Purine Metabolism

Raymond B. Birge, PhD
Biochemistry & Molecular Biology

Nucleotides play key roles in many, many cellular processes

1. Activated precursors of RNA and DNA

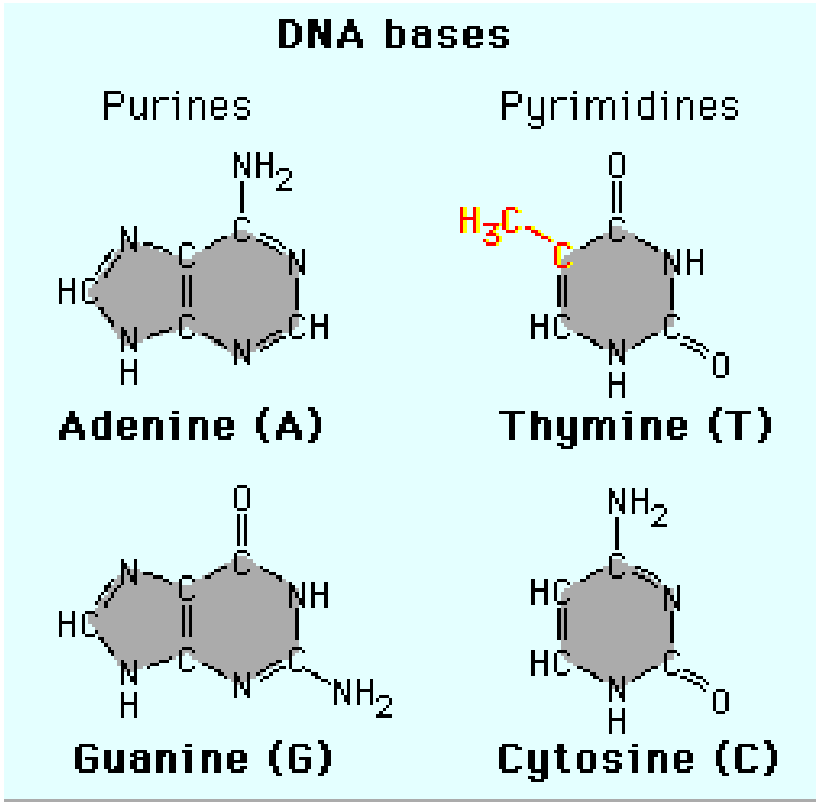
2. Adenine nucleotides are components of three major co-enzymes, NAD, FAD, and CoA

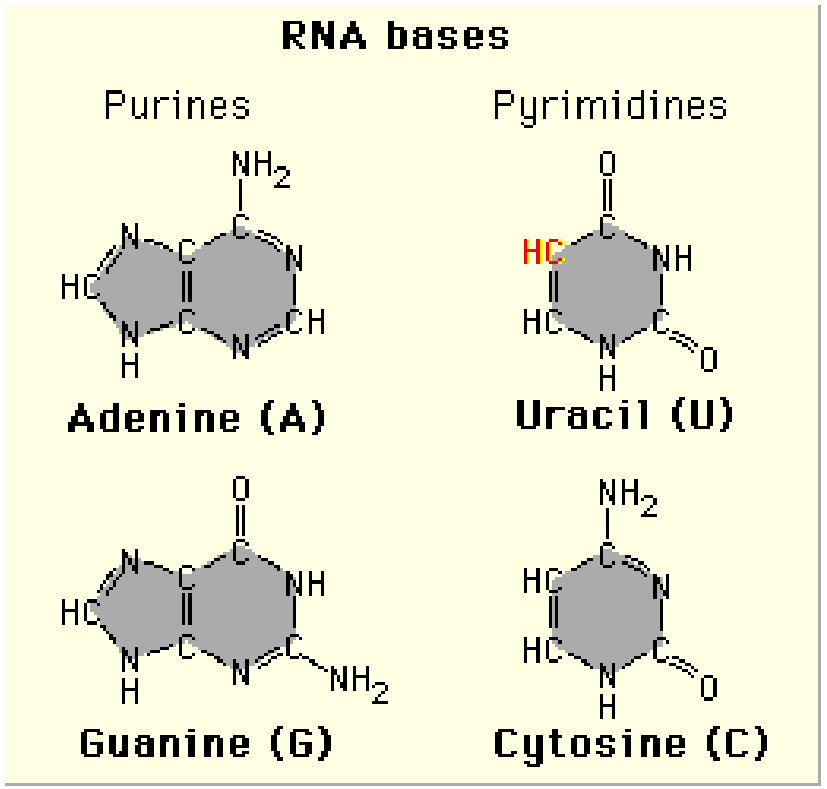
3. Nucleotide derivatives are activated intermediates in biosynthetic processes (UDP-glucose, SAM)

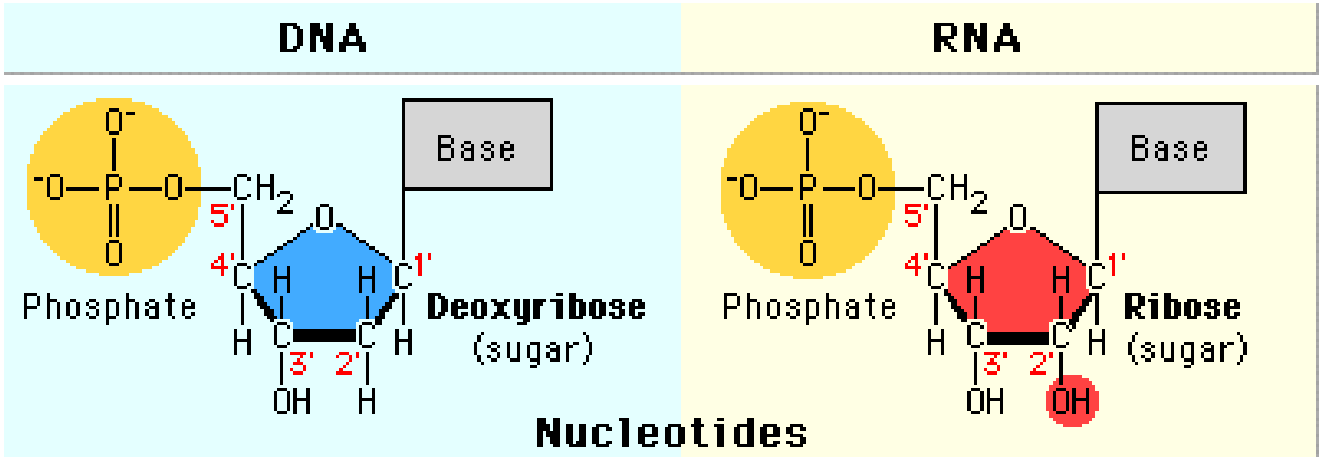
4. Serve as metabolic regulators (e.g cAMP and the activation of cell signaling).

5. Serve as major currency of energy in all cells (ATP and GTP).

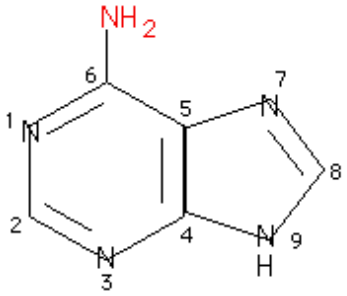
6. Many metabolic diseases have their etiology in nucleotide metabolism.

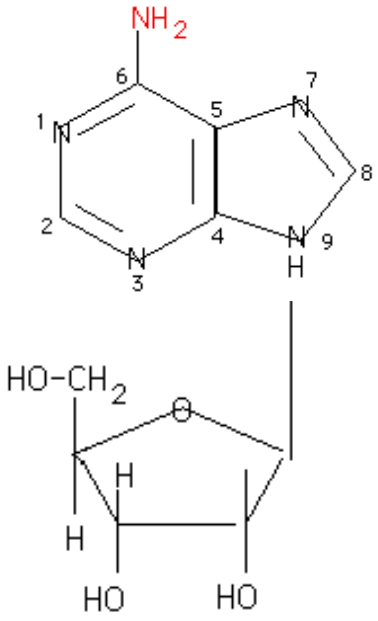

DNA bases
Purines
Pyrimidines
Adenine (A)
Thymine (T)
Guanine (G)
Cytosine (C)

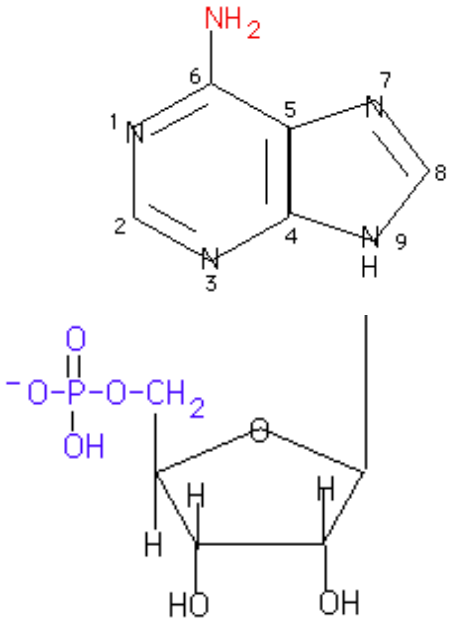

RNA bases
Purines
Pyrimidines
Adenine (A)
Uracil (U)
Guanine (G)
Cytosine (C)


DNA
RNA
Base
Phosphate
Deoxyribose
(sugar)
Ribose
(sugar)
Nucleotides

The nomenclature of purines depends on their linkage to a pentose







Adenine

Base

Adenosine

Nucleoside*
Base

Adenosine Monophosphate

Nucleotide
Base ($P0_4$ ester)

* when the base is purine, then the nucleoside ends in **OSINE (**Aden**OSINE,** Guan**OSINE,** In**OSINE)**

The active forms of nucleotides in biosynthesis and energy conversions are di-and tri-phosphates

(i) Nucleoside Monophosphate Kinase

$$CMP + ATP \rightleftharpoons CDP + ADP$$

(ii) Nucleoside Diphosphate Kinase

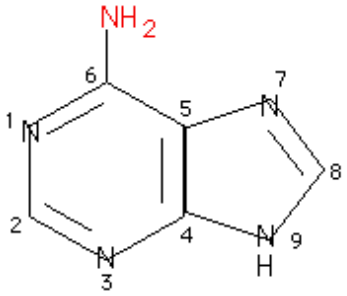
$$XDP + YTP \rightleftharpoons XTP + YDP$$

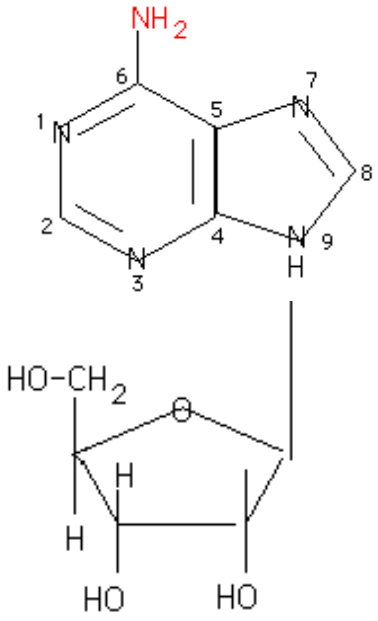
What do nucleosides and nucleotides do?

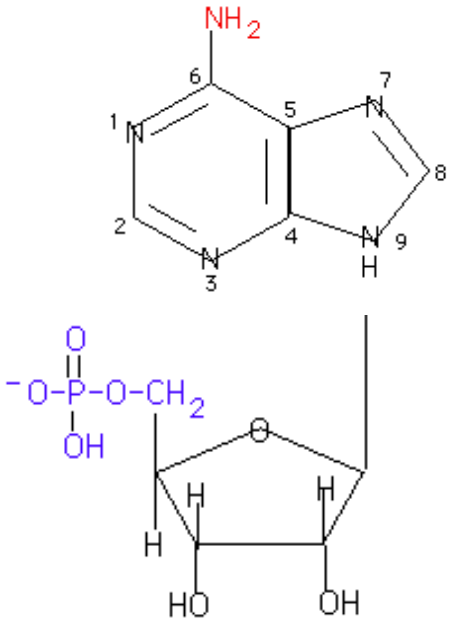
Purine binding proteins (“the purine proteome”) comprise a family of 3-4,000 Proteins and as much as 50% of all druggable targets in biology.

Kinases
Helicases
Reductases
Transferases
Synthetases
Dehydrogenases
Chaperones
Metabolic Enzymes
DNA and RNA processing
Etc

The nomenclature of purines depends on their linkage to a pentose







Adenine

Base

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Base

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* when the base is purine, then the nucleoside ends in **OSINE (**Aden**OSINE,** Guan**OSINE,** In**OSINE)**

Nucleoside Function in extracellular signal transduction

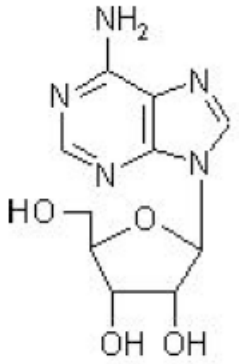
Adenosine nucleoside-increased during ATP degradation.

