Nucleotide Metabolism

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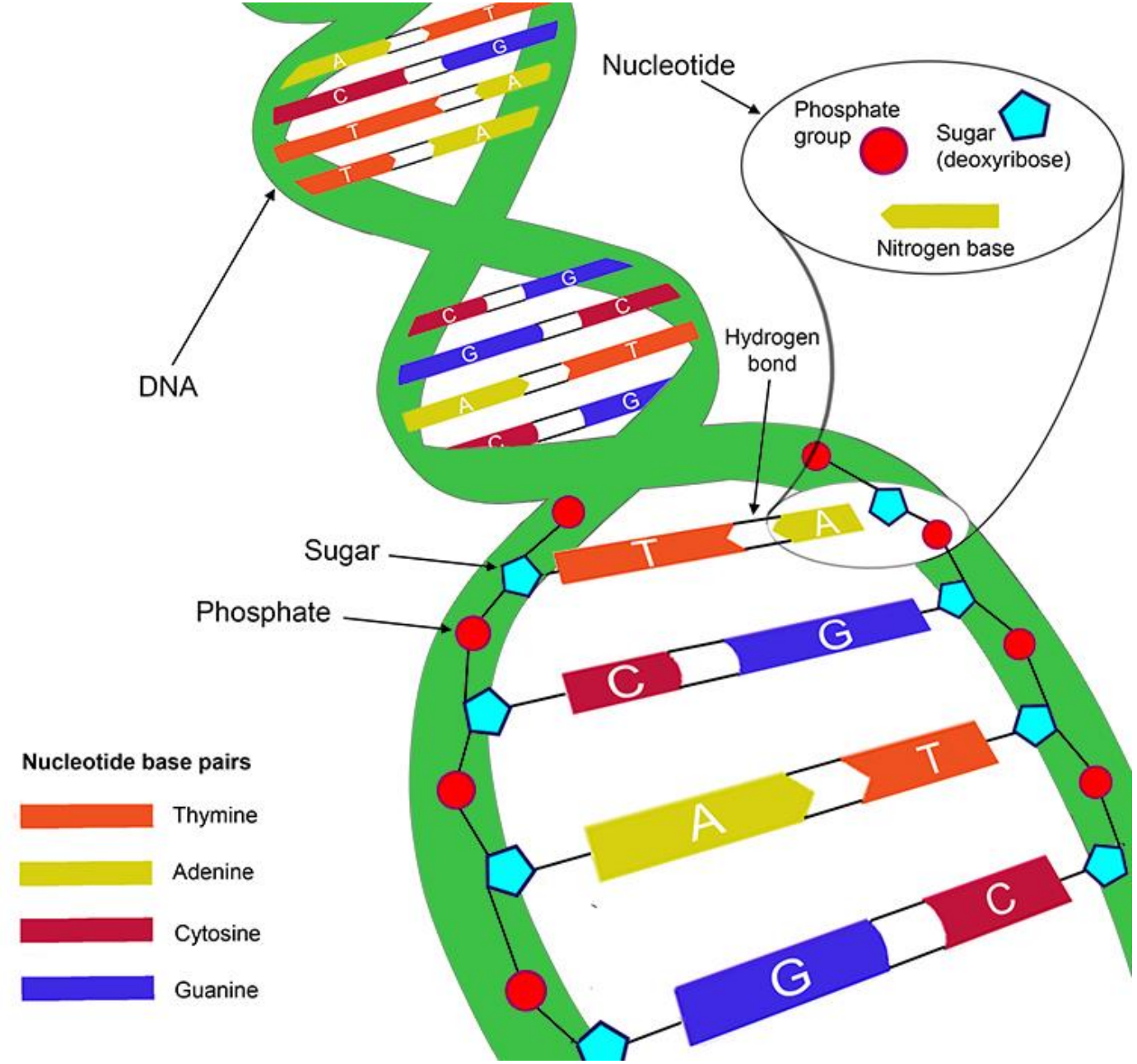
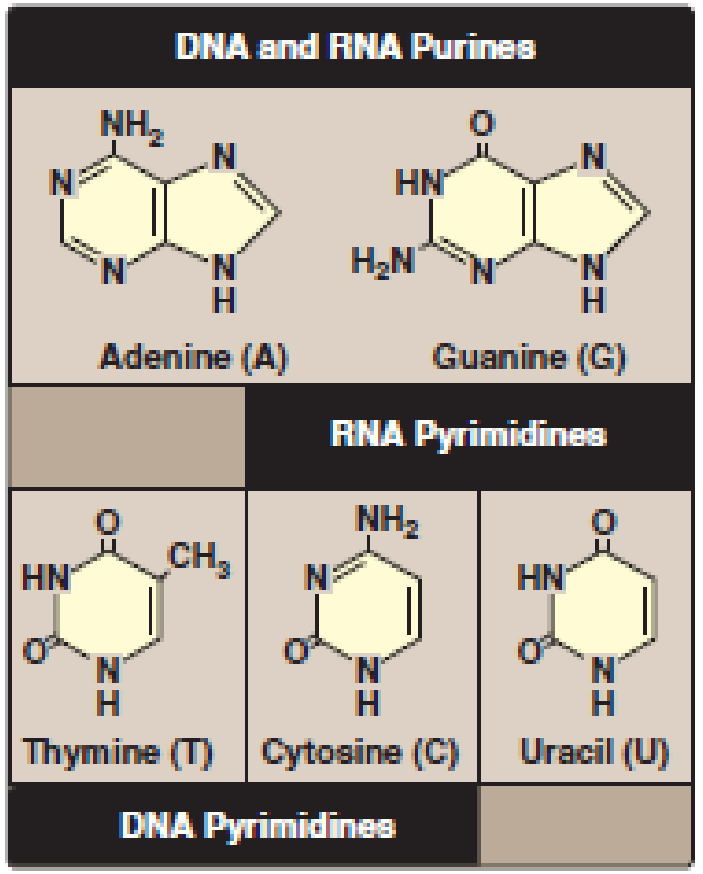


All images were taken from Lippincott's Biochemistry textbook except where noted

(mammothmemory.net)

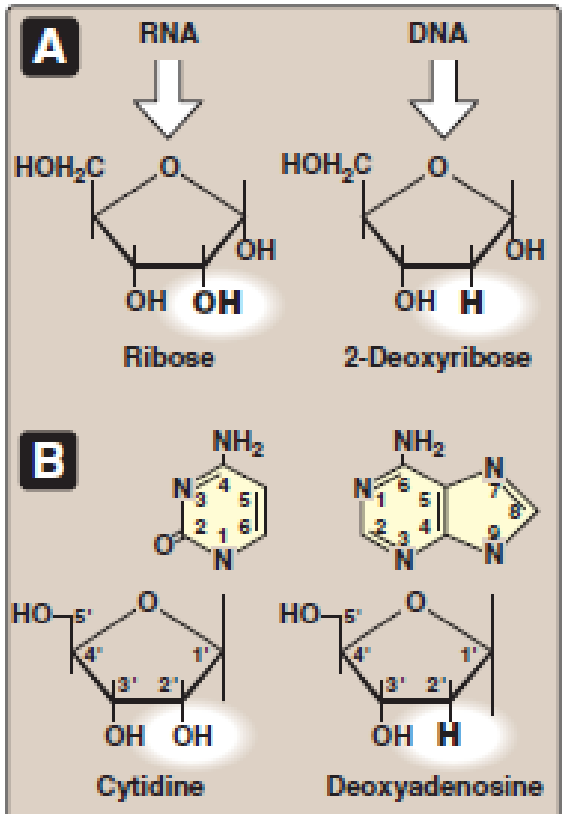
Purine and pyrimidine structures and roles

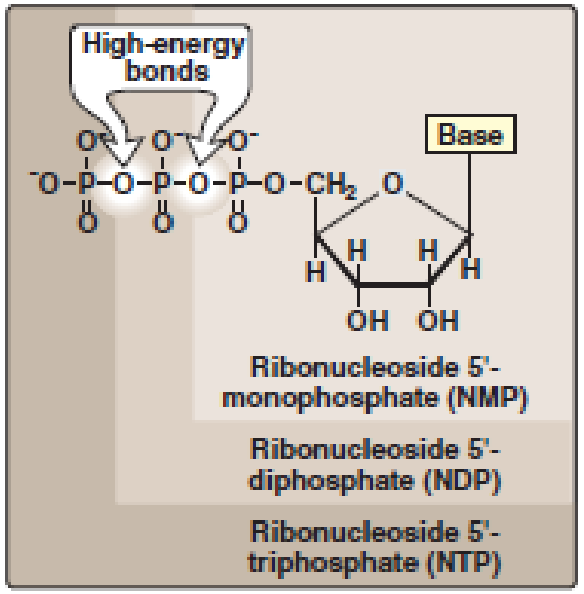
- Essential for RNA and DNA synthesis.
- They serve as carriers of activated intermediates in the synthesis of some carbohydrates, lipids, and conjugated proteins, such as, UDP-glucose and CDP-choline
- They are structural components of several essential coenzymes, such as coenzyme A, FAD, NAD+, and NADP+.
- They serve as second messengers in signal transduction pathways, such as cAMP and cGMP
- They are “energy currency” in the cell.
- They act as regulatory compounds for many metabolic pathways by inhibiting or activating key enzymes.



