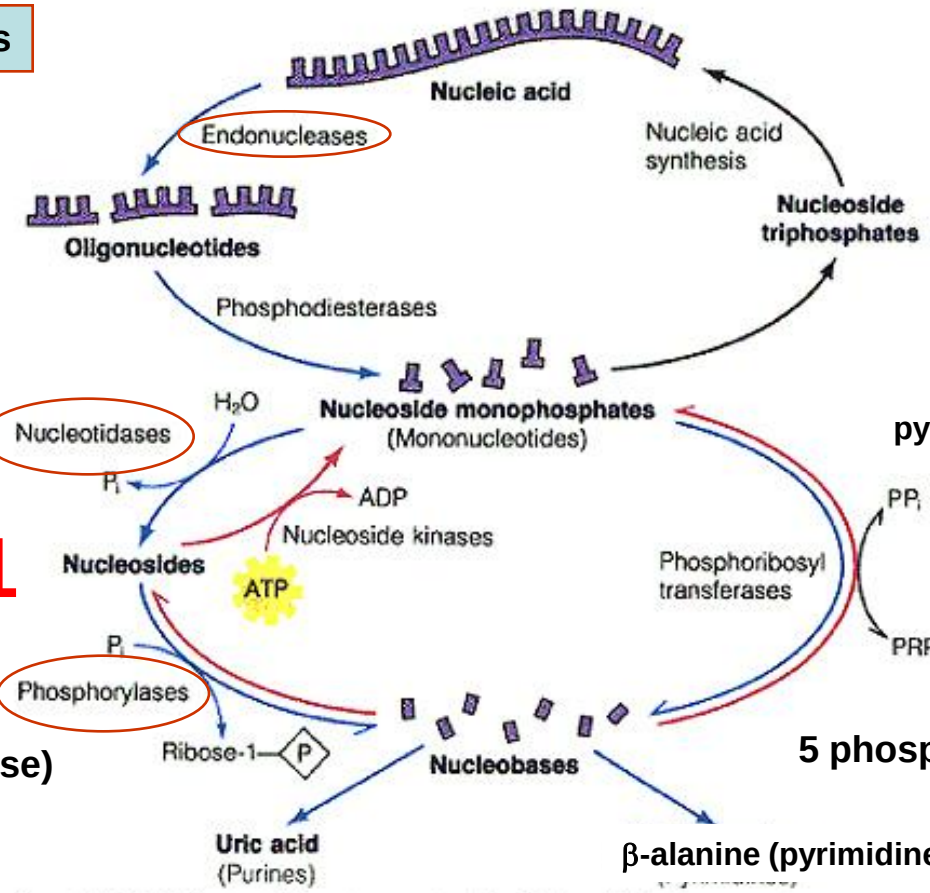
Adenine

2. Monophosphate nucleosides are converted to nucleobases.

3. Nucleobases have two fates:
   A. Conversion to Uric acid/β-alanine
   B. Conversion to monophosphate nucleosides for reuse

Salvage pathways 1 and 2

From pancreas



Intestinal mucosa

1

2

REVERSIBLE

(Nucleoside Phosphorylase)

5 phosphoribosyl-1-pyrophosphate

1. Pathway 1 possible but not all animal cells have enzymes for all nucleotides (eg. Guanosine kinase or uridine phosphorylase not in animals)
2. Therefore pathway 2 more common

Salvage pathway:

Phosphoribosyl transferases convert free bases to nucleotides

AMP catabolism

adenine phosphoribosyl transferase

Adenine + PRPP ⟶ Adenylate + PPi

hypoxanthine-guanine phosphoribosyl transferase (HGPRT)