Released in cells when there is low O_2 concentration

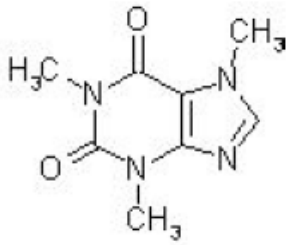
Binds to purinogenic receptors A_1, A_{2A}, A_{2B}, A_3

Slows the heart down, at the same time increases capillary dilation

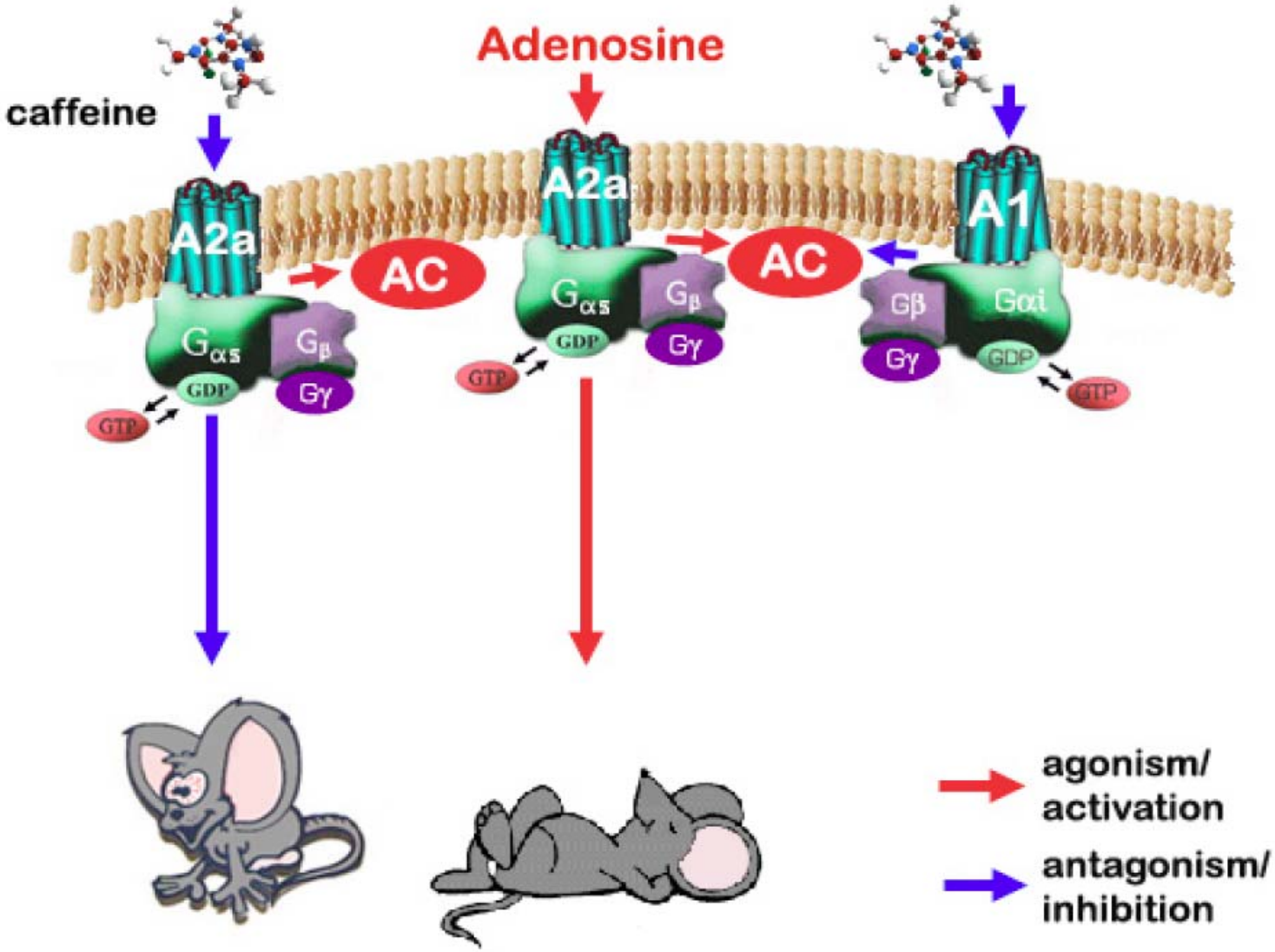
Caffeine is a adenine derivative, and antagonizes the effects of adenine.

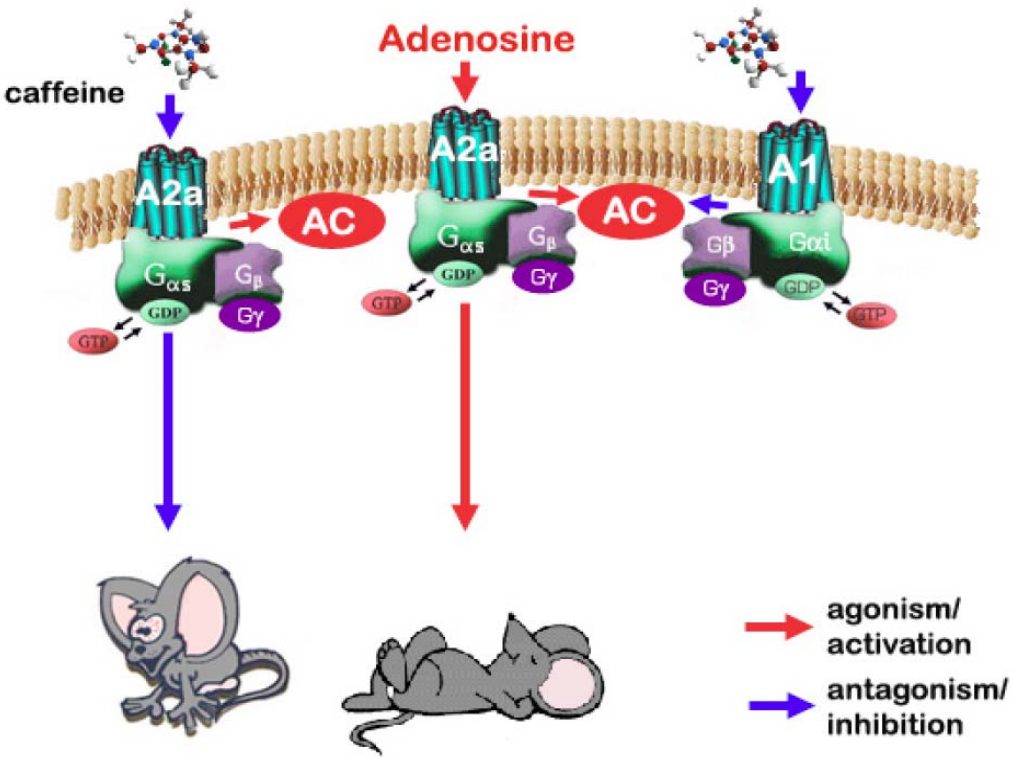


adenosine



caffeine


caffeine
Adenosine
A2a
A2a
A1
AC
AC
Gαs
Gβ
Gγ
GDP
GTP
Gβ
Gαi
Gγ
GDP
GTP
agonism/
activation
antagonism/
inhibition


caffeine
Adenosine
A2a
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A1
Gαs
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Gαi
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agonism/
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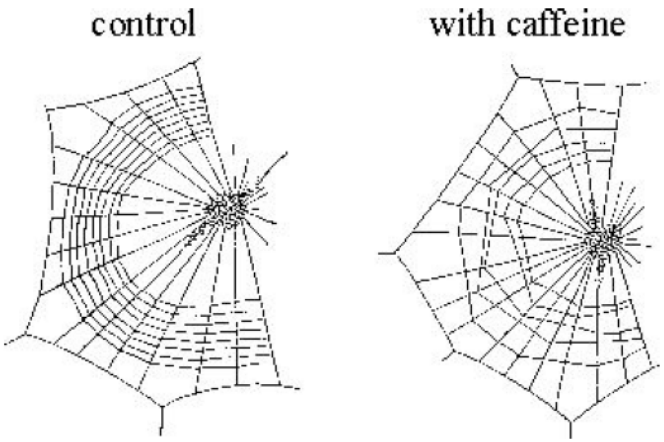
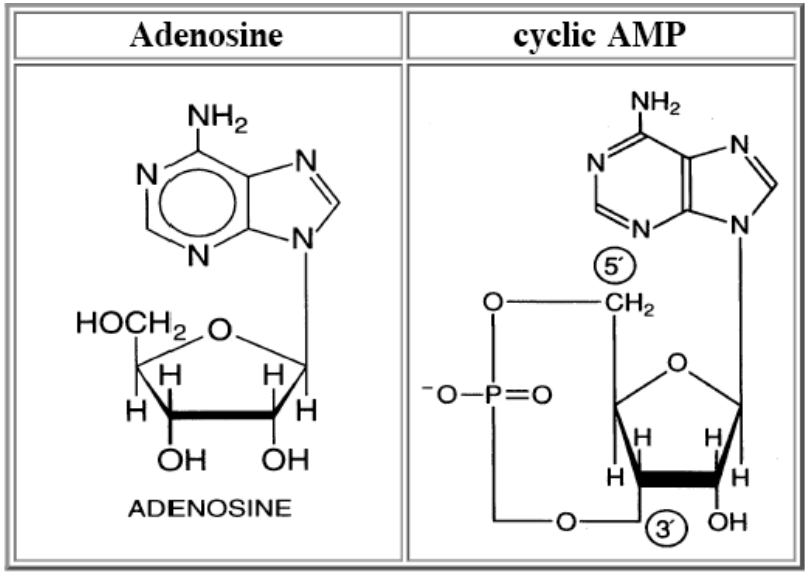
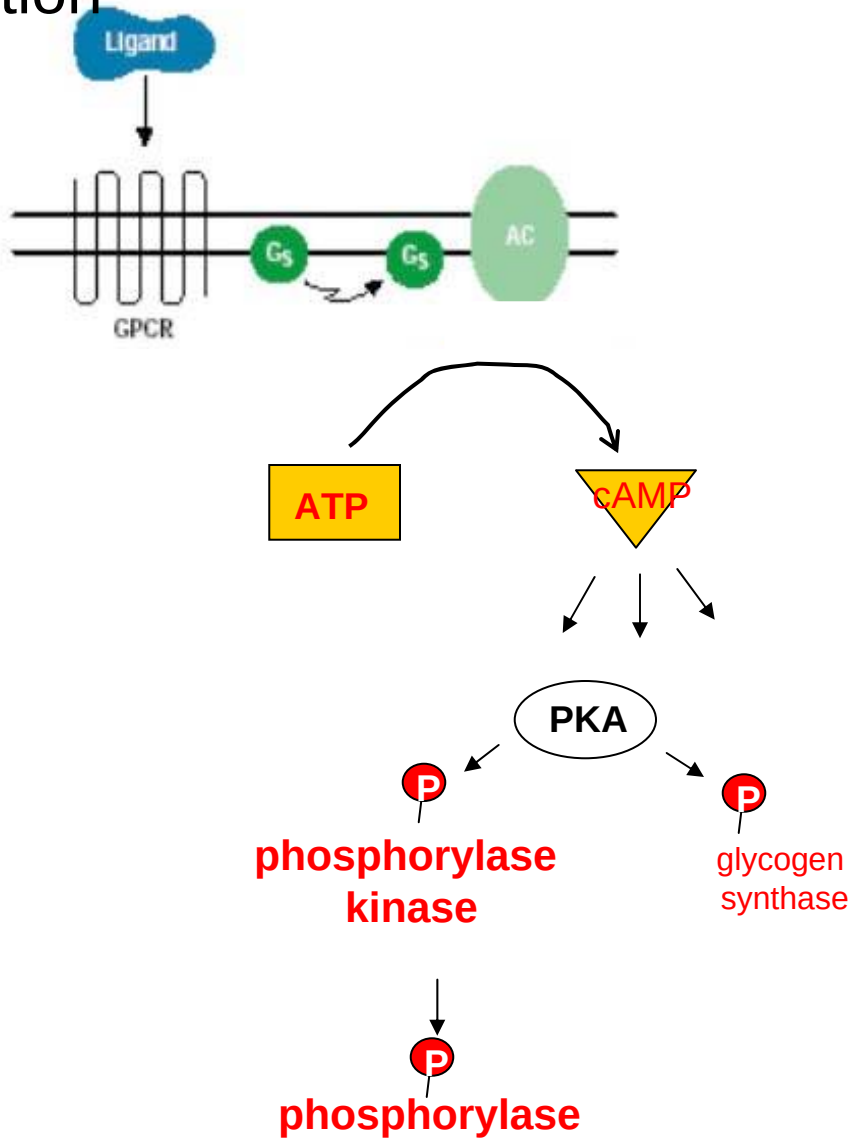
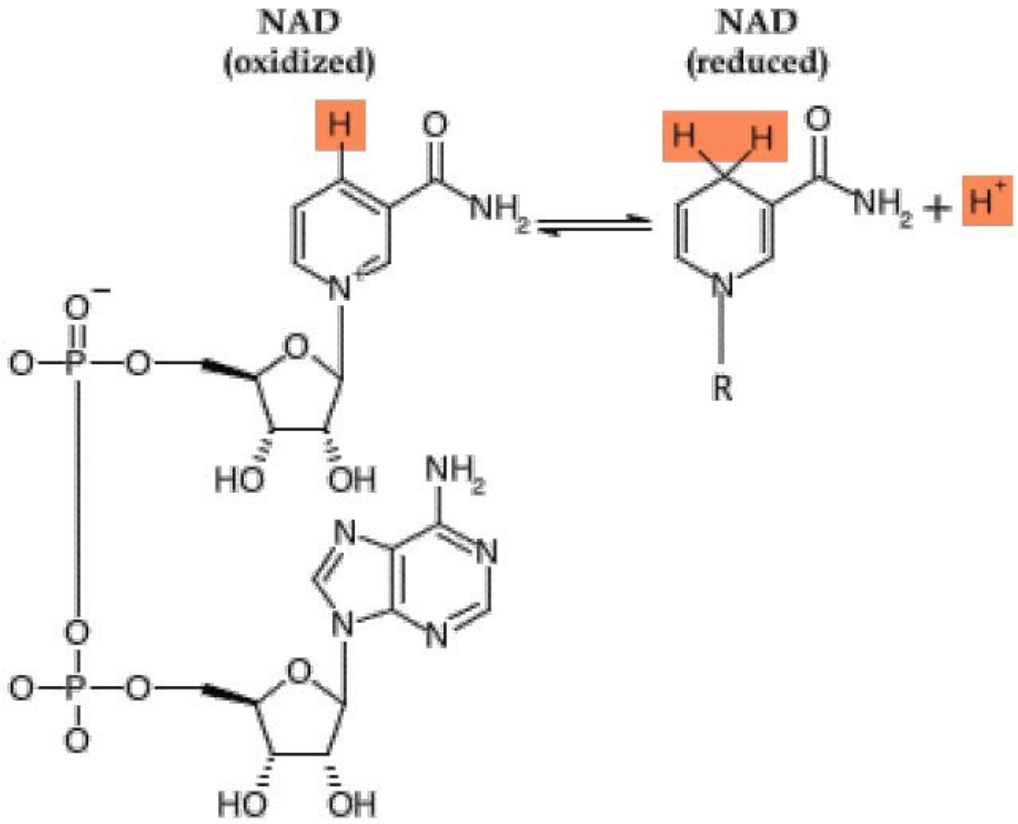