Nucleosides vs Nucleotides

- ✓ Nucleoside= Pentose sugar + Base
- ✓ Ribose + base = Ribonucleoside (adenosine, guanosine, cytidine, and uridine)
- ✓ 2-deoxyribose + base = deoxyribonucleoside (deoxyadenosine, deoxyguanosine, deoxycytidine, and deoxythymidine)
- ✓ Nucleoside + one or more phosphate groups= Nucleotide
- ✓ The first P group is attached by an ester linkage to the
- ✓ 5'-OH of the pentose forming a nucleoside 5'-phosphate or a 5'-nucleotide.
- ✓ The second and third phosphates are each connected to the nucleotide by a "high-energy" bond.
- ✓ The phosphate groups are negatively charged causing DNA and RNA to be nucleic acids.





Sources of purines and pyrimidines

- The purine and pyrimidine bases can be synthesized de novo
- Or can be obtained through salvage pathways (reuse of the preformed bases resulting from normal cell turnover).
- Little of the purines and pyrimidines supplied by diet are utilized, and are degraded instead

Purine synthesis- the contributing compounds

The atoms of the purine ring are contributed by a number of compounds:

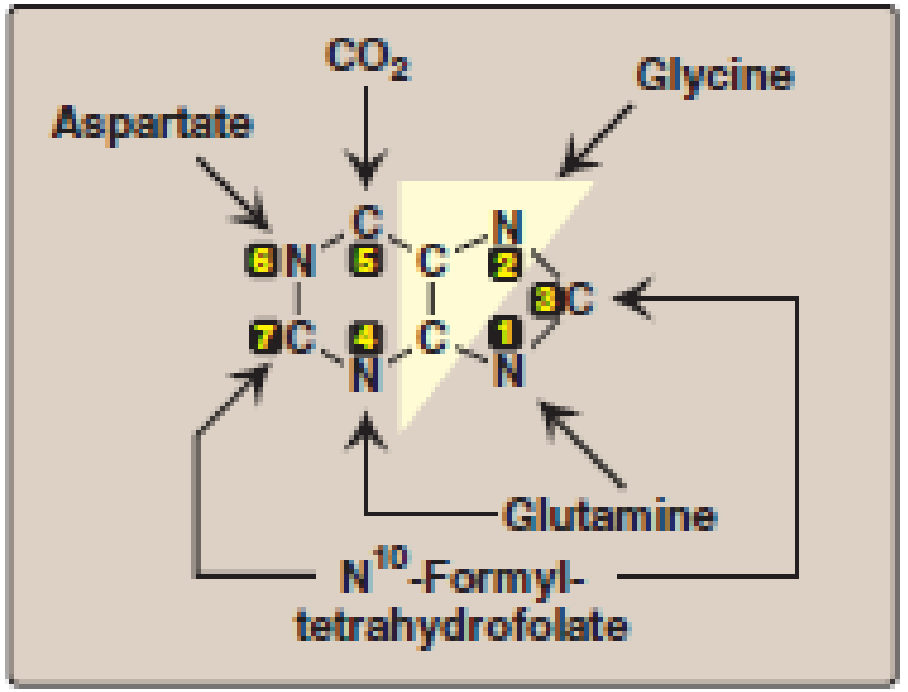
1. Amino acids (aspartic acid, glycine, and glutamine)

2. CO2

3. N10–formyltetrahydrofolate

The purine ring is constructed primarily in the liver by a series of reactions that add the donated carbons and nitrogens to a preformed ribose 5-phosphate.

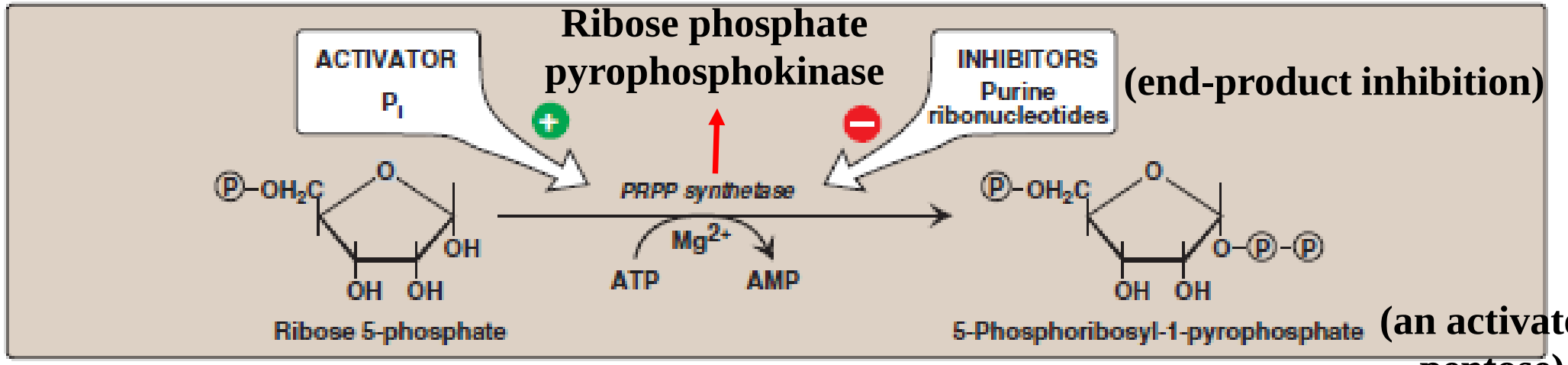
Ribose 5-phosphate is synthesized by the pentose phosphate pathway



Sources of the individual atoms in the purine ring. The order in which the atoms are added is shown by the numbers in the black boxes

Synthesis of Purine Nucleotides

Steps:



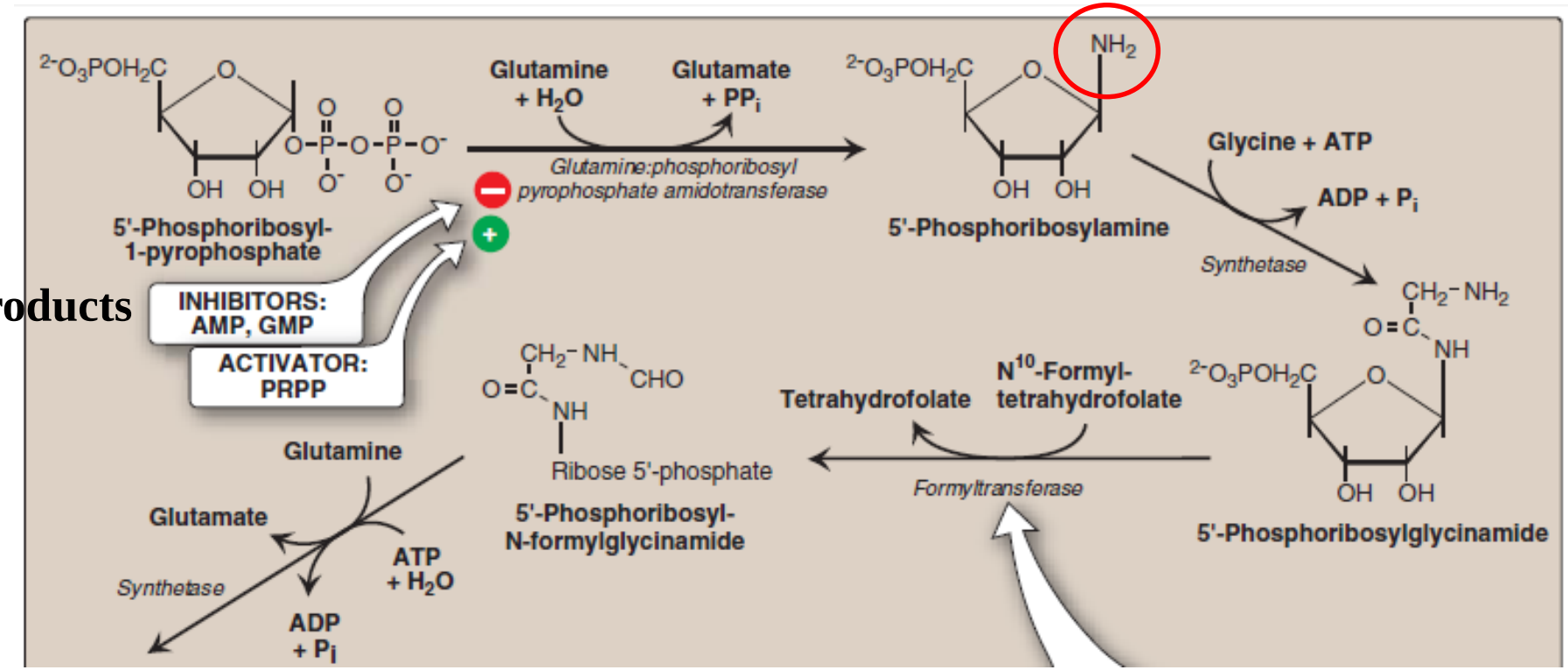
1. Synthesis of 5-phosphoribosyl-1-pyrophosphate (PRPP)

The sugar moiety of PRPP is ribose, therefore, ribonucleotides are the end products of de novo purine synthesis.

