Nucleotide Metabolism

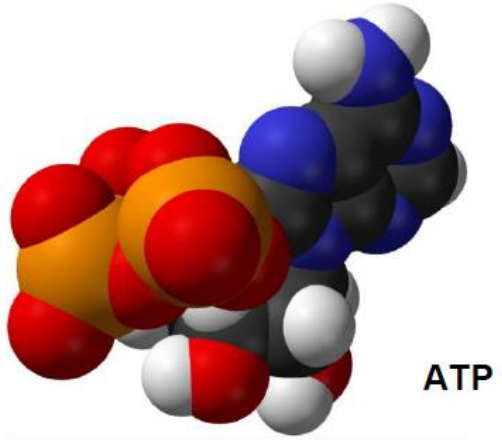
From basics to clinic

MOLE-203 | Metabolism

Rafa Najumudeen, FIMM, Helsinki Institute of Life Science

Overview

1. Basic Concepts
2. Main Features of Nucleotide Metabolism
3. De Novo Biosynthesis of Purines
4. De Novo Biosynthesis of Pyrimidines
5. Nucleotides & their importance in clinical use
6. Biosynthesis of Deoxyribonucleotides
7. Recycling and Catabolism
8. Other Roles of Nucleotides
9. Important Function as Carriers of Free Energy
10. Summary