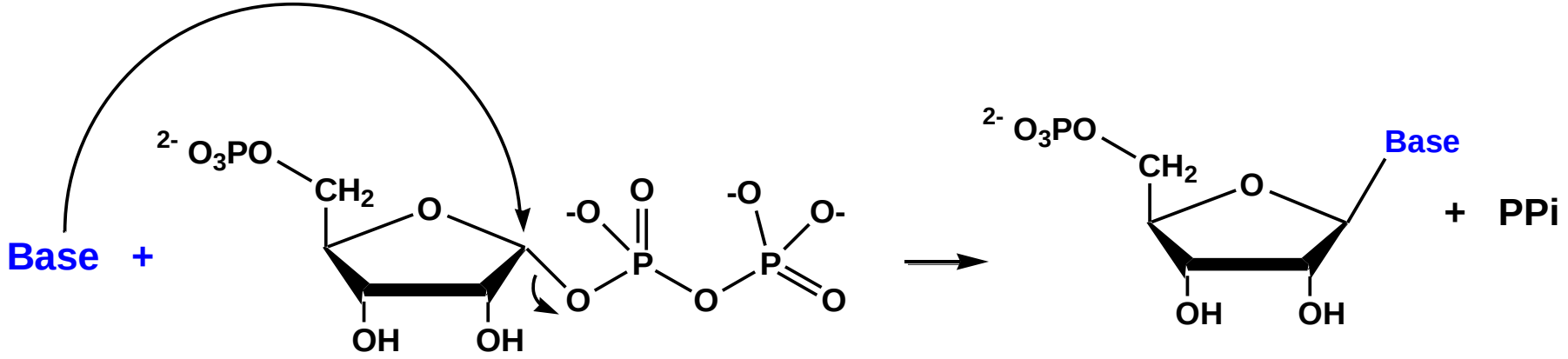
Guanine + PRPP ⟶ Guanylate + PPi

Hypoxanthine + PRPP ⟶ Inosinate + PPi

pyrimidine anabolism

orotate phosphoribosyl transferase

Orotate + PRPP ⟶ OMP + PPi



5-phosphoribosyl-1-pyrophosphate (PRPP)

There are six functional classes of enzymes

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TABLE 6–3 International Classification of Enzymes

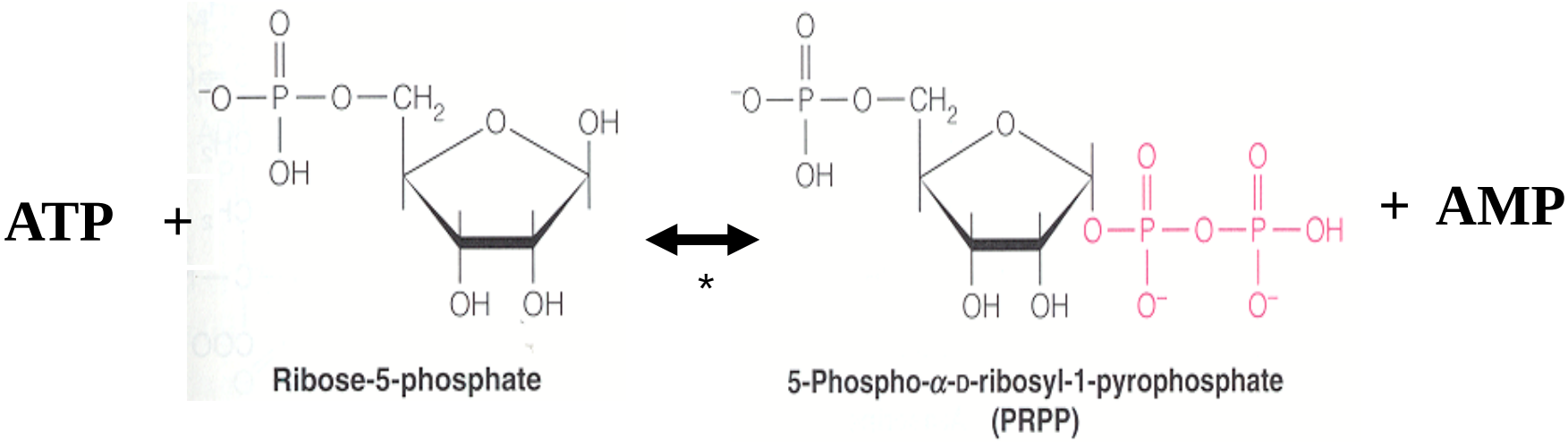
No.	*Class*	*Type of reaction catalyzed*	
1	Oxidoreductases	Transfer of electrons (hydride ions or H atoms)	
2	Transferases	Group transfer reactions	Reaction 1,3,6,9
3	Hydrolases	Hydrolysis reactions (transfer of functional groups to water)	
4	Lyases	Addition of groups to double bonds, or formation of double bonds by removal of groups	Reaction 8
5	Isomerases	Transfer of groups within molecules to yield isomeric forms	
6	Ligases	Formation of C—C, C—S, C—O, and C—N bonds by condensation reactions coupled to ATP cleavage	Reaction 2,4,5,7,10

Note: Most enzymes catalyze the transfer of electrons, atoms, or functional groups. They are therefore classified, given code numbers, and assigned names according to the type of transfer reaction, the group donor, and the group acceptor.