control
with caffeine

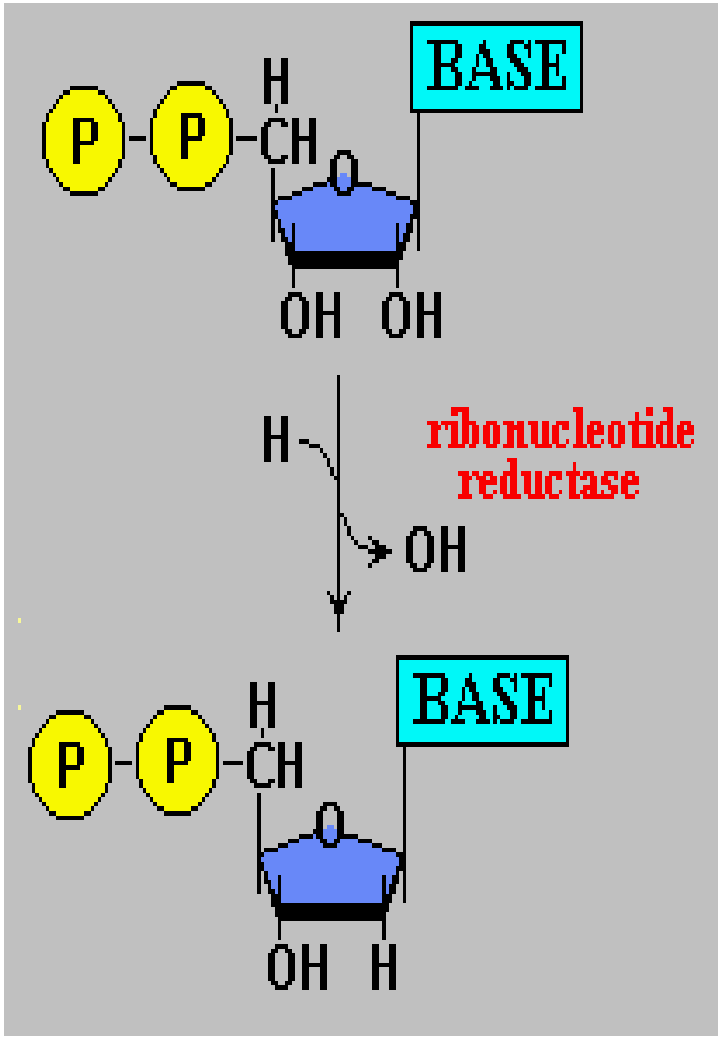
Cyclic nucleotides are important mediators for Intracellular signal transduction





Modified nucleotide mono and di phosphates have important in electron transfer and Redox control





RIBONUCLEOTIDE REDUCTASE

1. Complex enzymatic reaction whereby electrons are transferred from NADPH through a series of sufhydryl groups at the catalytic site of Ribonucleotide Reductase.

2. Active site of RR contains thioredoxin, a 12 kD proteinwith two exposed cysteines, which become oxidized.

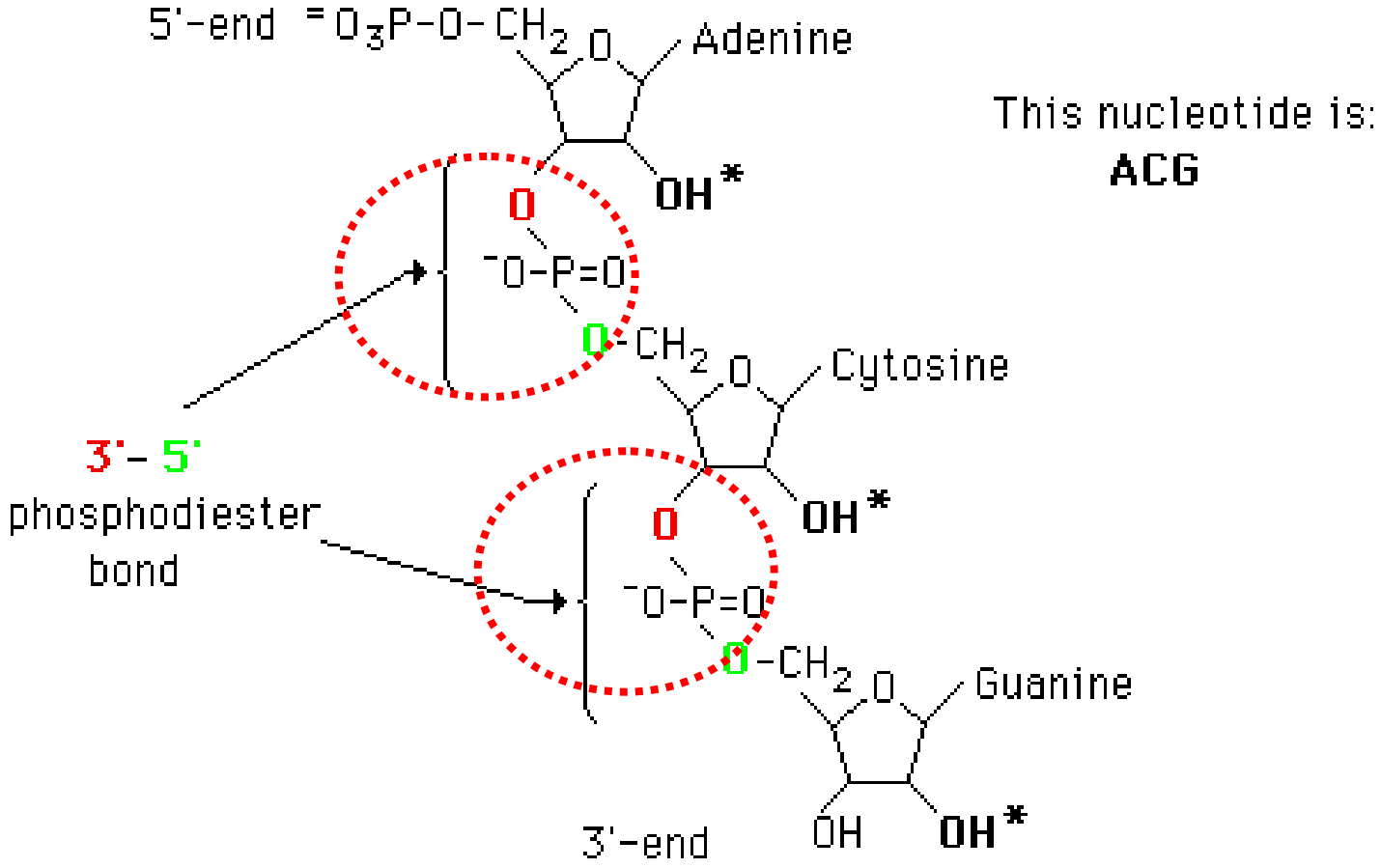
3. This ultimately allows for the reduction of ribose.

REGULATION

1. Based on the response to cellular need for dATPs.

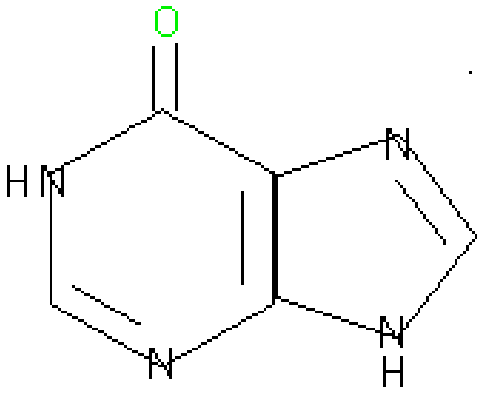
dATP is general inhibitor
ATP is a general activator

Nucleotides are linked by 5’ to 3’ phosphodiester bonds to generate DNA and RNA

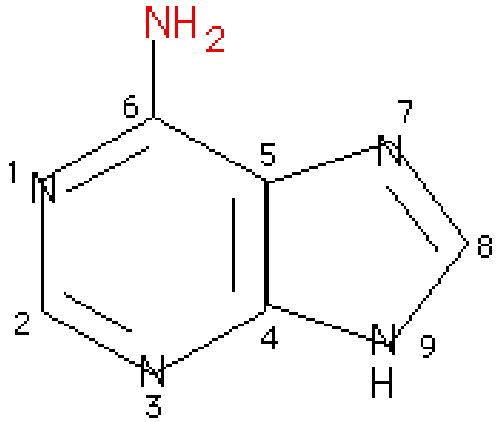


* In DNA, this atom would be H instead of OH.

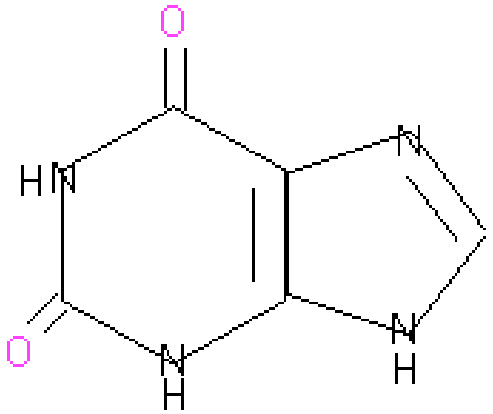
Structures of Common Purine Bases.