When deoxy ribonucleotides are required for DNA synthesis, the ribose sugar moiety is reduced

Synthesis of Purine Nucleotides

End products



Steps:

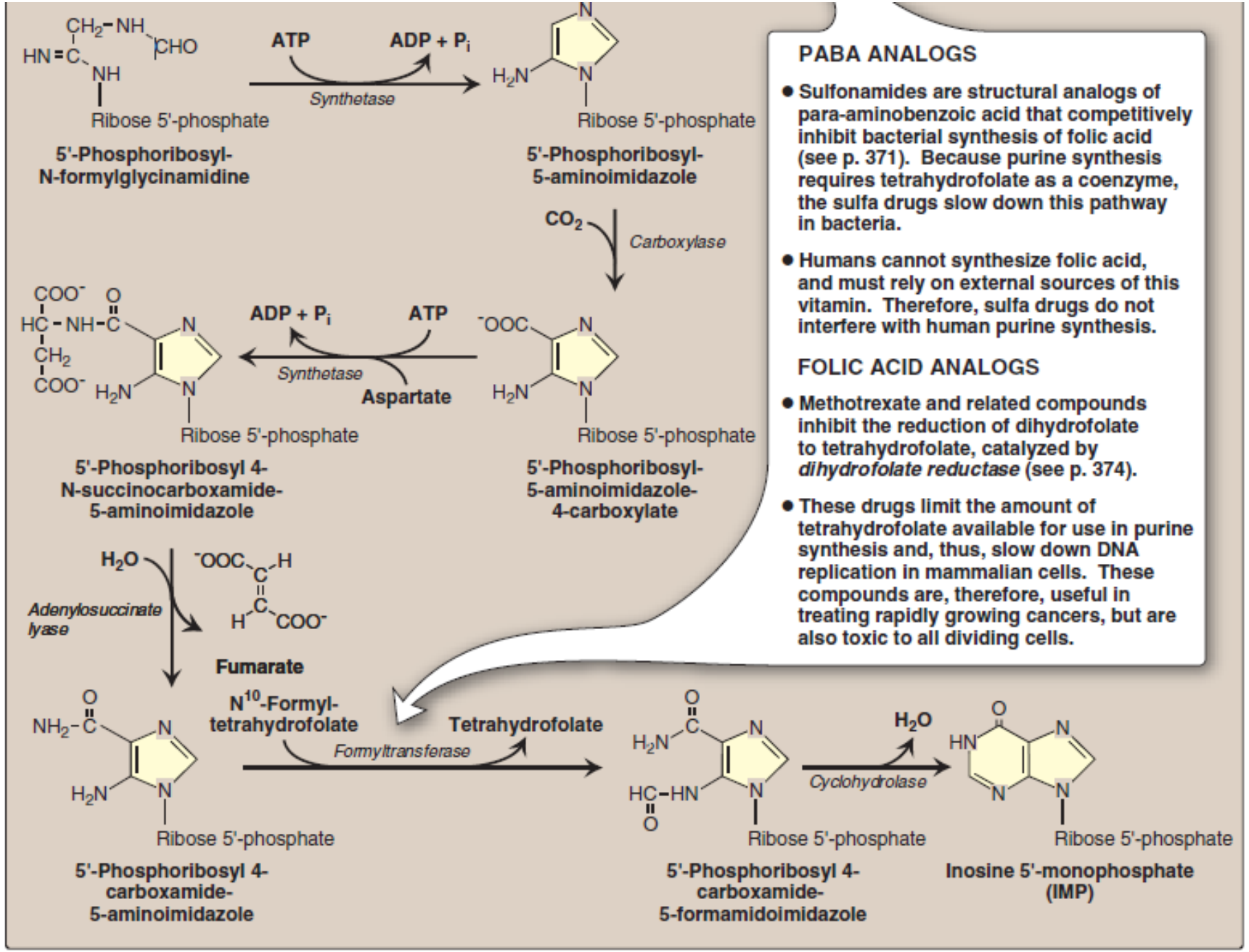
2. Synthesis of 5'-phosphoribosylamine (the committed step in purine nucleotide biosynthesis).

3. Synthesis of inosine monophosphate, the "parent" purine nucleotide

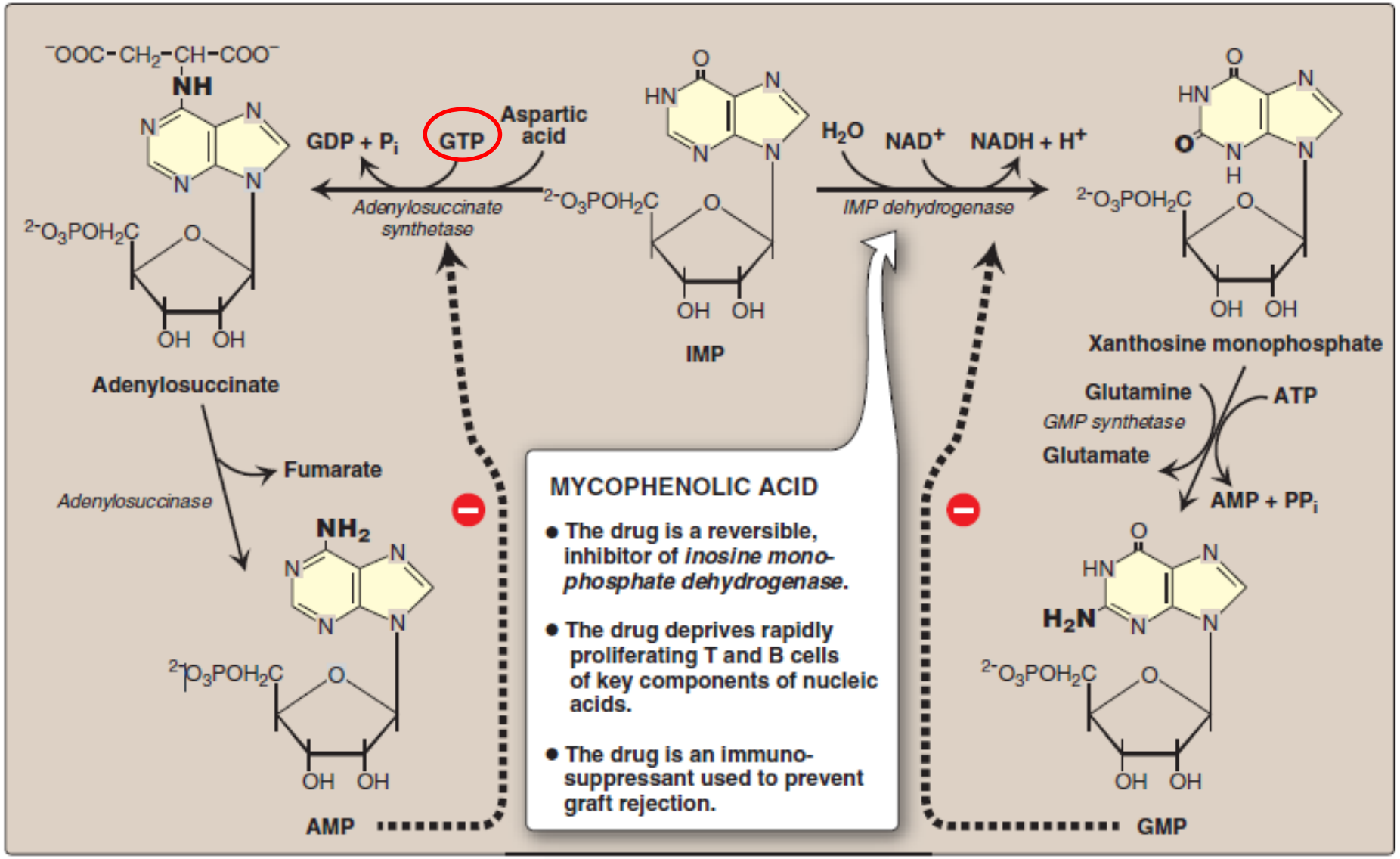
The next nine steps lead to the synthesis of IMP, whose base is hypoxanthine

This pathway requires ATP as an energy source.

Synthesis of Purine Nucleotides

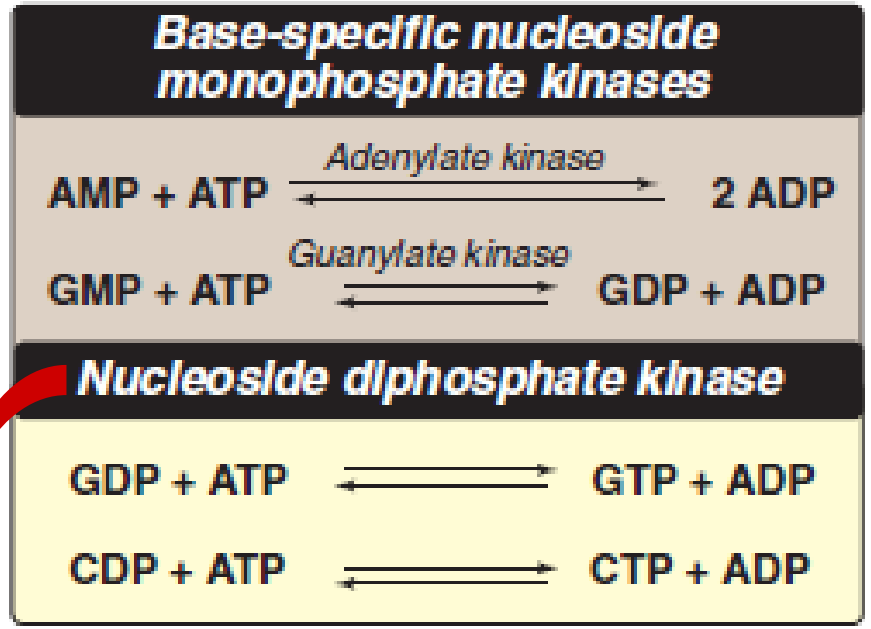


Synthesis of Purine Nucleotides
4. Conversion of IMP to AMP and GMP



Synthesis of Purine Nucleotides

5. Conversion of nucleoside monophosphates to nucleoside diphosphates and triphosphates



Broad specificity not like the monophosphate kinases

Base-specific nucleoside monophosphate kinases do not discriminate between ribose or deoxyribose in the substrate

ATP is the general source of the phosphate, since it is present in higher concentrations than the other nucleoside triphosphates.