A lyase that catalyses an addition reaction = synthase (no ATP)

Also known as synthetase (requires ATP)

PRPP is a Central Metabolite in de Novo Nucleotide Synthesis



The first step for all nucleotide synthesis involves activation of ribose-5-phosphate making PRPP.

The synthesis of PRPP is a key regulation point in de novo nucleotide synthesis.

***Ribose phosphate pyrophosphokinase = PRPP synthetase**

Purine synthesis: de novo

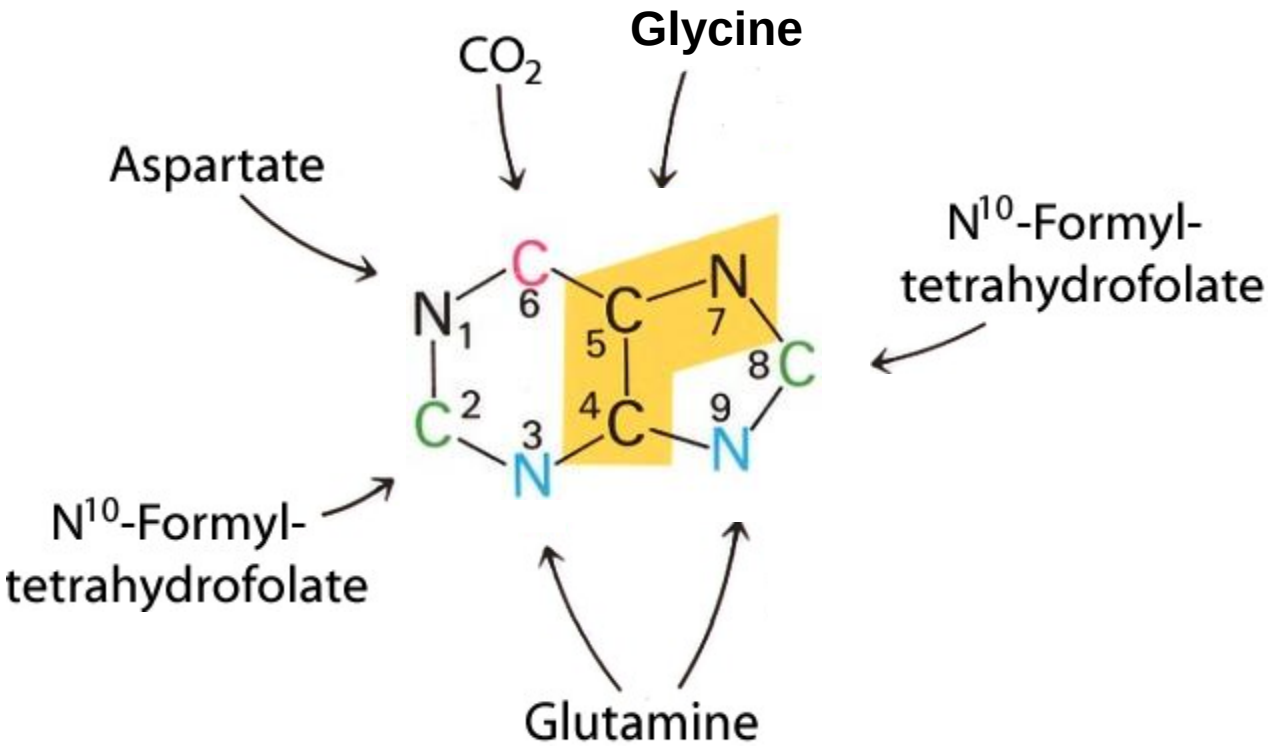
- 10 enzyme-catalyzed steps
- Each reaction uses different metabolic intermediates to acquire different atoms in the base ring of nucleotides
- Overall equation:

PRPP + 2Gln + Gly + 2 N^{10}formyltetrahydrofolate + HCO3-+ Asp + 4ATP

inosine monophosphate + 2Glu + 2 tetrahydrofolate + fumarate +4ADP + 4Pi

de novo Purine synthesis starts from PRPP. The purine ring atoms are added step by step to the ribose ring.