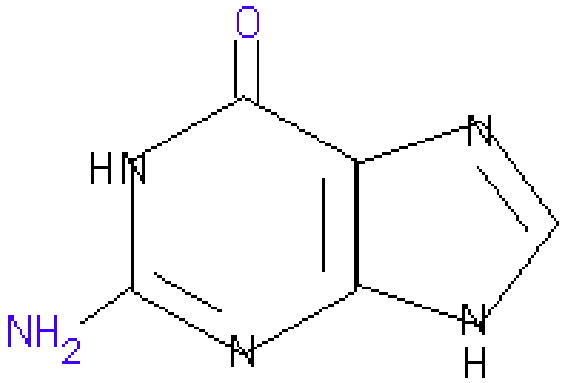
Hypoxanthine



Adenine



Xanthine



Guanine

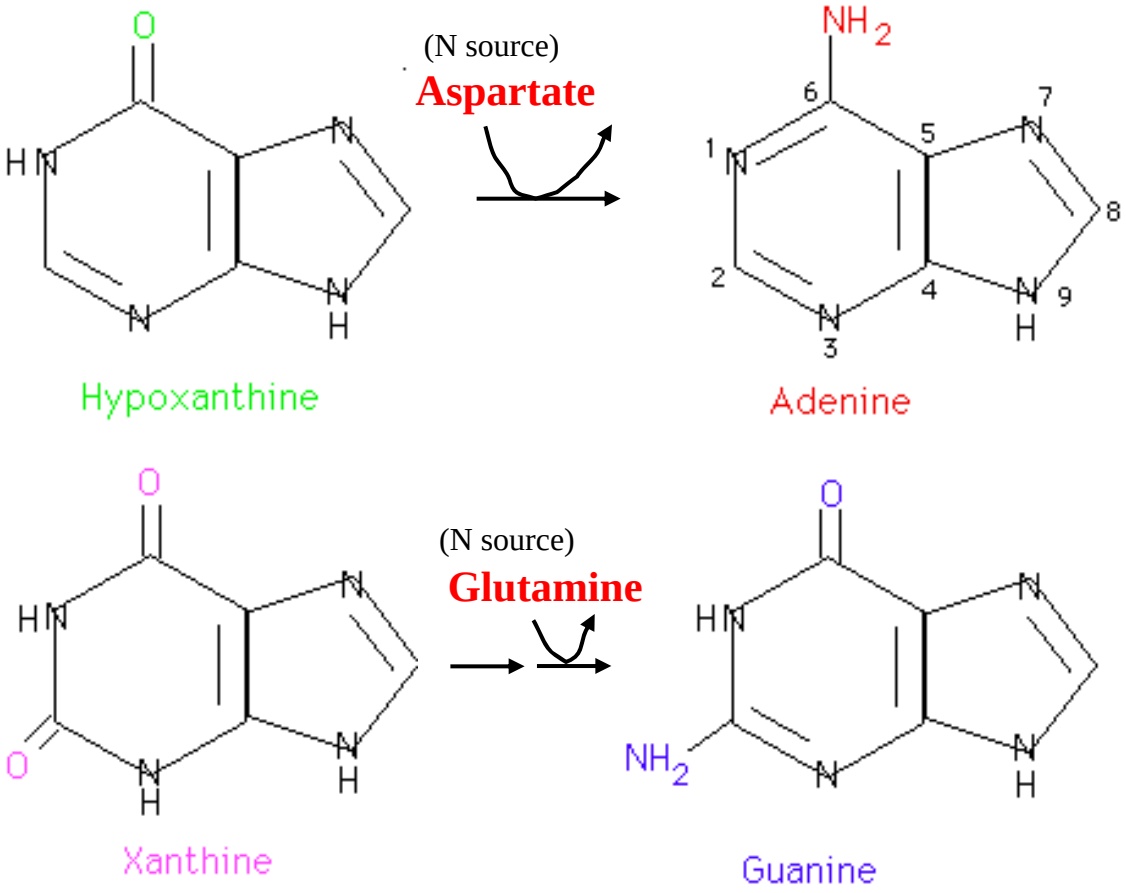
H= 6 oxy purine

X= 2,6 dioxy purine

A= 6 amino purine

G= 2 amino, 6-oxy purine

Hypoxanthine is an intermediate for Adenine and Guanine



The common mechanistic them for the conversion of A and G is the conversion of a carbonyl oxygen to an amino group

There are two basic mechanisms to generate purines and pyrimidines

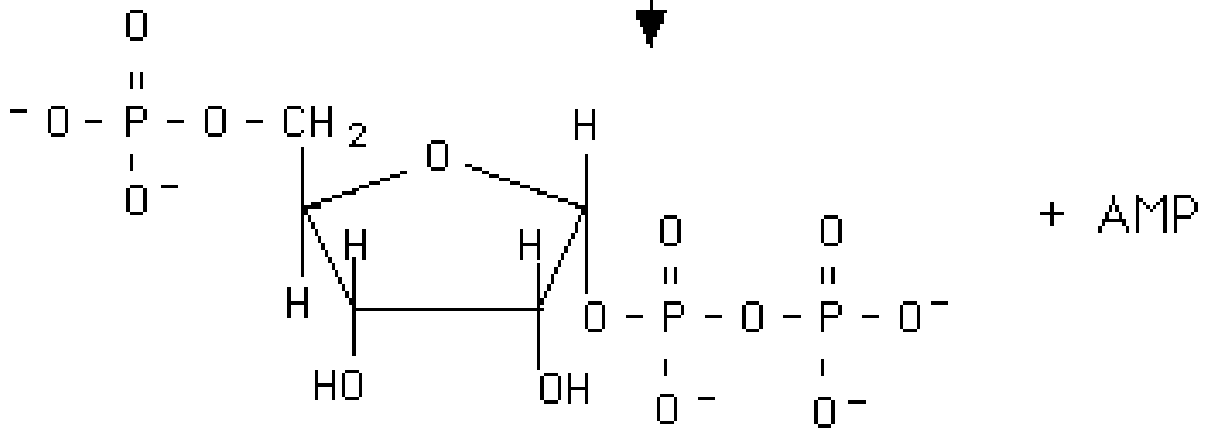
1. *DE NOVO* BIOSYNTHETIC PATHWAYS
(building the bases from simple building blocks)

2. SALVAGE PATHWAYS
(the reutilization of bases from dietary
or catabolic sources)

The biosynthesis of purine (A and G) begins with the synthesis of the ribose-phosphate

Pentose phosphate pathway → Ribose 5- phosphate + ATP

↓ **Ribose phosphate pyrophospho-KINASE**

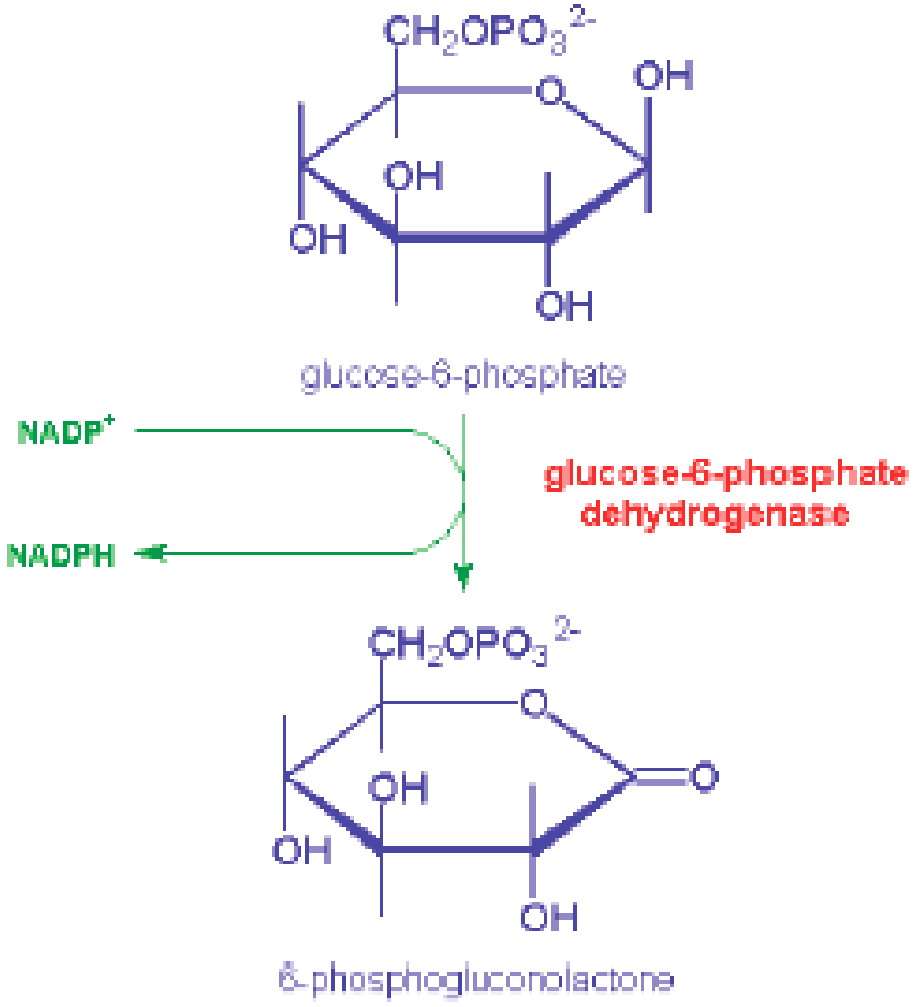


+ AMP

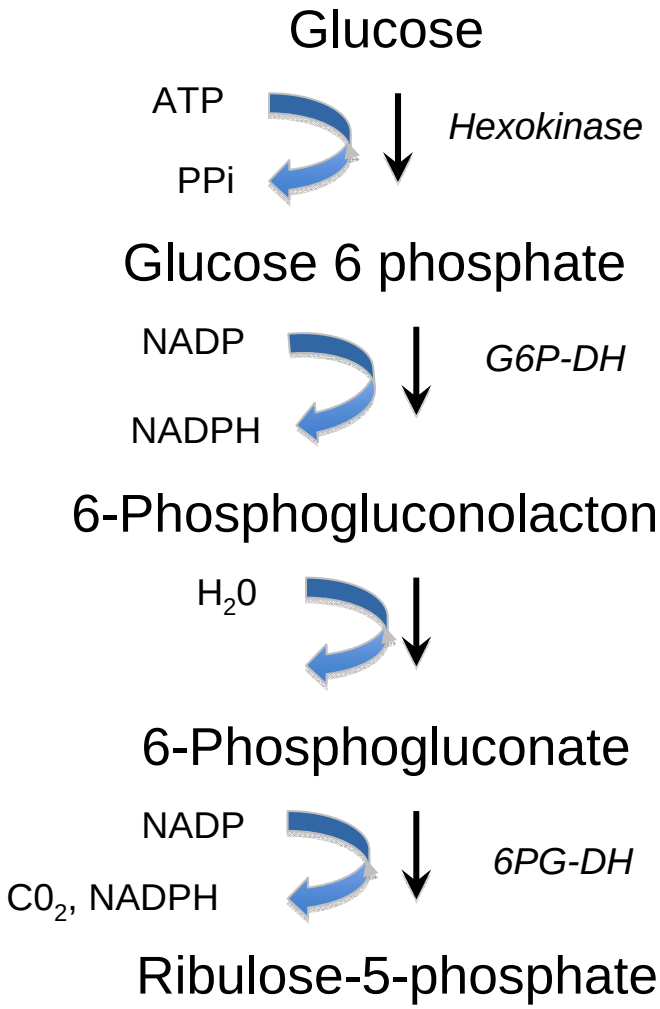
5-Phosphoribosyl-1-pyrophosphate

PRPP

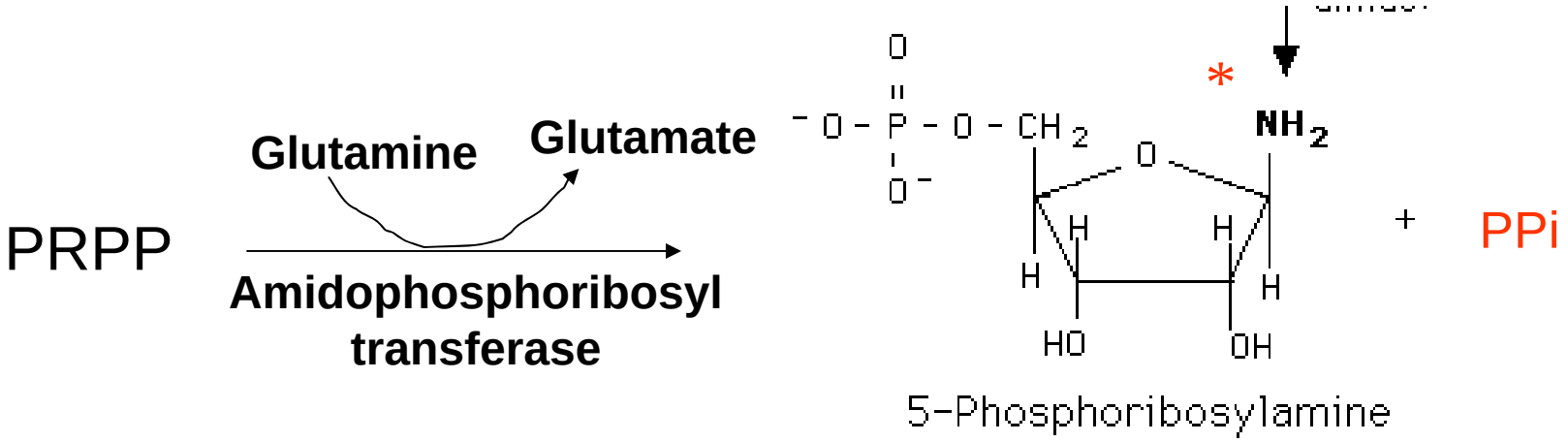
Oxidative Stage of Pentose Phosphate Pathway