Adenylate kinase (AK) is particularly active in liver and muscle

AK maintains an equilibrium among AMP, ADP, and ATP

Application: Synthetic inhibitors of purine synthesis

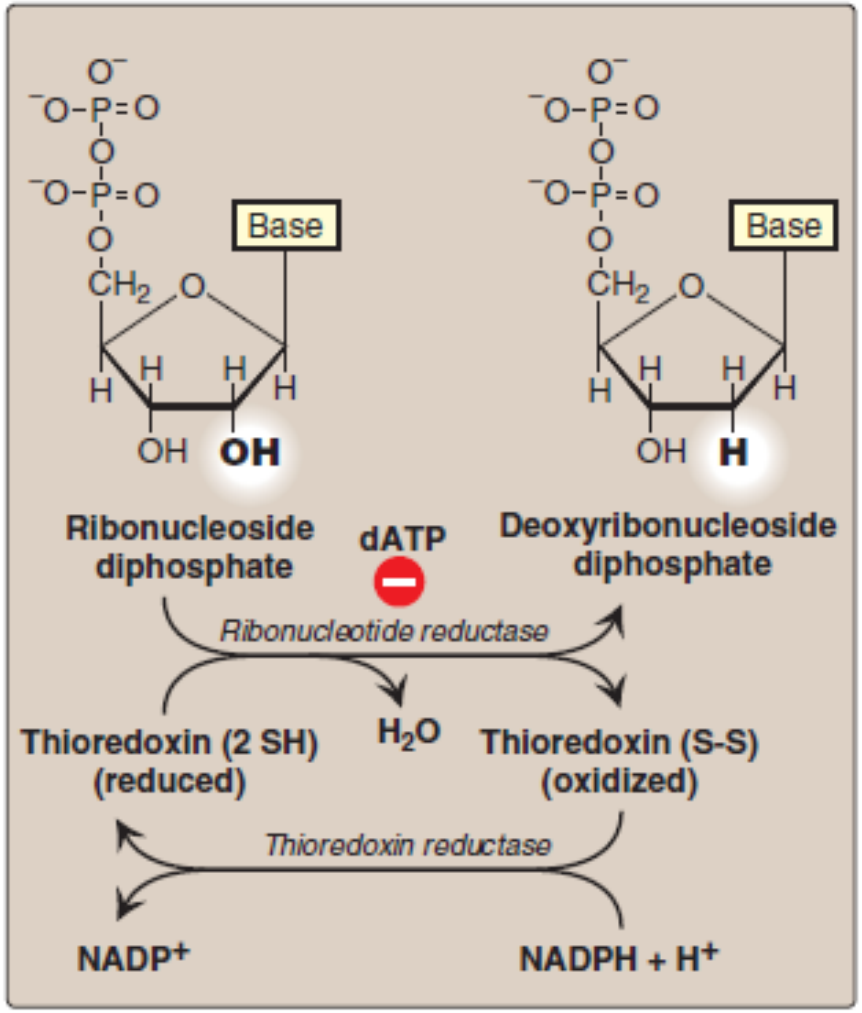
Synthetic inhibitors of purine synthesis (the sulfonamides1), are designed to inhibit the growth of rapidly dividing microorganisms without interfering with human cell functions

Other purine synthesis inhibitors, such as structural analogs of folic acid (such as, methotrexate2), are used as drugs that control the spread of cancer by interfering with the synthesis of nucleotides and, therefore, of DNA and RNA.

Inhibitors of human purine synthesis are extremely toxic to tissues, especially to developing structures such as in a fetus, or to cell types that normally replicate rapidly, including those of bone marrow, skin, GI tract, immune system, or hair follicles.

Thus, anticancer drugs result in adverse effects, including anemia, scaly skin, GI tract disturbance, immunodeficiencies, and hair loss.

Synthesis of Deoxyribonucleotides



2'-deoxyribonucleotides are produced from ribonucleoside diphosphates by the enzyme ribonucleotide reductase during the S-phase of the cell cycle

The same enzyme acts on pyrimidine ribonucleotides

1. Ribonucleotide reductase (RR)

RR is specific for the reduction of:

A. Purine nucleoside diphosphates (ADP and GDP) to their deoxyforms (dADP and dGDP).

B. Pyrimidine nucleoside diphosphates, cytidine diphosphate (CDP) and uridine diphosphate (UDP) to their deoxyforms (dCDP, and dUDP).

2. Regeneration of reduced enzyme:

Thioredoxin—a peptide coenzyme of RR

3. Regeneration of reduced thioredoxin:

Thioredoxin must be converted back to its reduced form

NADPH + H+ are needed

The reaction is catalyzed by thioredoxin reductase

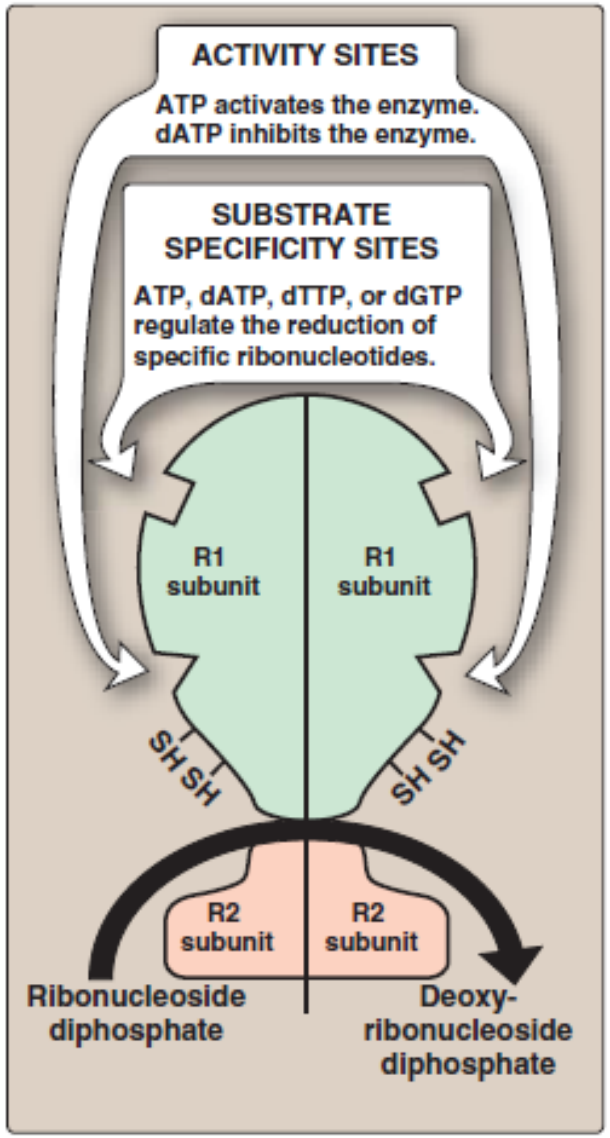
Regulation of deoxyribonucleotide synthesis

Ribonucleotide reductase is composed of two non identical dimeric subunits, R1 and R2

RR is responsible for maintaining a balanced supply of the deoxyribonucleotides required for DNA synthesis.

1. Activity sites (allosteric sites):
a. dATP inhibits the enzyme and prevents the reduction of any of the four nucleoside diphosphates resulting in preventing DNA synthesis.
b. ATP activates the enzyme.

2. Substrate specificity sites (allosteric sites):
Nucleoside triphosphates regulate substrate specificity, causing an increase in the conversion of different species of ribonucleotides to deoxyribonucleotides.
dTTP binding activates the reduction of GDP to dGDP at the catalytic site.