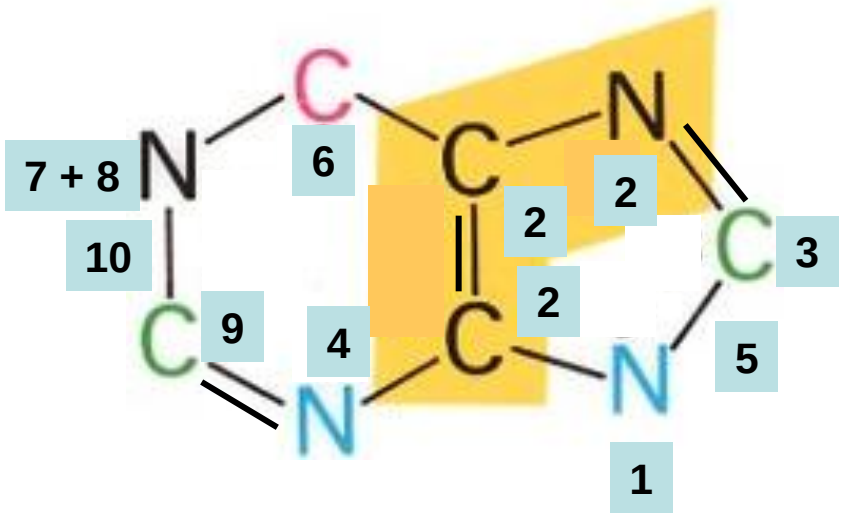
The biosynthetic origins of purine ring atoms



Double bonds not indicated

ORDER OF ADDITION

1.	Glutamine	N
2.	Glycine	C=C-N
3.	Formate	C
4.	Glutamine	N
5.	Ring closure	
6.	Carbon dioxide	C
7.	Aspartate	N
8.	Removal of fumarate (half of aspartate)	
9.	Formate	C
10.	Ring closure	