Oxidative Stages of Pentose Phosphate Pathway

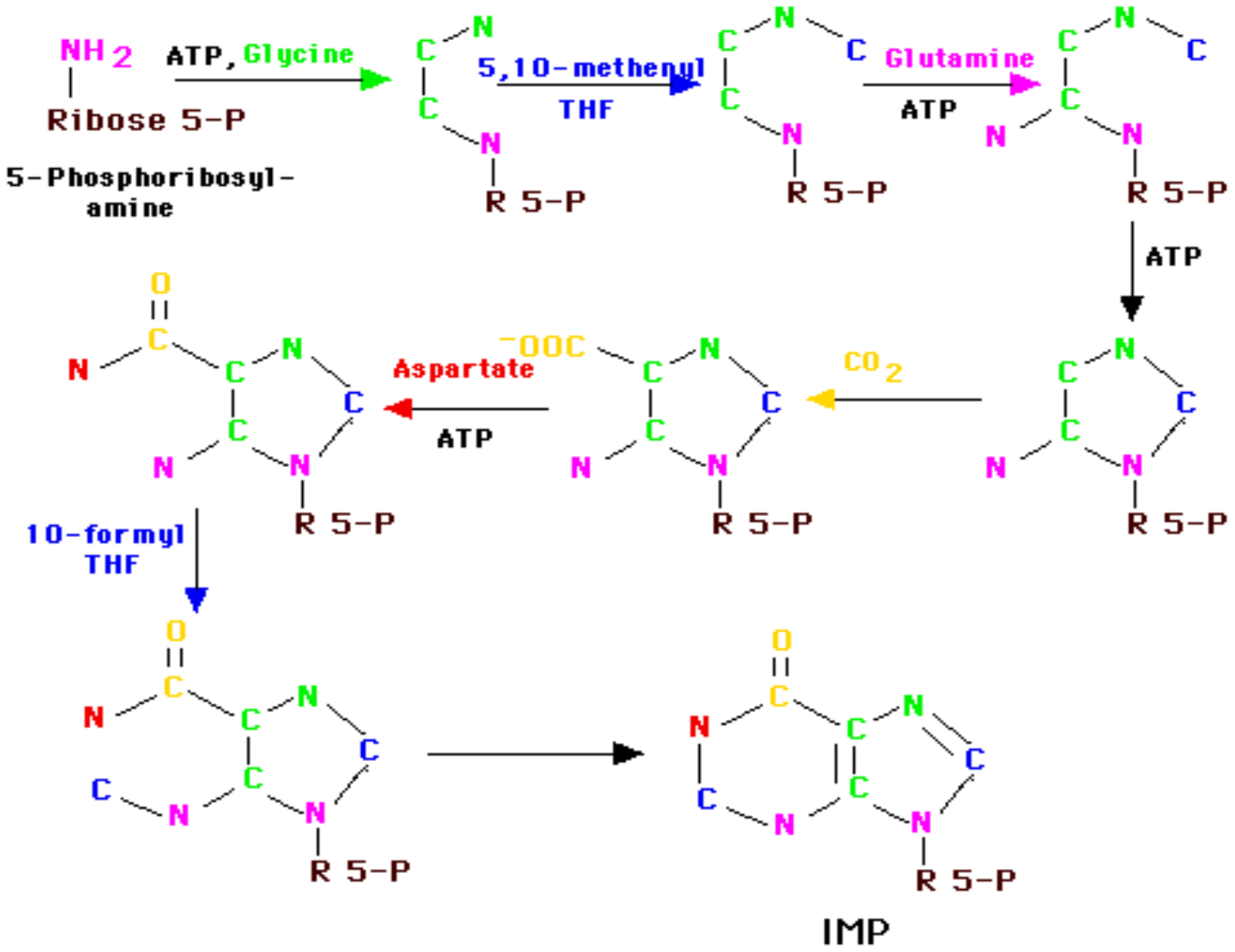


The major regulatory step in purine biosynthesis is the conversion of PRPP to 5-Phosphoribosyl-1-amine



Amidophosphoribosyl transferase is an important regulatory enzyme in purine biosynthesis. It is strongly inhibited by the end products IMP, AMP, and GMP. This type of inhibition is called FEEDBACK INHIBITION.

Several amino acids are utilized in purine biosynthesis,



IMP is the precursor for both AMP and GMP, the base is also called hypoxanthine

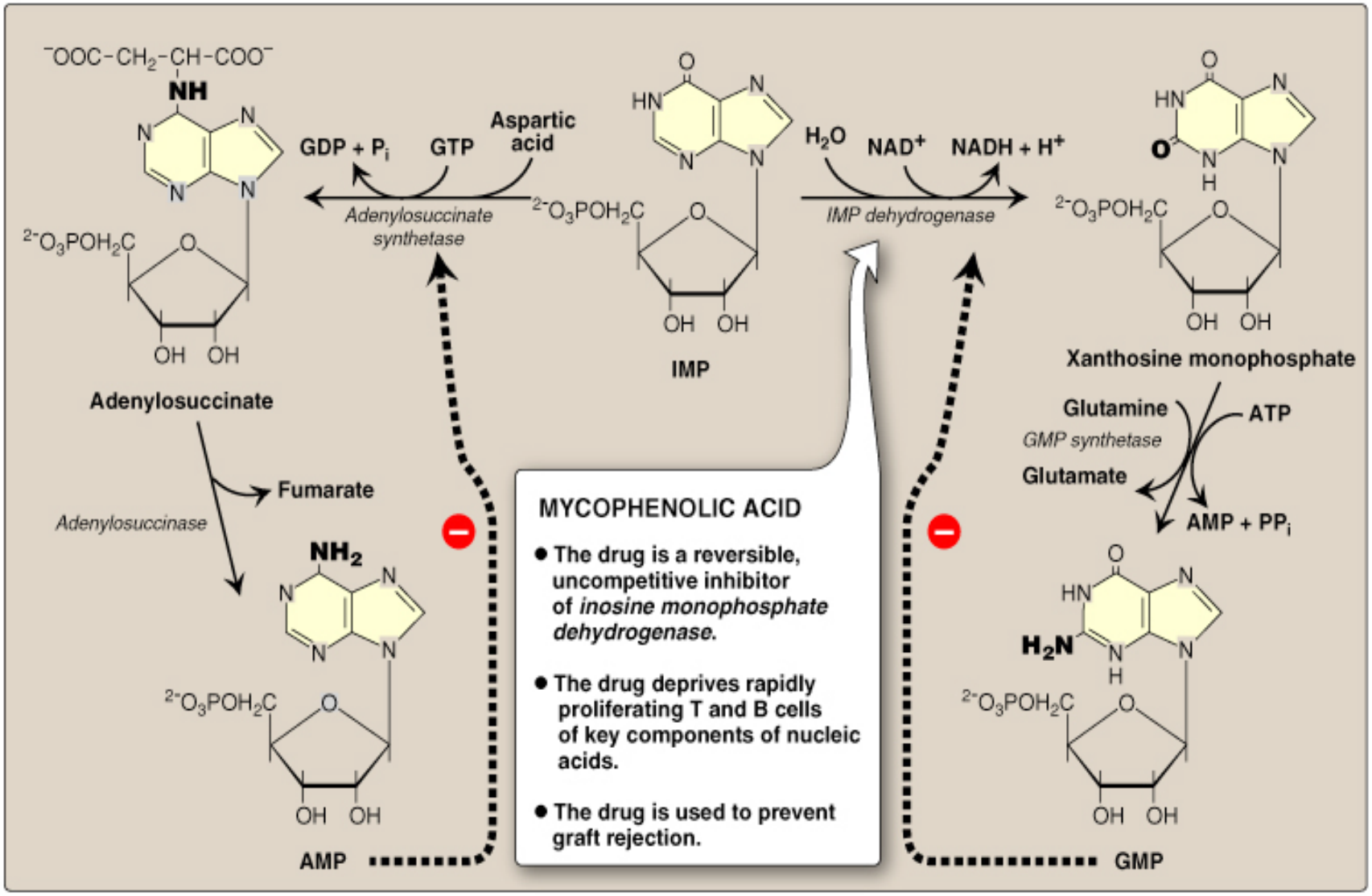
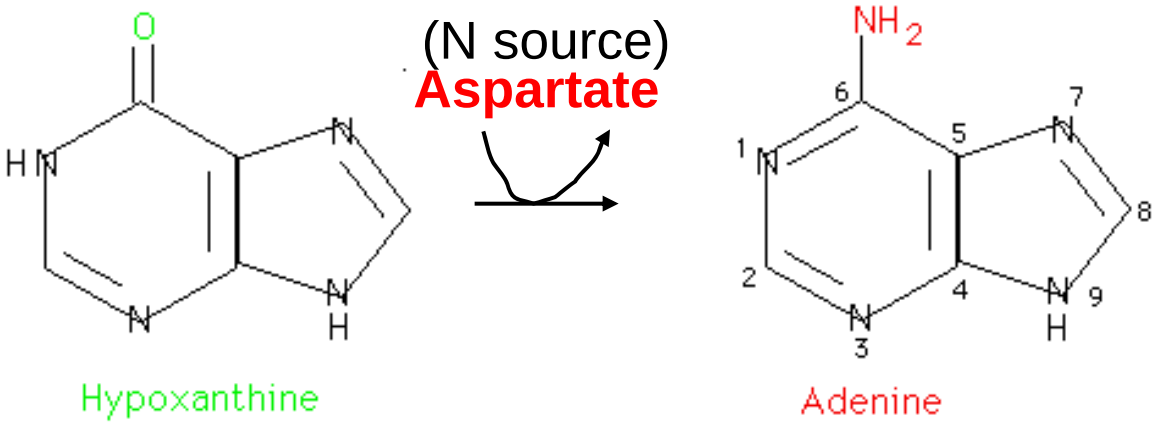
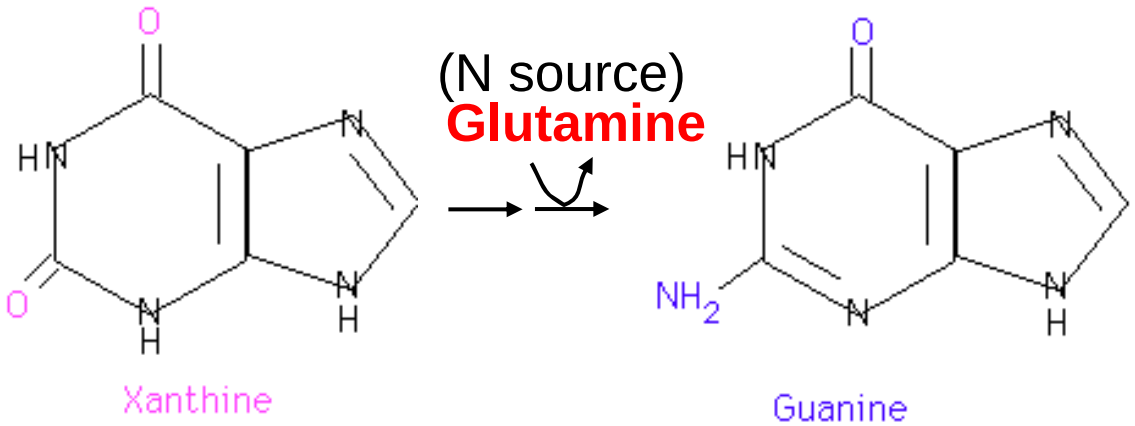


Figure 22.8

Conversion of IMP to AMP and GMP showing feedback inhibition.