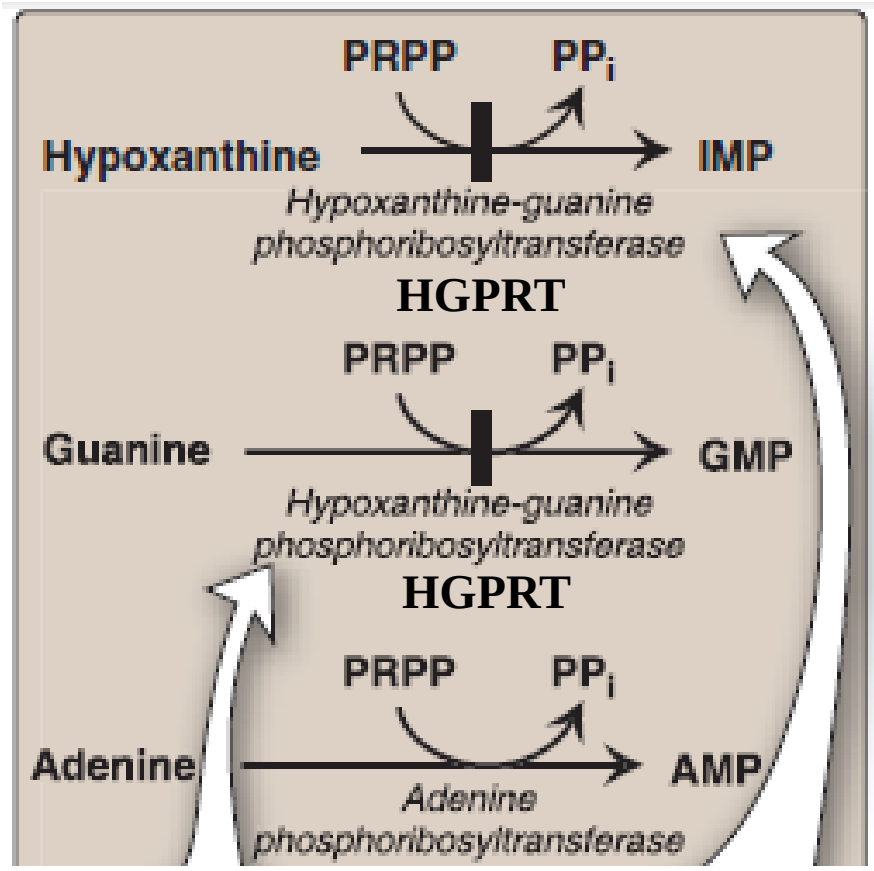
Application: Hydroxyurea and ribonucleotide reductase

The drug hydroxyurea destroys the free radical required for the activity of ribonucleotide reductase

Hydroxyurea inhibits the generation of substrates for DNA synthesis.

Hydroxyurea has been used in the treatment of cancers such as CML

Salvage pathway for purines



Salvage pathway for purines is purine synthesis from:

1. The normal turnover of cellular nucleic acids
2. Diet purines that are not degraded (small amounts)

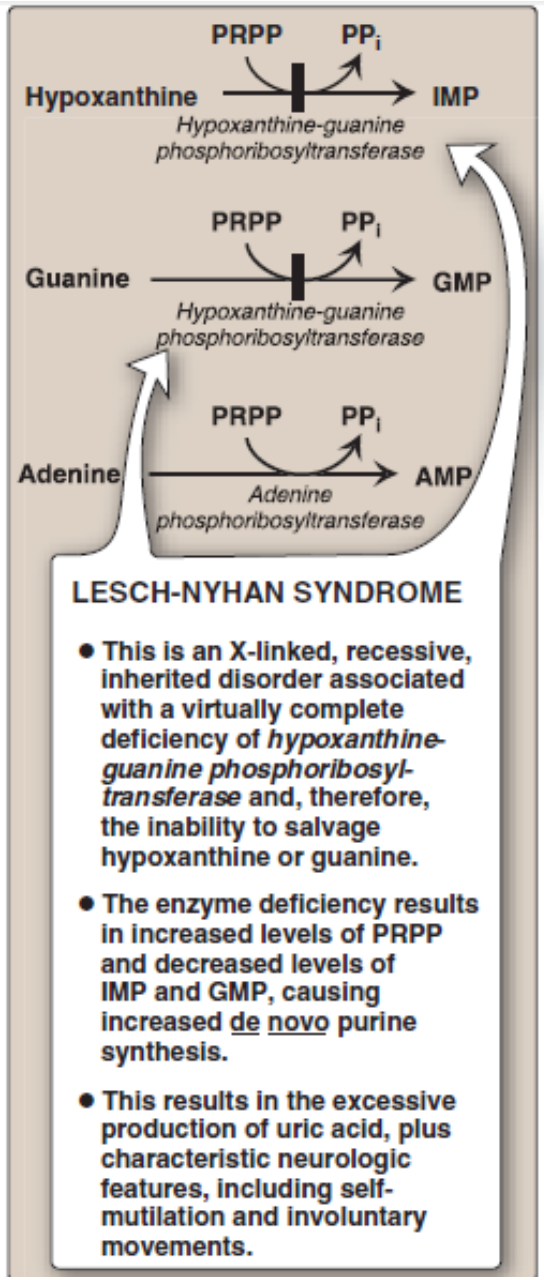
Conversion of purine bases to nucleotides:

Both APRT and HGPRT use PRPP as the source of the ribose 5-phosphate group.

PP is released and hydrolyzed by pyrophosphatase making these reactions irreversible.

Adenosine is the only purine nucleoside to be salvaged.
It is phosphorylated to AMP by adenosine kinase.

Application: Salvage pathway for purines- Lesch-Nyhan syndrome



- A rare, X-linked, recessive
- HGPRT deficiency.
- **Inability to salvage hypoxanthine or guanine** resulting in high amounts of uric acid (the end product of purine degradation)

- Increased PRPP levels and decreased IMP and GMP levels.
- The committed step in purine synthesis has excess substrate and decreased inhibitors available, and **de novo purine synthesis is increased.**

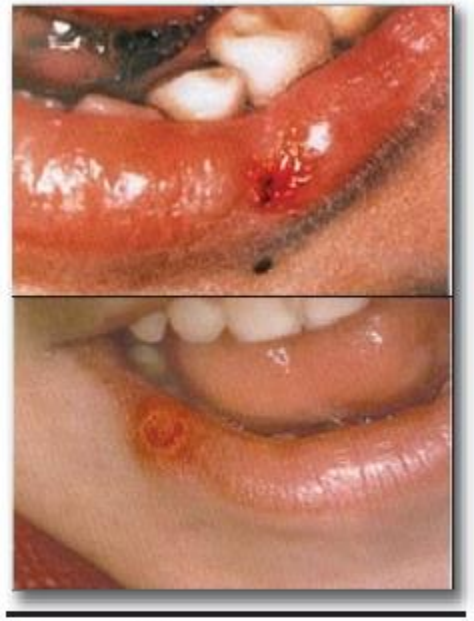


Figure 22.11
Lesions on the lips of Lesch-Nyhan patients caused by self-mutilation.

- The decreased purine reutilization and increased purine synthesis results in increased degradation of purines and the production of large amounts of uric acid (hyperuricemia)

- Hyperuricemia results in uric acid stones in the kidneys (urolithiasis) and the deposition of urate crystals in the joints (gouty arthritis) and soft tissues.

- The syndrome is characterized by motor dysfunction, cognitive deficits and behavioral disturbances that include self-mutilation (biting of lips and fingers)

Degradation of Purine Nucleotides

A. Degradation of dietary nucleic acids in the small intestine

-Ribonucleases and deoxyribonucleases, secreted by the **pancreas**, hydrolyze dietary RNA and DNA to oligonucleotides.

-Oligonucleotides are further hydrolyzed by **pancreatic** phosphodiesterases, producing a mixture of 3'- and 5'-mononucleotides.

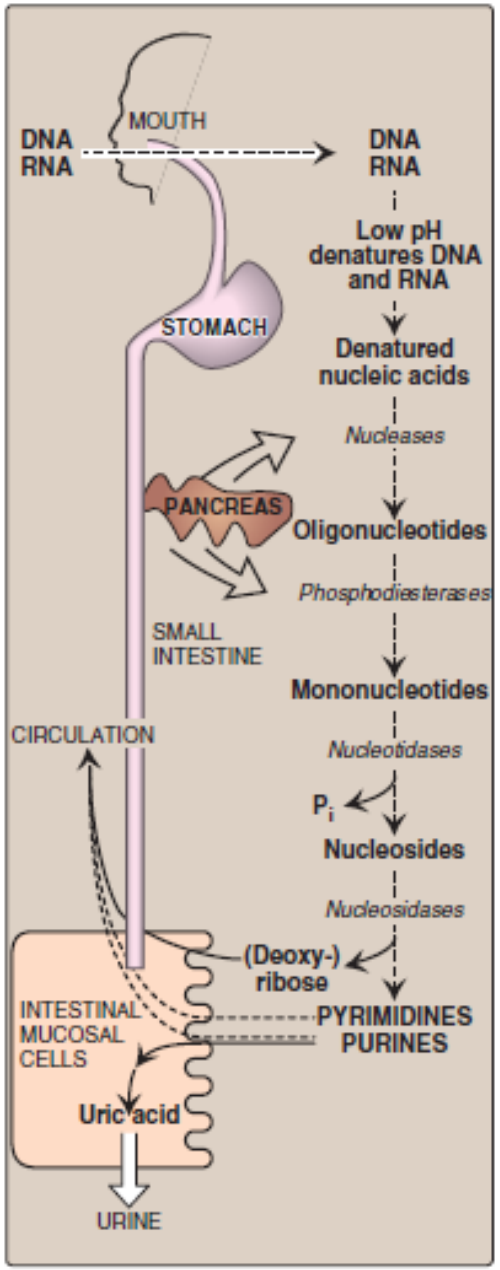
-In the intestinal mucosal cells, nucleotidases remove the phosphate groups hydrolytically, releasing nucleosides that are further degraded to free bases.

-Dietary purine bases are not an appreciable source for the synthesis of tissue nucleic acids.

-Dietary purines are generally converted to uric acid (excreted in urine) in intestinal mucosal cells.