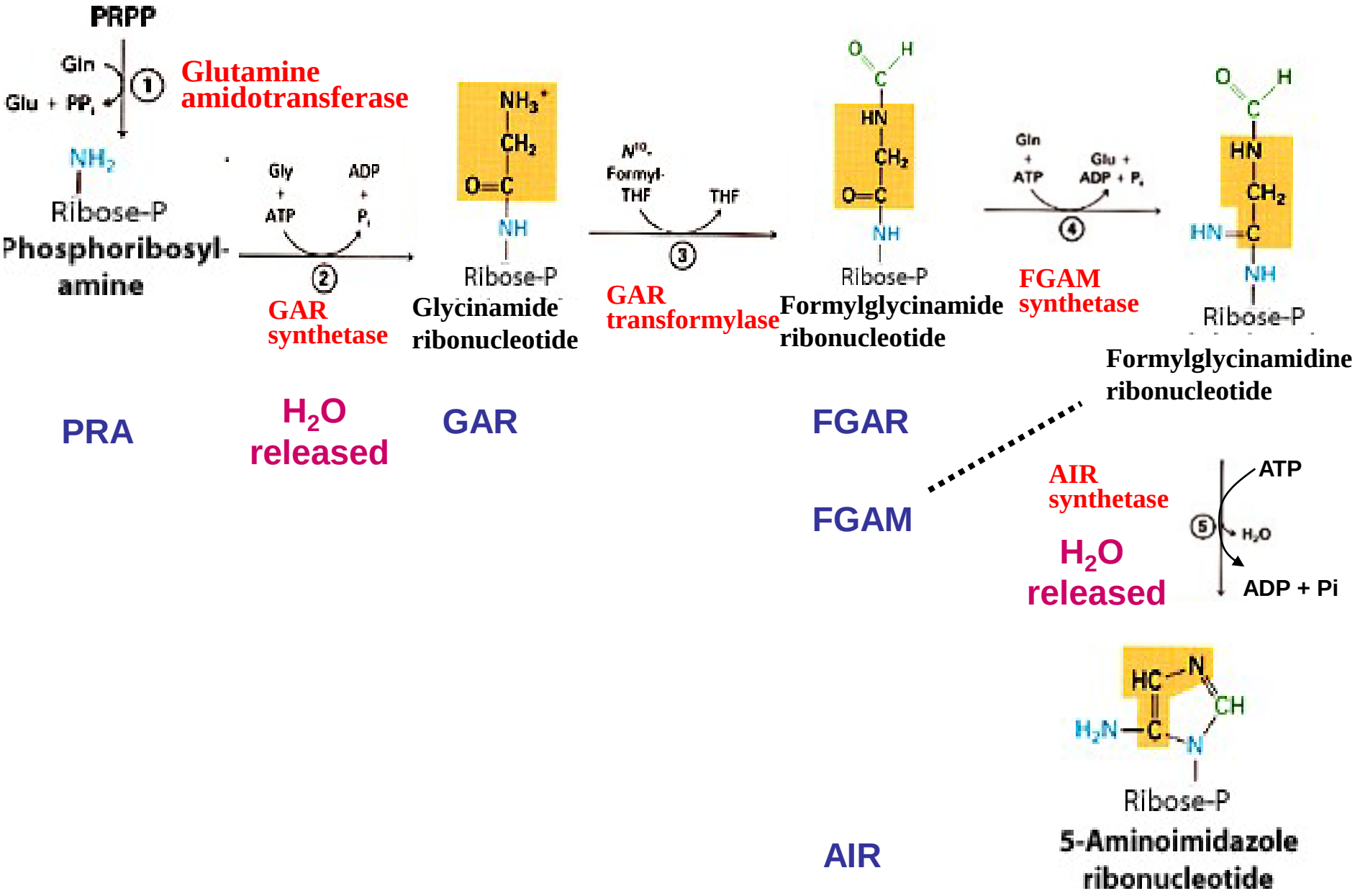


Know in full:

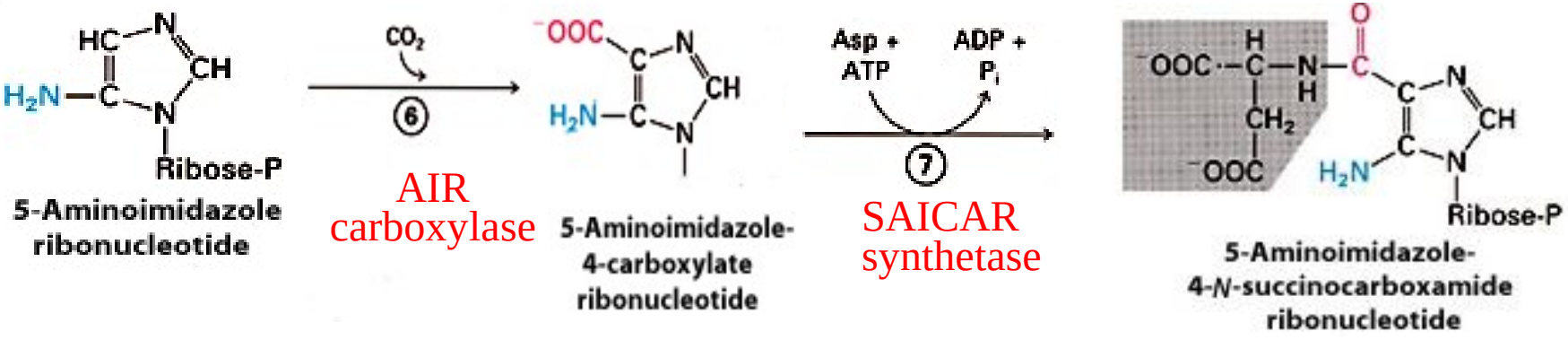
The intermediates are as follows in order:

PRPP
5-phospho-ribosylamine (PRA)
glycinamide ribonucleotide (GAR)
formylglycinamide ribonucleotide (FGAR)
formylglycinamidine ribonucleotide (FGAM)
aminoimidazole ribonucleotide (AIR)
carboxyaminoimidazole ribonucleotide (CAIR)
succinylo-aminoimidazole-carboxamide ribonucleotide (SAICAR)*
aminoimidazole-carboxamide ribonucleotide (AICAR)
formylaminoimidazole-carboxamide ribonucleotide (FAICAR)*
inosinate (inosine monophosphate) (IMP)