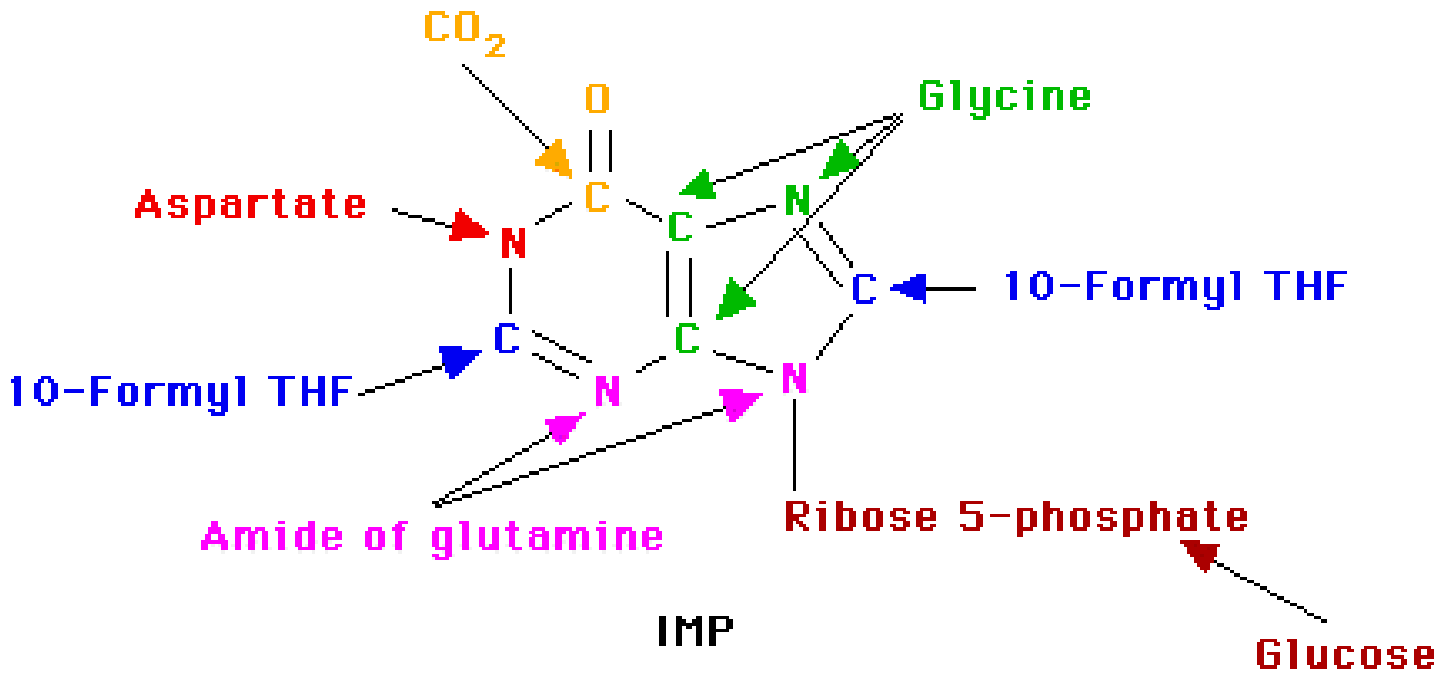
Conversion of Hypoxanthine to Adenine/Guanine.





The common mechanistic theme for the conversion of A and G is the conversion of a carbonyl oxygen to an amino group

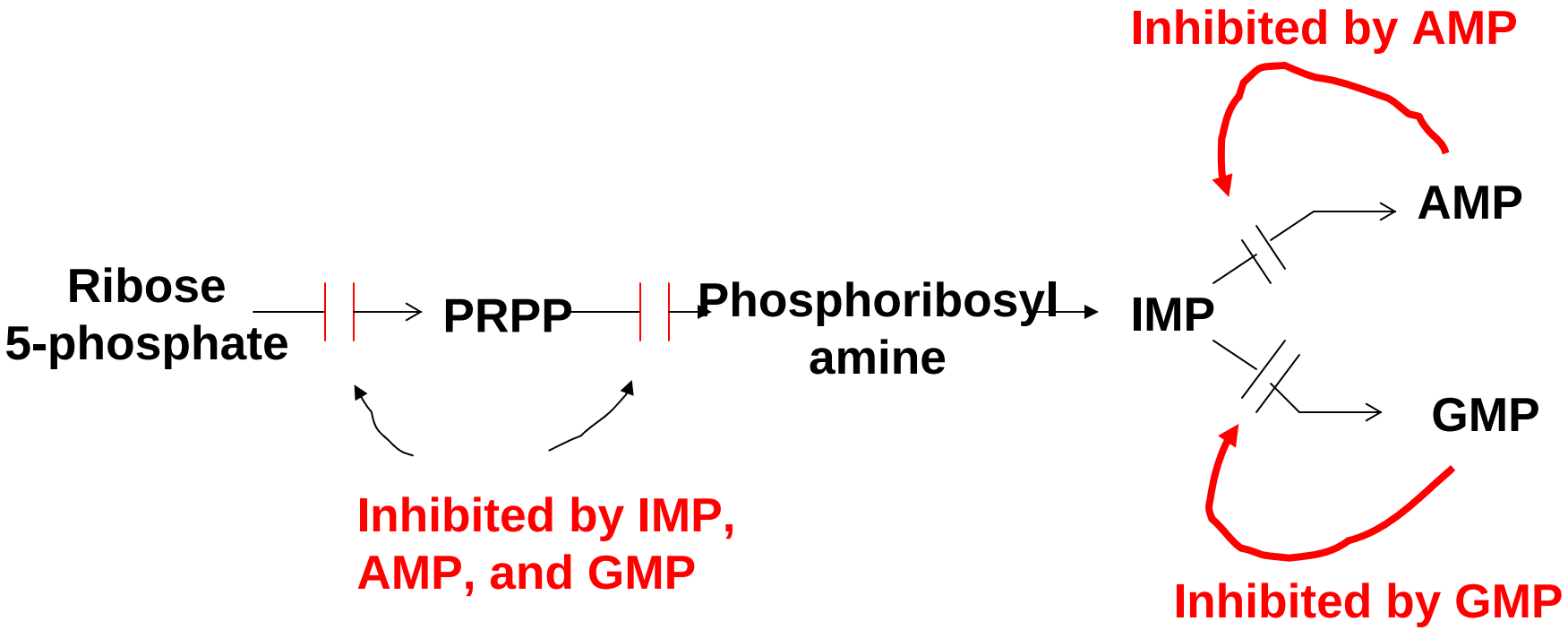
Purines:where do the atoms come from?



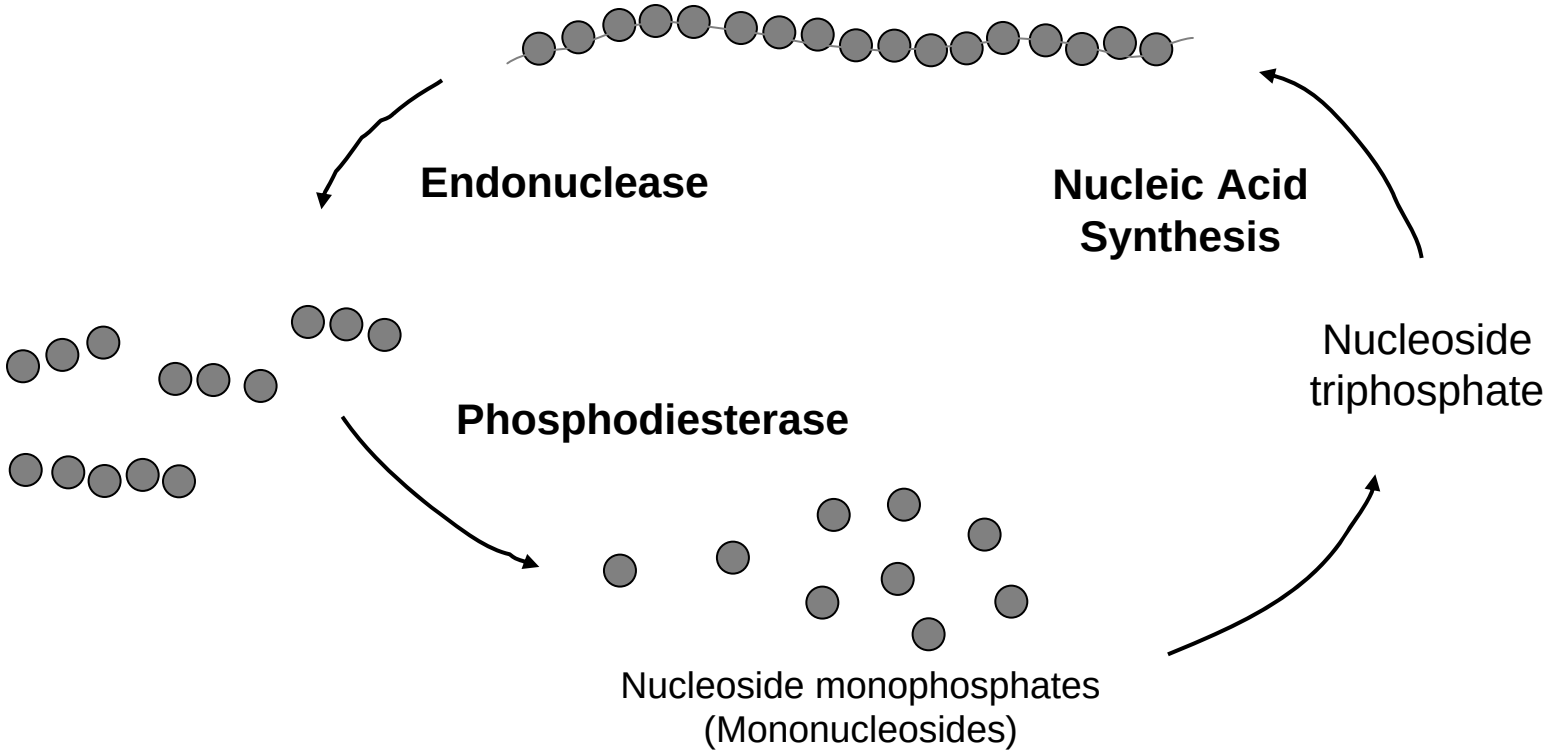
Purine intermediates include:

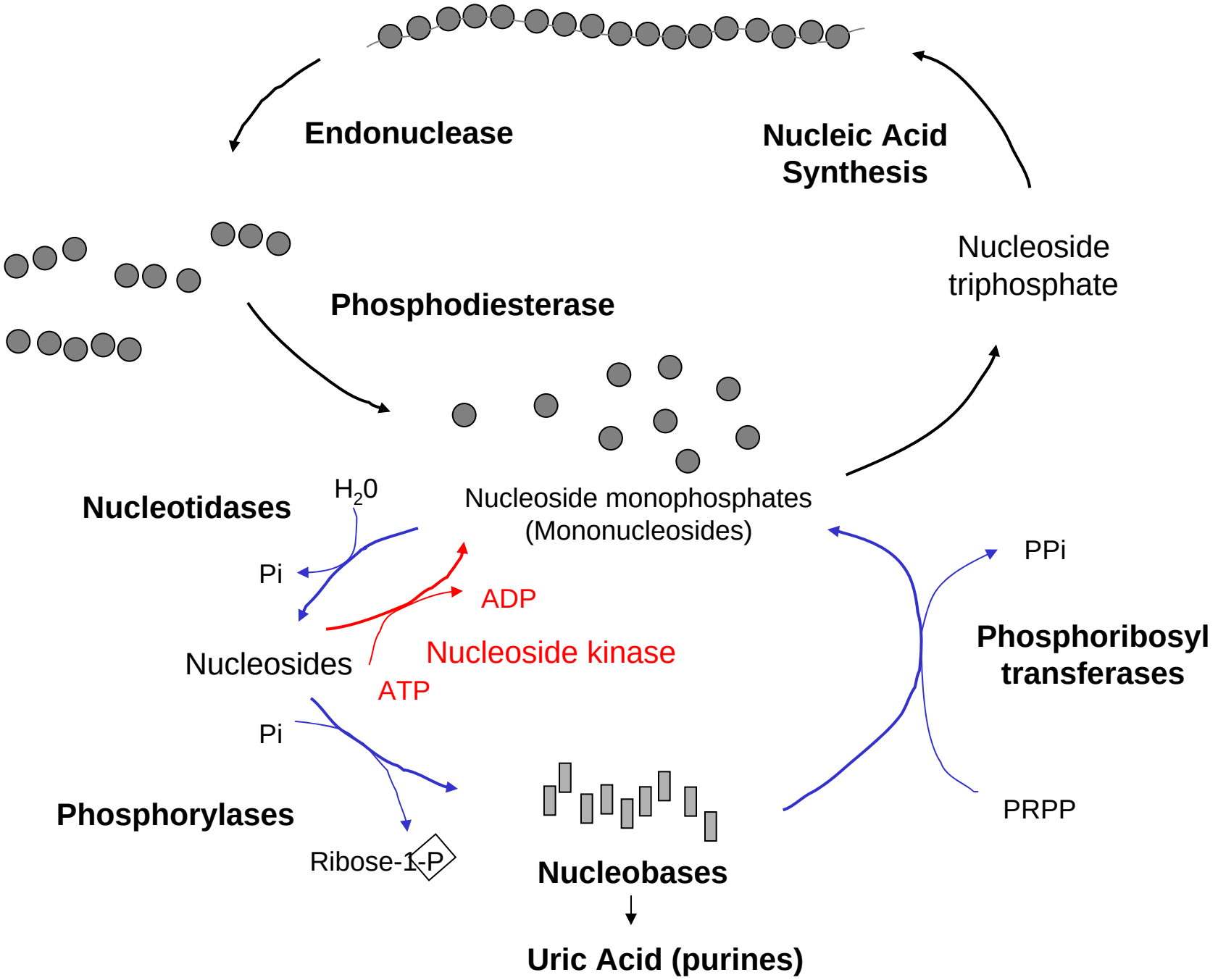
1. Glycine
2. 1 C units of 5,10 mTHF
3. Glutamine
4. Asparate