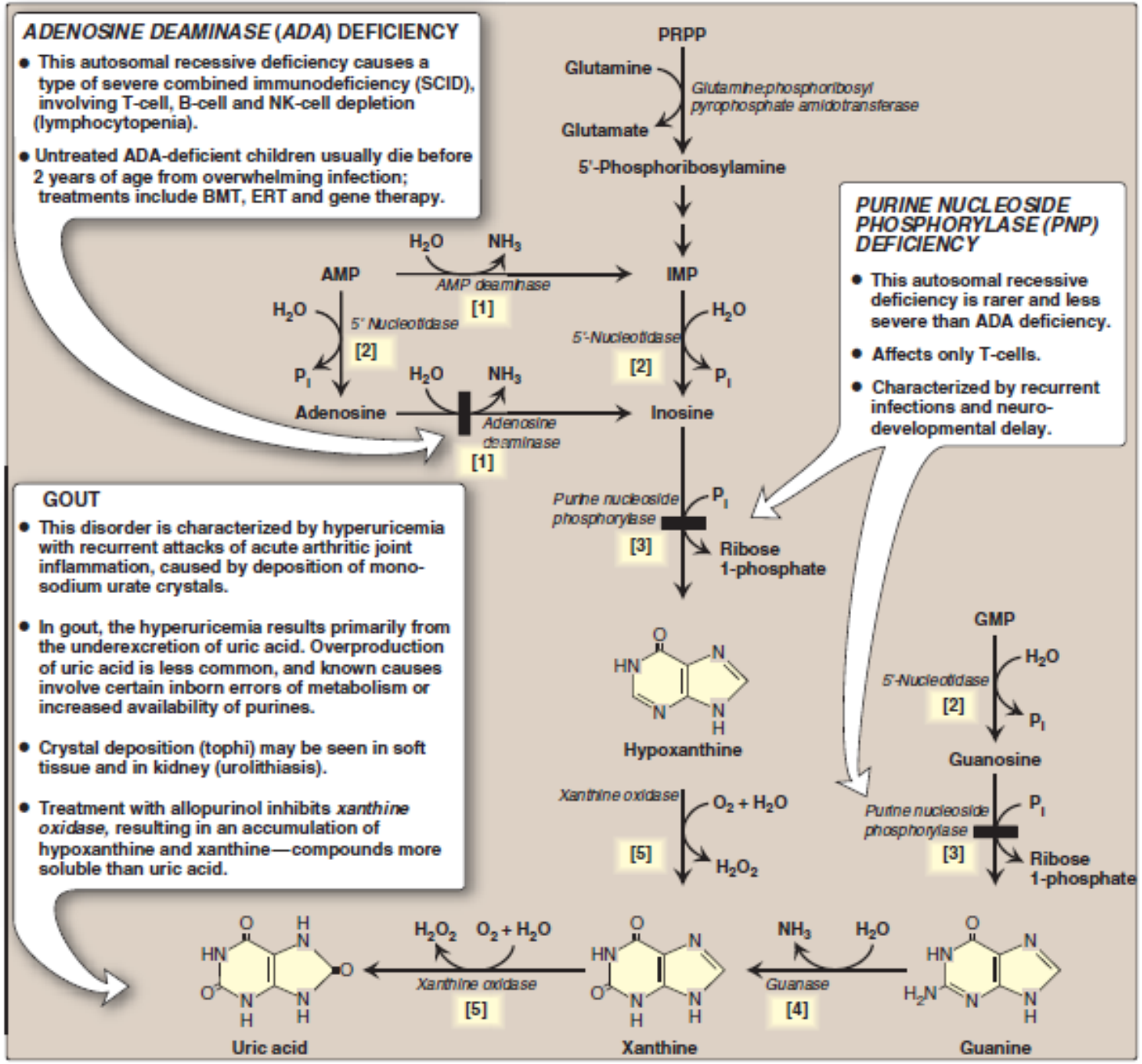
-Purine nucleotides <u>from de novo synthesis</u> are degraded in the <u>liver</u> primarily.

-The free bases are sent out from liver and salvaged by peripheral tissues



Degradation of Purine Nucleotides

[**1**] An amino group is removed from AMP to produce IMP by AMP deaminase, or from adenosine to produce inosine (hypoxanthineribose) by adenosine deaminase.
[**2**] IMP and GMP are converted into their nucleoside forms—inosine and guanosine—by the action of 5'-nucleotidase.
[**3**] Purine nucleoside phosphorylase converts inosine and guanosine into their respective purine bases, hypoxanthine and guanine.
Note: A mutase interconverts ribose 1- and ribose 5-phosphate.
[**4**] Guanine is deaminated to form xanthine.
[**5**] Hypoxanthine is oxidized by xanthine oxidase to xanthine, which is further oxidized by xanthine oxidase to uric acid, the final product of human purine degradation.



Application: Diseases associated with purine degradation

- ✓ **Gout:** high levels of uric acid in blood (hyperuricemia)
- ✓ Hyperuricemia due to either the overproduction or underexcretion of uric acid.
- ✓ Hyperuricemia lead to the deposition of monosodium urate crystals in the joints, leading to inflammation, causing first acute and then chronic gouty arthritis.
- ✓ Nodular masses of monosodium urate crystals (tophi) may be deposited in the soft tissues, resulting in chronic tophaceous gout

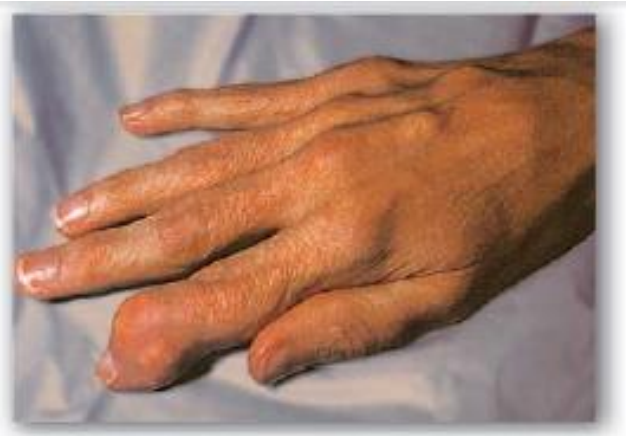


Figure 22.16
Tophaceous gout.

- ✓ Formation of uric acid stones in the kidney (urolithiasis)
- ✓ Hyperuricemia is typically asymptomatic and does not lead to gout, but gout is preceded by hyperuricemia.
- ✓ Diagnosis requires aspiration and examination of synovial fluid from an affected joint (or material from a tophus) using polarized light microscopy to confirm the presence of needle-shaped monosodium urate crystals

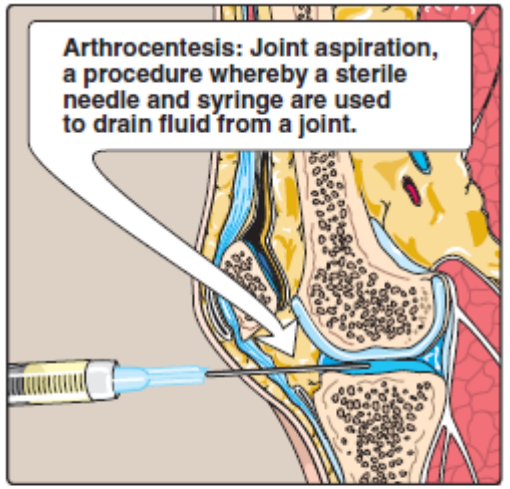


Figure 22.17
Analysis of joint fluid can help to define causes of joint swelling or arthritis, such as infection, gout, and rheumatoid disease.

Figure 22.18
Gout can be diagnosed by the presence of negatively birefringent monosodium urate crystals in aspirated synovial fluid examined by polarized-light microscopy. Here, crystals are within poly-morphonuclear leukocytes.

- ✓ **Causes of hyperuricemia**

1. Underexcretion of uric acid (in most gout patients)

-Primary (due to unidentified inherent excretory defects)

-Or secondary to: a. A known disease that affects the kidney function in handling urate, such as lactic acidosis (lactate and urate compete for the same renal transporter), b. Environmental factors such as drugs (thiazide diuretics), c. Exposure to lead (saturnine gout)

2. Overproduction of uric acid: less common.

Several identified mutations in the X-linked PRPP synthetase gene that increase PRPP production

Pyrimidine Synthesis

The pyrimidine ring is synthesized before being attached to ribose 5-phosphate

Ribose 5-phosphate is donated by PRPP.

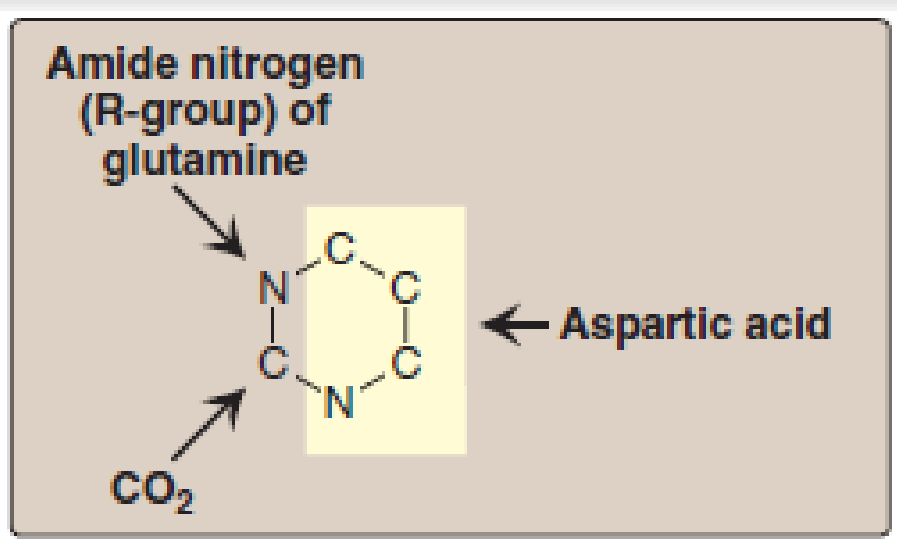


Figure 22.19
Sources of the individual atoms in the pyrimidine ring.