The Basics

Nucleotide

A biomolecule composed of a pentose sugar, a nitrogenous base, and a phosphate

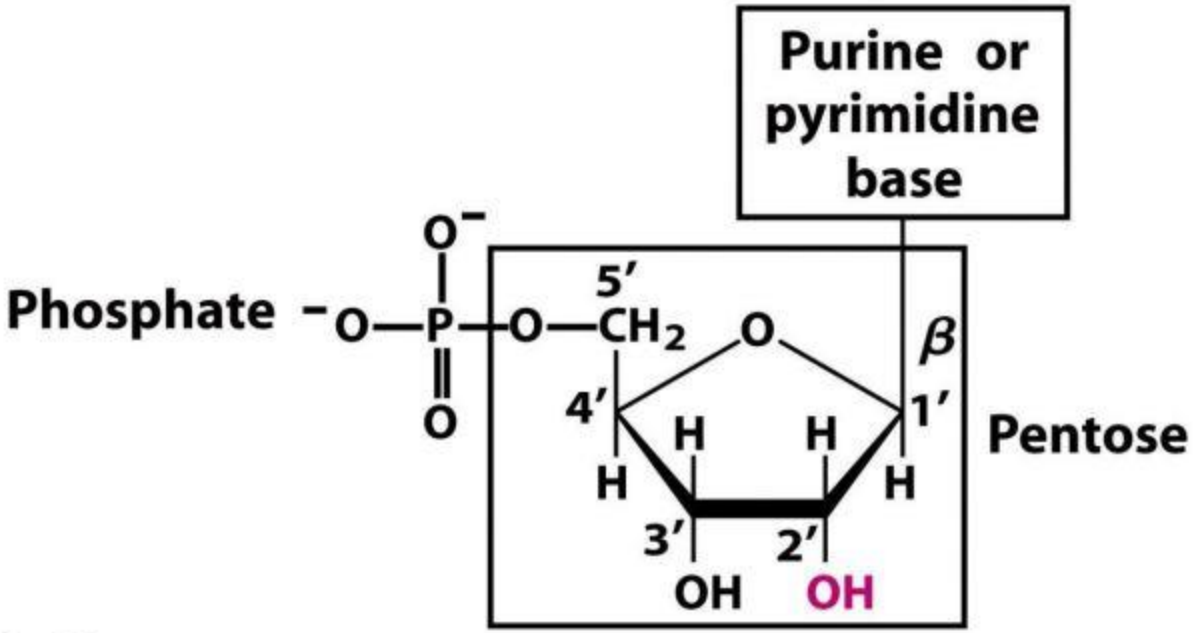


Figure 8-1a
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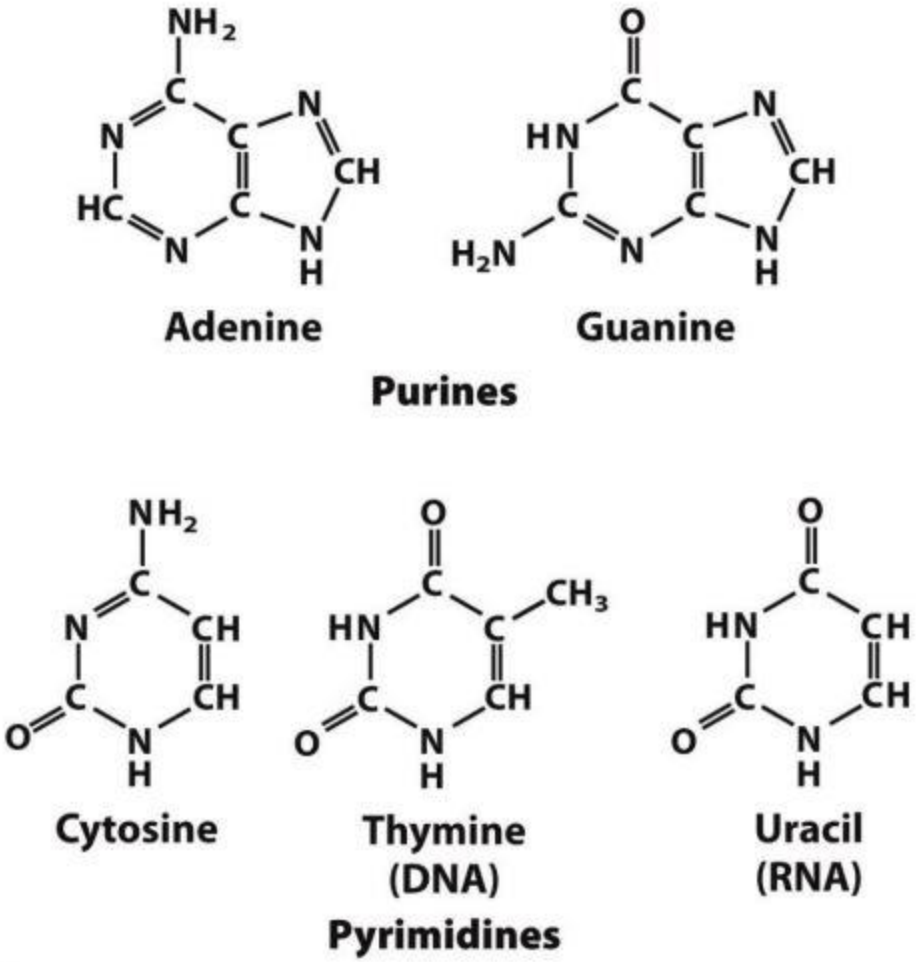

Figure 8-2
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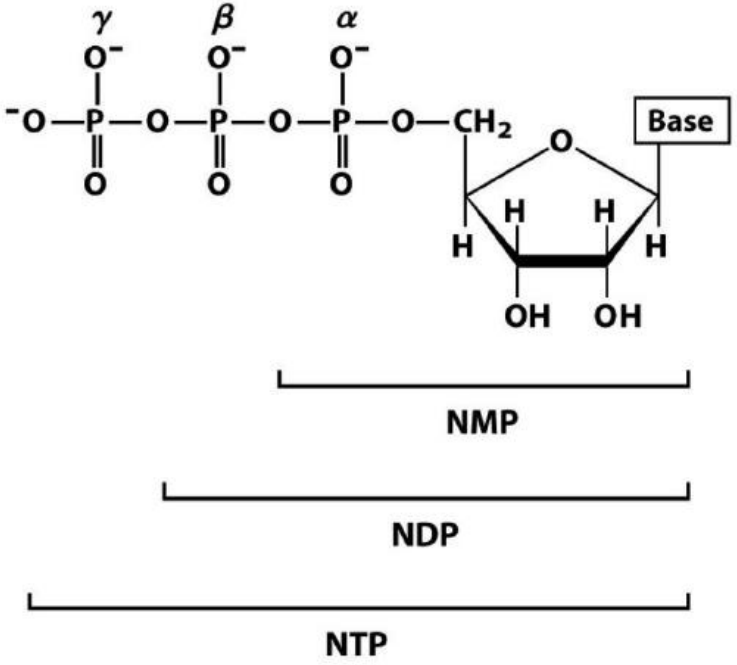
Nucleoside

- Nucleotide without phosphate part = pentose + base
- Nucleotides are nucleosides with one, two, or three phosphate groups attached.
- Prefix "d" in deoxynucleotides Indicates the absence of an oxygen atom in the sugar component (deoxyribose).
- Abbreviations According to the Base

  dNTP: Deoxynucleoside triphosphates (e.g., dATP, dGTP, dTTP, dCTP).