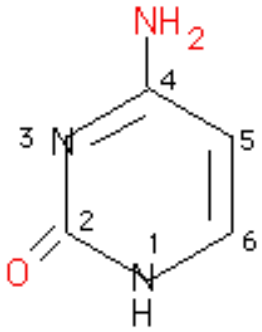
The regulation of purine biosynthesis is a classic example of negative feedback



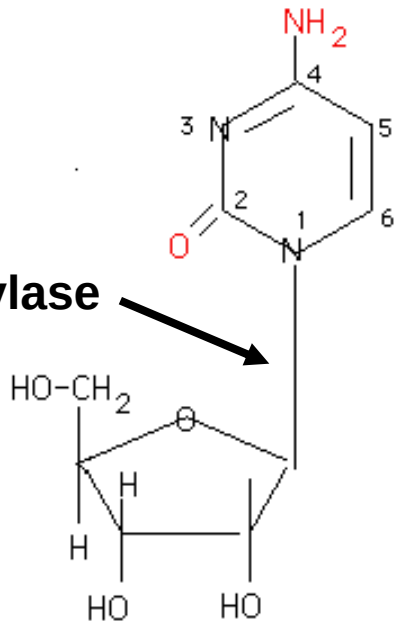
Stages of nucleotide metabolism



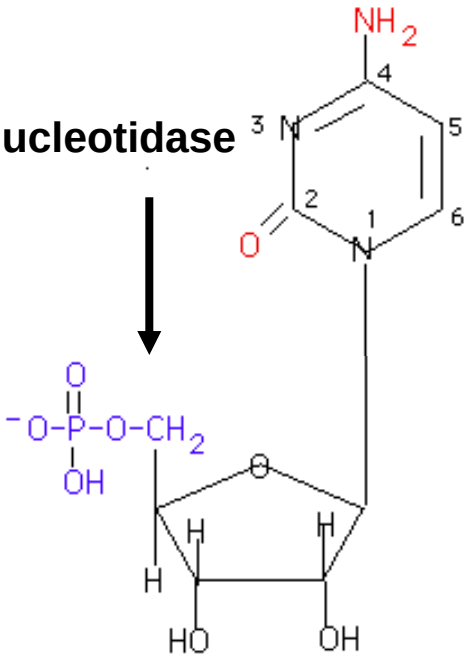

Endonuclease
Nucleic Acid Synthesis
Nucleoside triphosphate
Phosphodiesterase
Nucleotidases
H_20
Nucleoside monophosphates (Mononucleosides)
Pi
ADP
Nucleoside kinase
Nucleosides
ATP
PPi
Phosphoribosyl transferases
Pi
Phosphorylases
PRPP
Ribose-1-P
Nucleobases
Uric Acid (purines)



Phosphorylase



Nucleotidase



Cytosine

Base

Cytidine

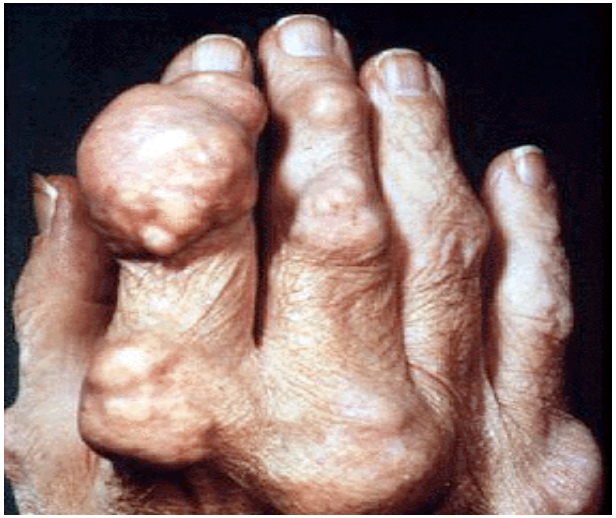
Nucleoside*
Base

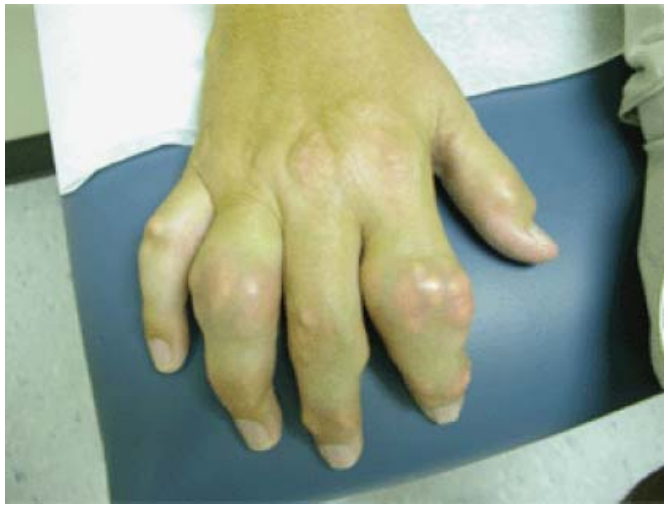
Cytidine Monophosphate

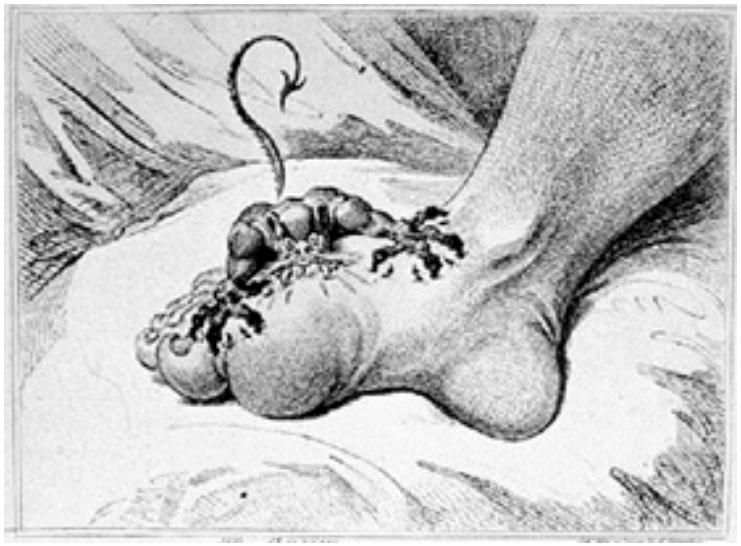
Nucleotide
Base ($P0_4$ ester)

Gout-Gouty Arthritis

(a metabolic condition of abnormal purine metabolism)



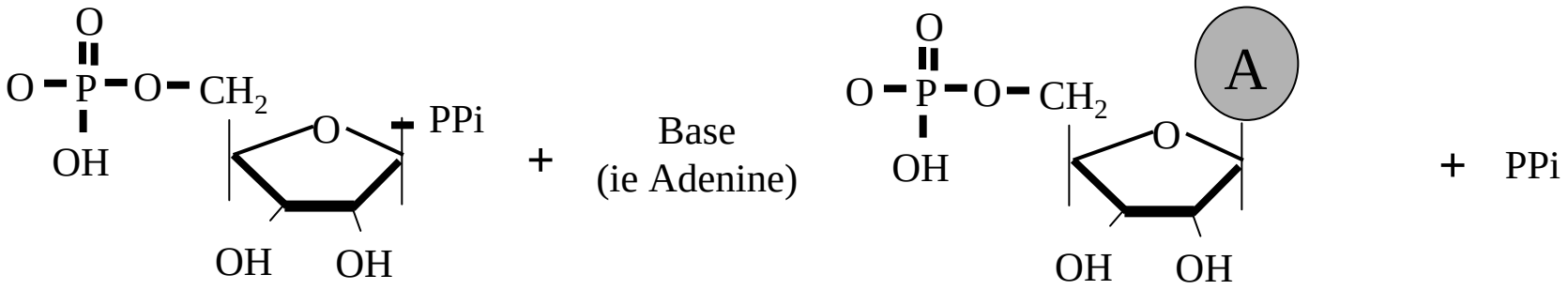


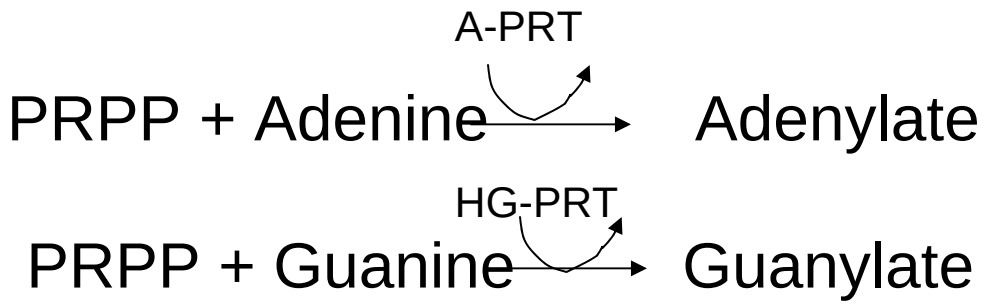


Salvage pathways for the re-utilization of purines;

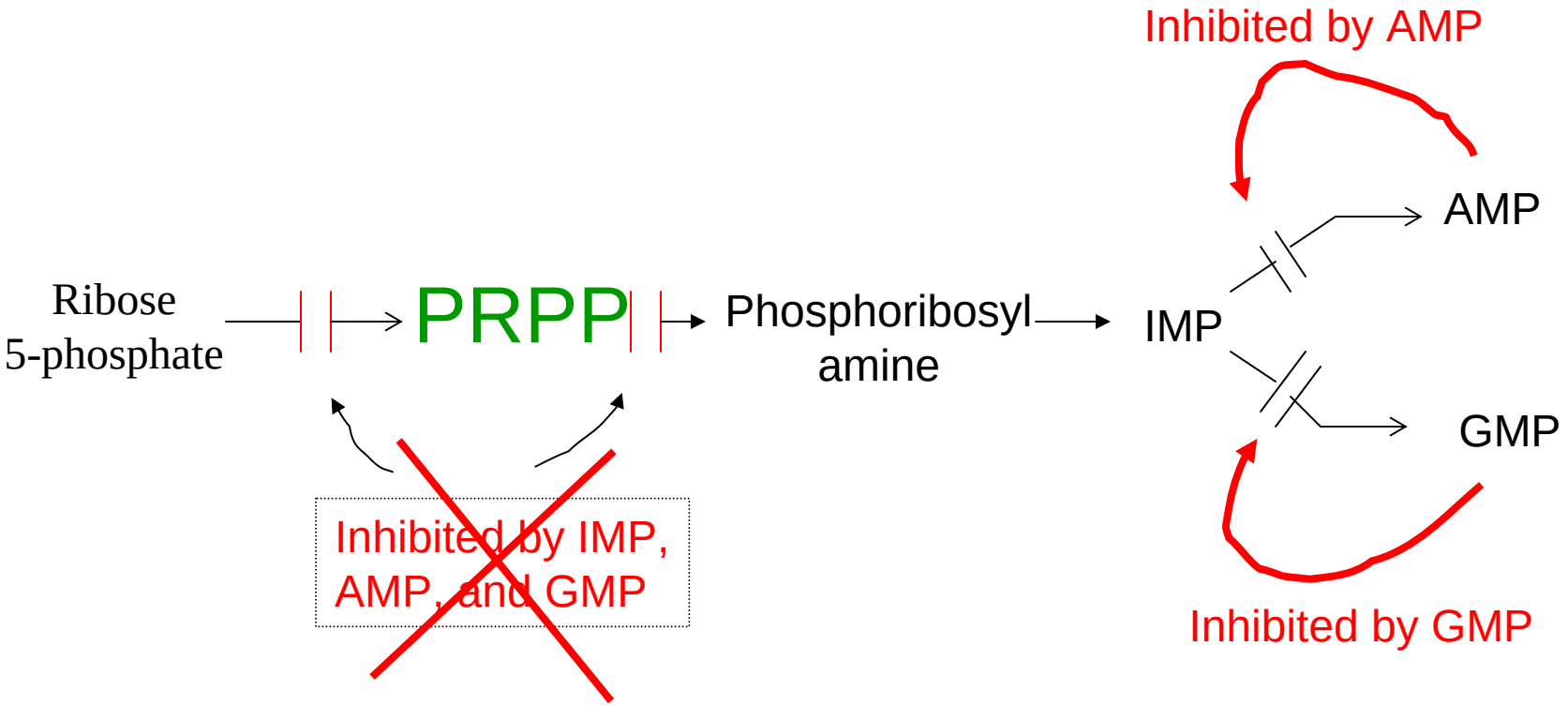
There are 2 salvage enzymes with different specificity;