Pyrimidine Synthesis

A. Synthesis of carbamoyl phosphate

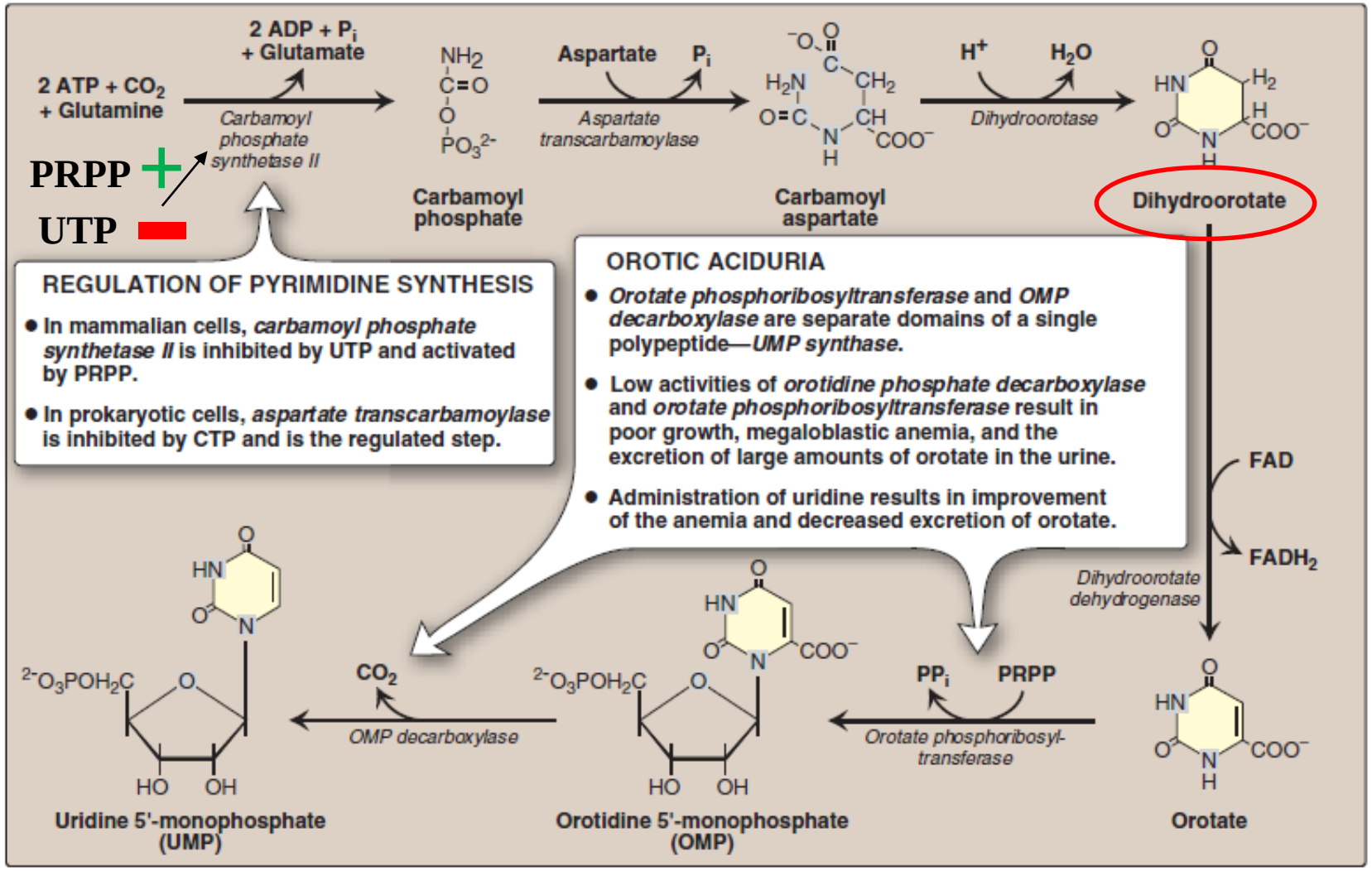
-The regulated step of this pathway in mammalian cells is the synthesis of carbamoyl phosphate from glutamine and CO2

B. Synthesis of orotic acid

-The enzyme that produces orotate, dihydroorotate dehydrogenase, is associated with the **inner mitochondrial membrane**.

-All other enzymes in pyrimidine biosynthesis are **cytosolic**.

-The first three enzymic activities in this pathway (**CPS II, aspartate transcarbamoylase, and dihydroorotase**) are three different catalytic domains of a single polypeptide chain



CPS I Versus CPS II

Carbamoyl phosphate, which is synthesized by CPS I, is a precursor of urea.

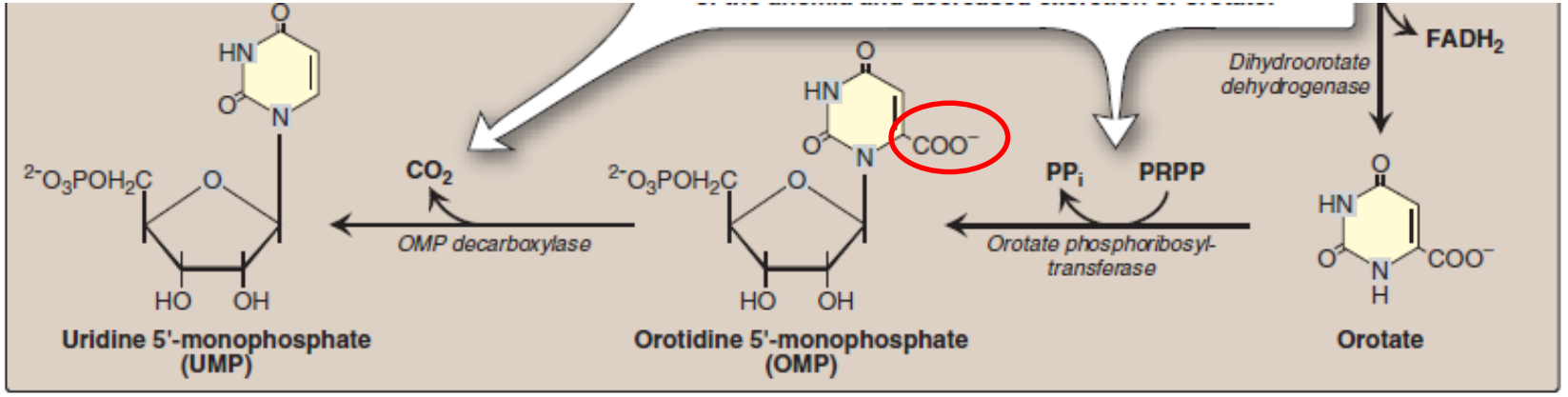
Defects in ornithine transcarbamylase of the urea cycle promote pyrimidine synthesis due to increased availability of carbamoyl phosphate.

	CPS I	CPS II
Cellular location	Mitochondria	Cytosol
Pathway involved	Urea cycle	Pyrimidine synthesis
Source of nitrogen	Ammonia	γ-Amide group of glutamine
Regulators	Activator: N–acetyl-glutamate	Activator: PRPP Inhibitor: UTP

Pyrimidine Synthesis

C. Formation of a pyrimidine nucleotide

-The completed pyrimidine ring is converted to the nucleotide orotidine 5'-monophosphate (OMP), or the parent pyrimidine mononucleotide.



-The reaction releases pyrophosphate, thus, it is irreversible.

-Both purine and pyrimidine synthesis require Gln, Asp, and PRPP as essential precursors.

-Orotate phosphoribosyl transferase and orotidylate decarboxylase are catalytic domains of a single polypeptide chain called UMP synthase.

-UMP is phosphorylated to UDP and then UTP.

-The UDP is a substrate for ribonucleotide reductase, which generates dUDP.

-The dUDP is phosphorylated to dUTP, which is rapidly hydrolyzed to dUMP by UTP diphosphatase (dUTPase).

-dUTPase reduces the available dUTP for DNA synthesis, thus preventing incorporation of uracil into DNA.

Pyrimidine Synthesis

D. Synthesis of UTP and cytidine triphosphate (CTP)

Some CTP is dephosphorylated to CDP (a substrate for ribonucleotide reductase)

dCDP can be phosphorylated to dCTP for DNA synthesis.