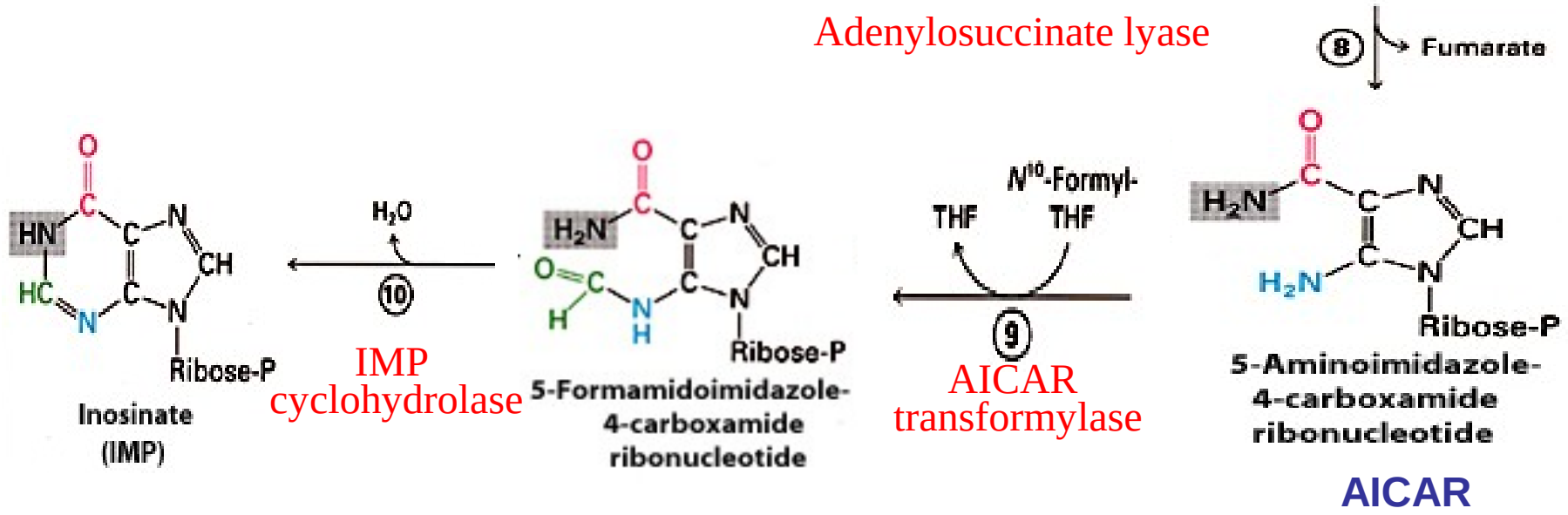
*Horton:
SAICAR: aminoimidazole succinylocarboxamide ribonucleotide
FAICAR: formaminoimidazole-carboxamide ribonucleotide (FAICAR)


PRPP
Gln
Glu + PPi
1
Glutamine amidotransferase
NH2
Ribose-P
Phosphoribosyl-amine
PRA
Gly + ATP
ADP + Pi
2
GAR synthetase
H2O released
NH3+
CH2
O=C
NH
Ribose-P
Glycinamide ribonucleotide
GAR
N10-Formyl-THF
THF
3
GAR transformylase
HN
CH2
O=C
NH
Ribose-P
Formylglycinamide ribonucleotide
FGAR
Gln + ATP
Glu + ADP + Pi
4
FGAM synthetase
HN
CH2
HN=C
NH
Ribose-P
Formylglycinamidine ribonucleotide
FGAM
AIR synthetase
ATP
5
H2O
ADP + Pi
H2O released
HC
N
CH
H2N
C
N
Ribose-P
5-Aminoimidazole ribonucleotide
AIR

H_2O released



Adenylosuccinate lyase



Enzymes and reactions

1. Glutamine-PRPP-amidotransferase (compare to 4)

amino group transfer

2. Glycinamide ribonucleotide synthetase

addition of a glycine

ATP drives the reaction

condensation reaction

3. Glycinamide ribonucleotide transformylase

formyl group transfer

4. Formylglycinamide ribonucleotide amido transferase

amino group transfer

ATP required

5. Aminoimidazole ribonucleotide synthetase

ATP dependent ring closure

condensation reaction

6. Aminoimidazole ribonucleotide carboxylase

carboxylation reaction

7/8. Succinyloaminoimidazole carboxamide ribonucleotide synthetase/lyase

transfer of amino group to imidazole ring in two steps

first aspartate is transferred to the carboxyl group (water released) followed by an elimination reaction (fumarate is eliminated)

9. Aminoimidazole carboxamine ribonucleotide transformylase

transfer of formyl group

10.IMP synthetase

ring closure

condensation reaction.

NOTE similarities between the following

reactions 1 and 4	2 and 4 and 5 and 7
2 and 4 and 7	3 and 9
5 and 10	2 and 5 and 7