1. Adenine phosphoribosyl transferase
2. Hypoxanthine-guanine phosphoribosyl transferase





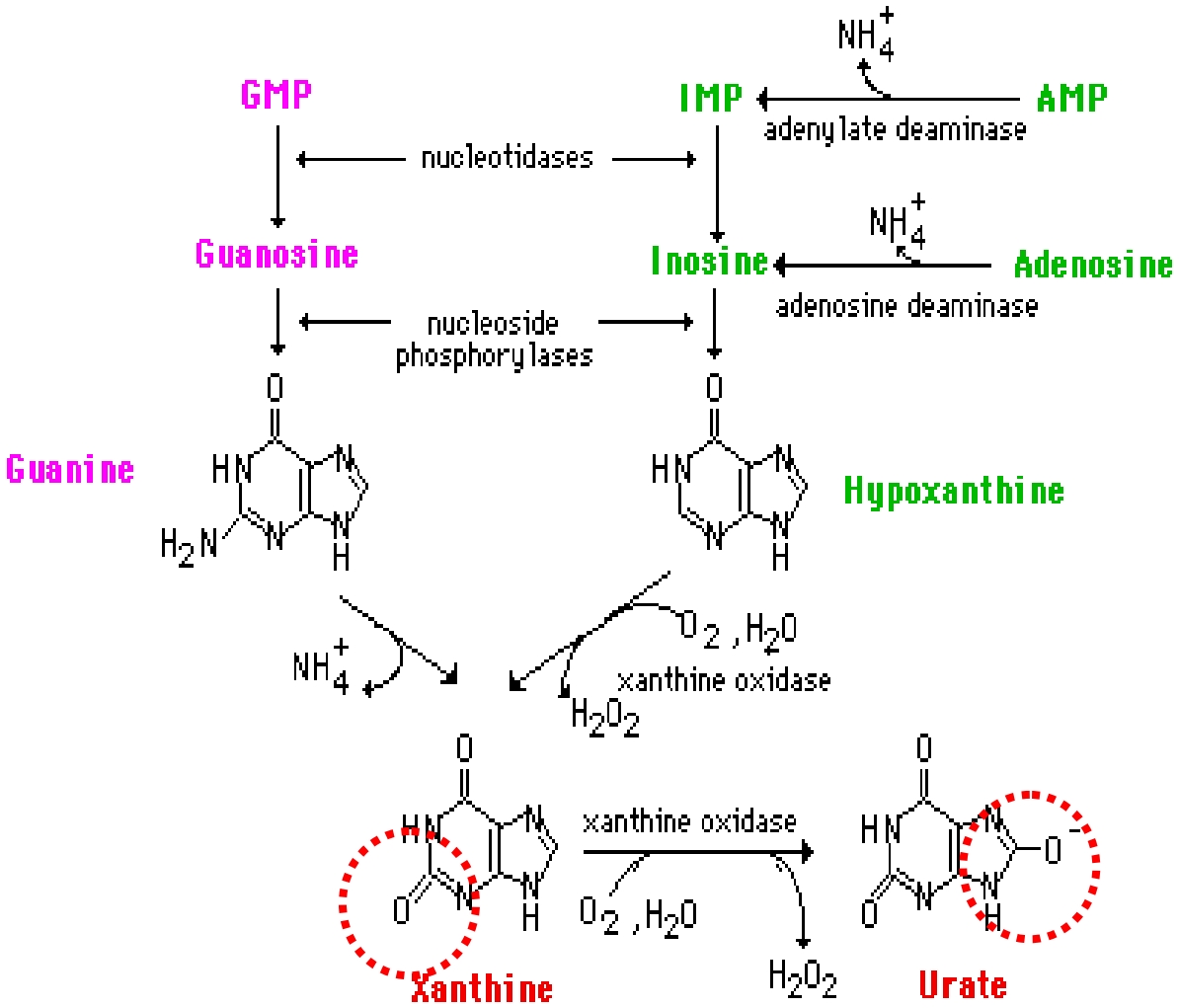
What happens in gout?



1. Negative regulation of PRPP Synthatase & PRPP Amidotransferase is lost
2. PRPP levels are increased because of defects in salvage pathways

Therefore, there is net increase in biosynthetic/degradation pathways!!

Purines in humans are degraded to Urate



Important points:

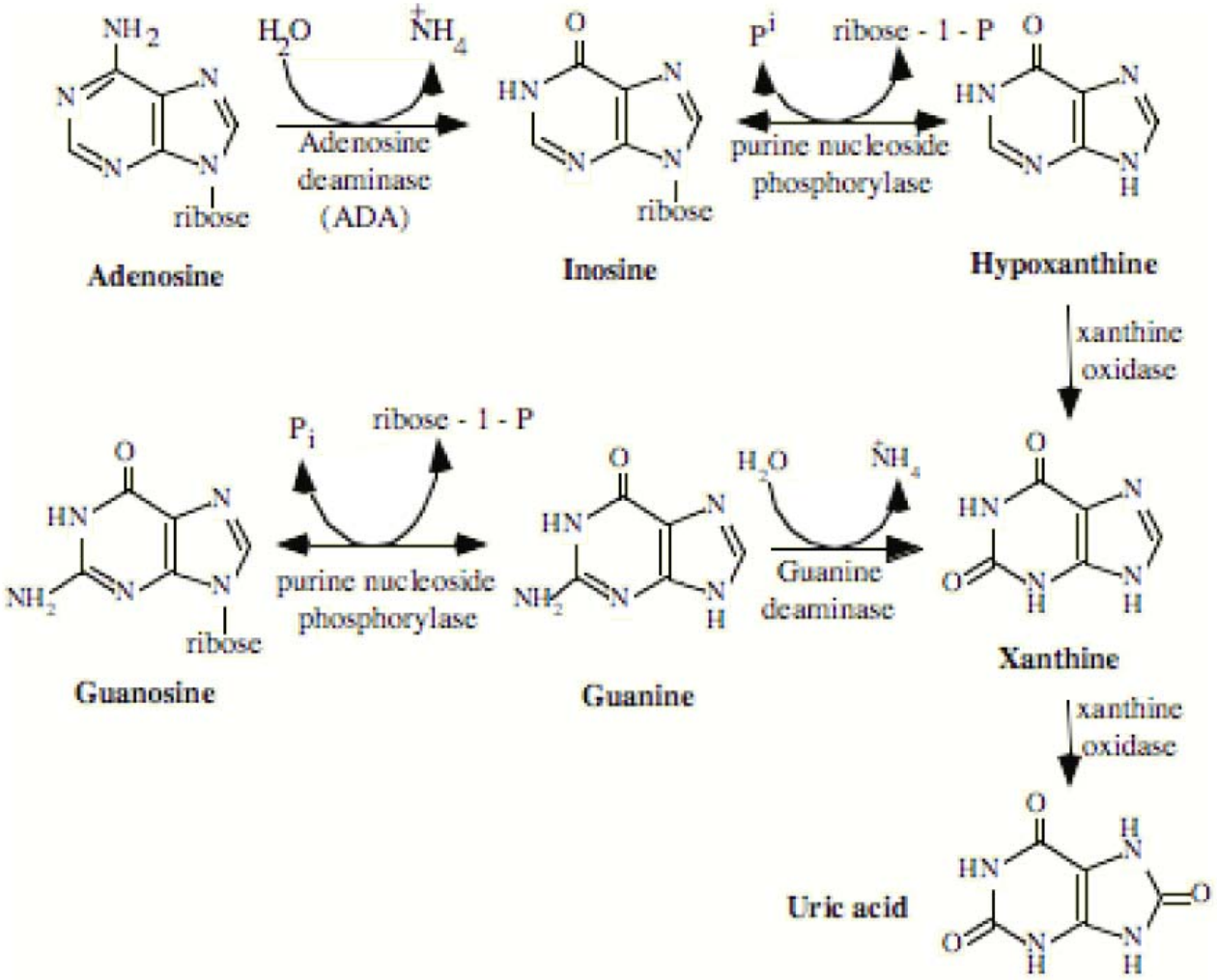
1. **Nucleotides are constantly undergoing turnover!**

2. **There are many enzymes involved;**
   Nucleotidases
   Nucleoside phosphorylases
   Deaminases
   Xanthine oxidases

3. **the final common intermediate in humans is Urate, which is excreted.**

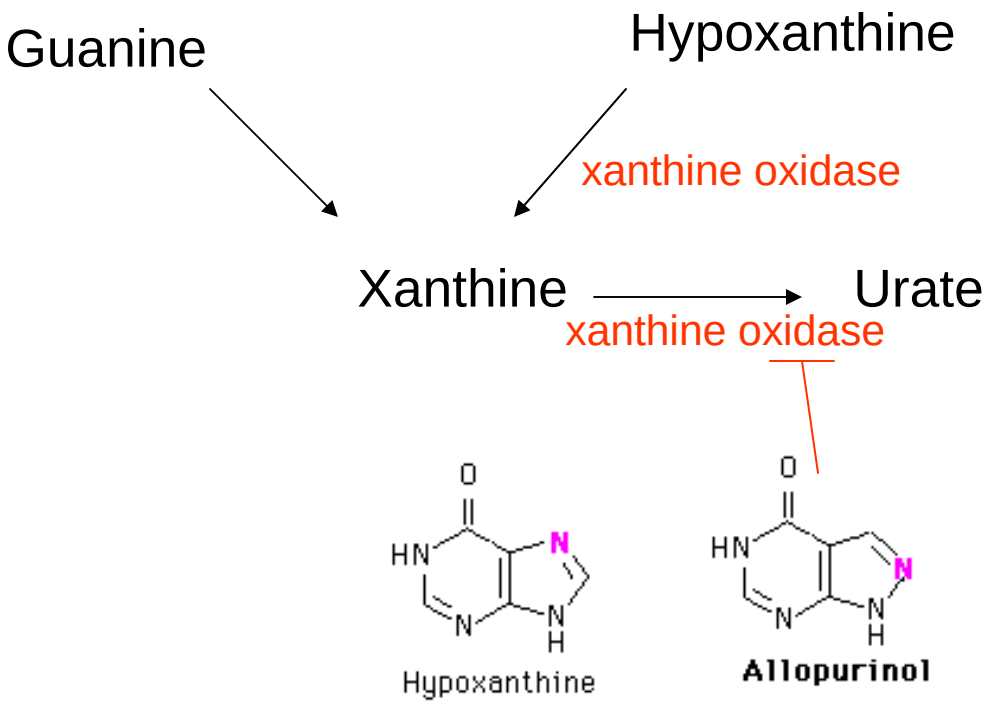
4. **there are several metabolic disorders resulting from defects in purine catabolism.**

Catabolism of Adenosine and Guanosine to Uric acid



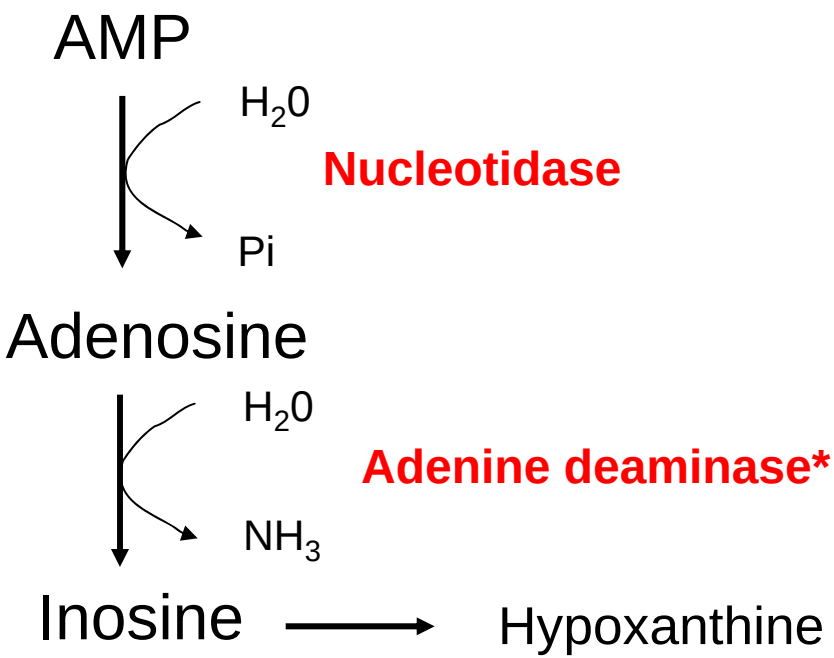
GOUT (Gouty Arthritis): A defect of purine metabolism

Serum Uric Acid Levels (mg/dl)	Incidence of Gout (% of cases)
>9.0	**~10%**
7-9	**0.5-3.5%**
<7.0	**0.1%**



Allopurinol:
a. decrease urate
b. increase xanthine & hypoxanthine
c. decrease PRPP

SCID-Severe Combined Immunodeficiency Syndrome



Autosomal recessive disorder
Mutations in ADA

Infants subject to bacterial, candidiasis, viral, protazoal infections

Both T and B cells are significantly reduced (dATP is toxic)

1995-AdV expressing ADA was successfully employed as gene therapy strategy

Disorders of Purine Metabolism:

Disorder	Defect	Comments
Gout	PRPP synthase/ HGPRT	Hyperuricemia
Lesch Nyhan syndrome	lack of HGPRT	Hyperuricemia
SCID	ADA	High levels of dAMP
von Gierke's disease	glucose -6-PTPase	Hyperuricemia