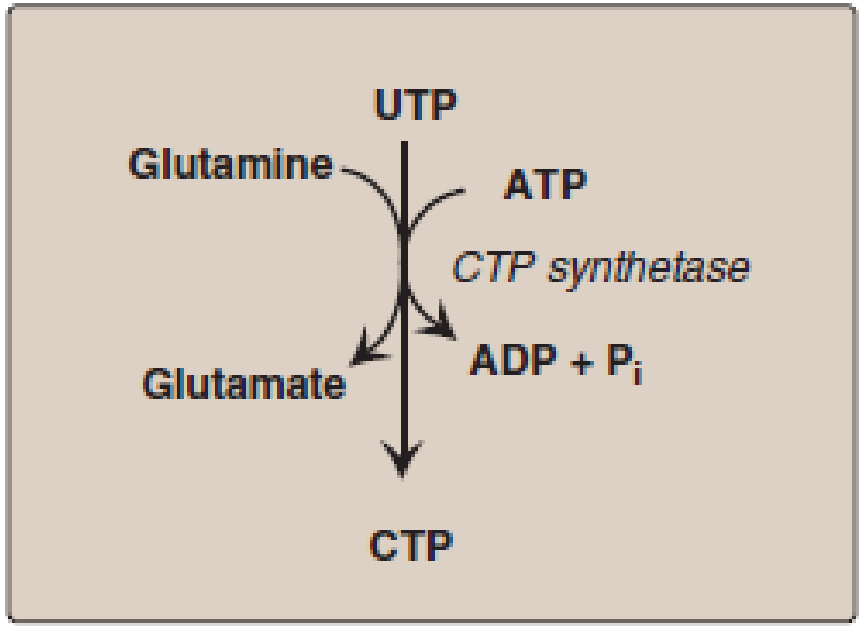


Figure 22.22
Synthesis of CTP from UTP. [Note: CTP, required for RNA synthesis, is converted to dCTP for DNA synthesis.]

Pyrimidine Synthesis

E. Synthesis of thymidine monophosphate (TMP) from dUMP

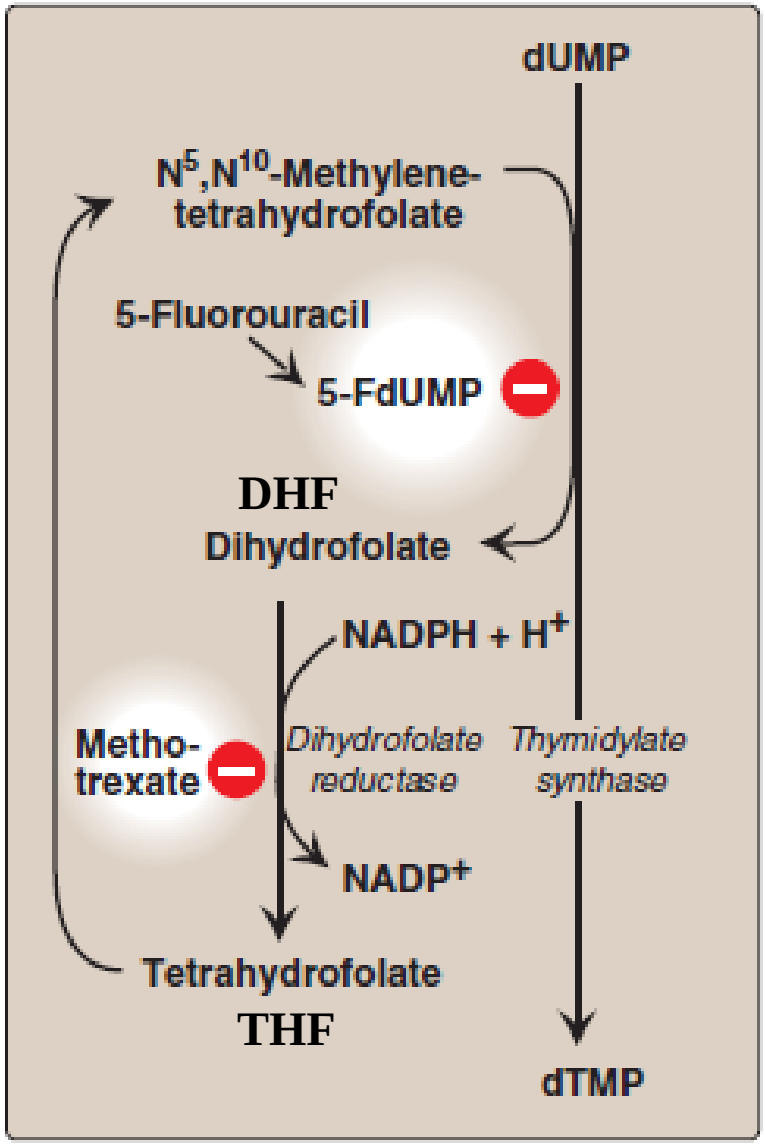
Thymidylate synthase inhibitors include thymine analogs such as 5-fluorouracil (antitumor agents).

5-Fluorouracil (**suicide inhibitor**) is converted to 5-FdUMP that **permanently** binds to the inactivated thymidylate synthase

Methotrexate inhibits dihydrofolate reductase

Methotrexate reduces THF, inhibits purine synthesis and prevents methylation of dUMP to dTMP, resulting in **DNA synthesis inhibition and cell growth slow down .**

5-Fluorouracil and Methotrexate are **anti cancerous agents**



Pyrimidine Salvage

Few pyrimidine bases are salvaged in human cells because of the very low concentration of the bases in plasma and tissues.

Mechanism: Phosphorylase then kinase

Specific Nucleoside kinases

Pyrimidine nucleosides ⟶ Nucleotides

ATP

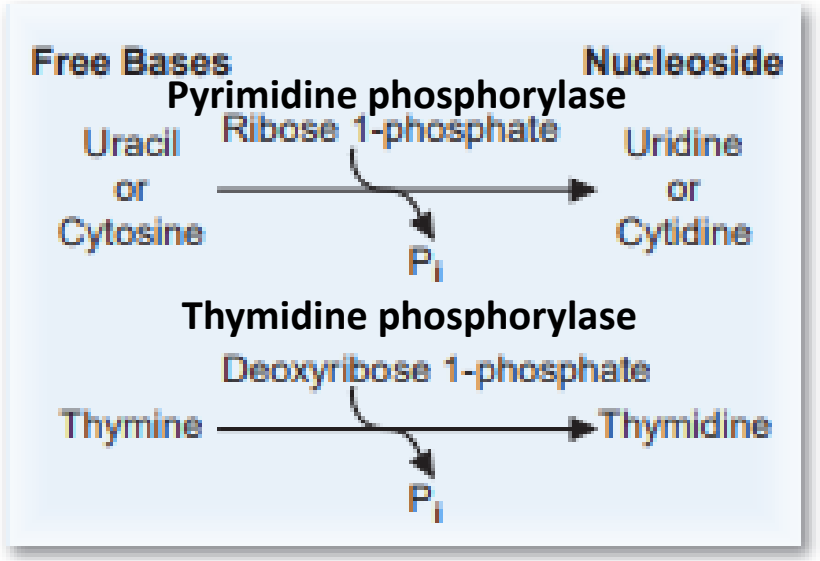


FIG. 41.17. Salvage reactions for pyrimidine nucleoside production. Thymine phosphorylase uses deoxyribose 1-phosphate as a substrate, so ribothymidine is rarely formed.

Thymidine kinase (TK) activity is closely related to the proliferative state of the cell as TK levels and activity increase dramatically as cells enter S-phase and in rapidly dividing cells.

Radiolabeled thymidine is widely used for isotopic labeling of DNA, in radioautographic investigations or to estimate rates of intracellular DNA synthesis.

Pyrimidine Degradation

- ✓ The pyrimidine nucleotides are dephosphorylated
- ✓ The nucleosides are cleaved to produce ribose 1-phosphate and the free pyrimidine bases cytosine, uracil, and thymine.
- ✓ Cytosine is deaminated, forming uracil, which is converted to CO2, NH3, and beta-alanine.
- ✓ Thymine is converted to CO2, NH3, and beta-aminoisobutyrate

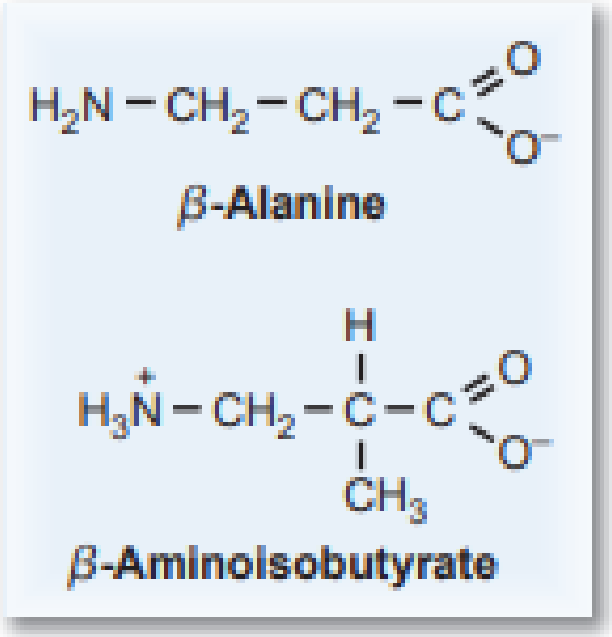


FIG. 41.20. Water-soluble end products of pyrimidine degradation.

- ✓ The highly soluble products β-alanine and β-aminoisobutyrate are excreted in the urine or converted to CO2, H2O, and NH3 (which forms urea).
- ✓ The products do not cause any problems for the body, in contrast to urate
- ✓ As with the purine degradation pathway, little energy can be generated by pyrimidine degradation