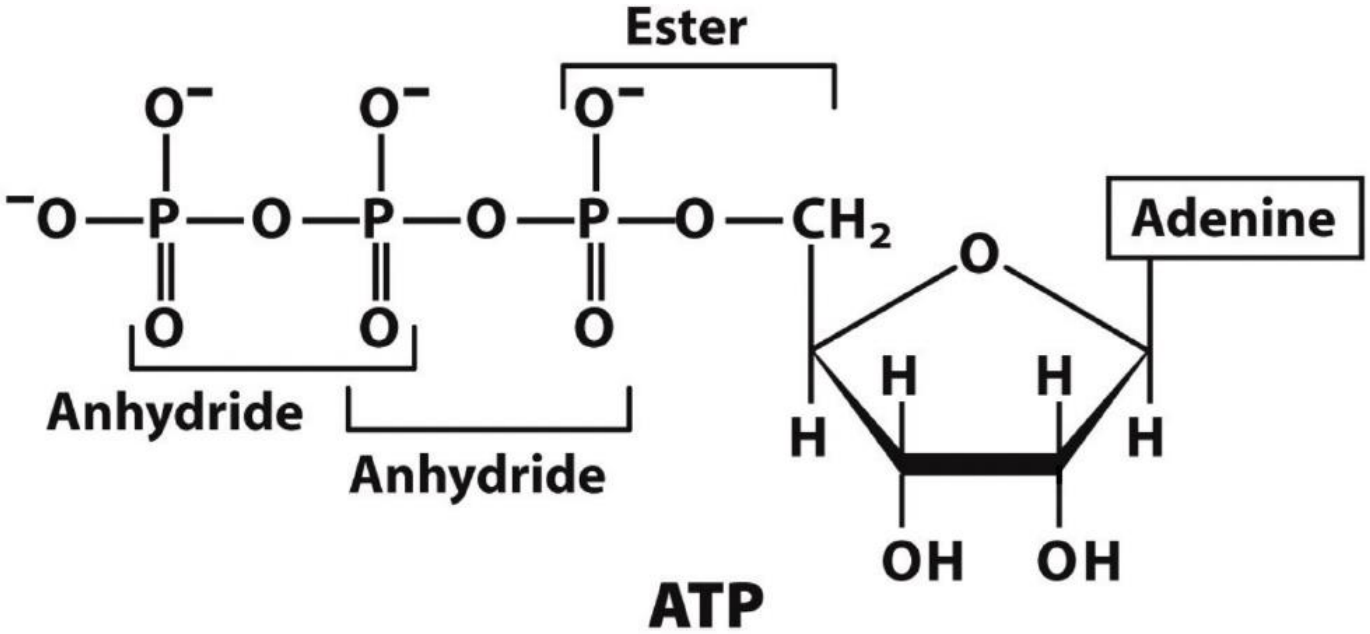
  NDP: Nucleoside diphosphates (e.g., ADP, GDP, UDP, CDP).

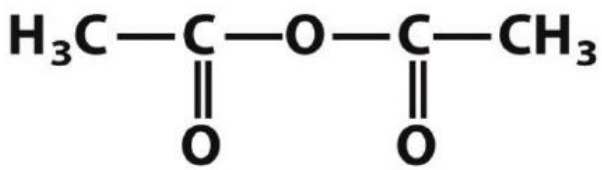
Base	Nucleoside
adenine	adenosine
guanine	cytidine
cytosine	guanidine
uracil	Uridine
thymine	thymidine



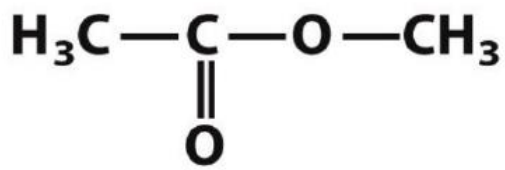
Abbreviations of ribonucleoside 5′-phosphates			
Base	**Mono-**	**Di-**	**Tri-**
Adenine	AMP	ADP	ATP
Guanine	GMP	GDP	GTP
Cytosine	CMP	CDP	CTP
Uracil	UMP	UDP	UTP

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





Acetic anhydride, a carboxylic acid anhydride



Methyl acetate, a carboxylic acid ester

Figure 8-37
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eneral features of nucleotide metabolis

Nucleotide Biosynthesis

1. *De Novo* Biosynthesis: Humans (like almost all organisms) can synthesize all nucleotides from simple metabolites (mainly sugars and amino acids).

2. Salvage Pathway:

- Nucleosides and free bases from DNA and RNA degradation can be recycled.
- primary method for nucleotide synthesis under normal conditions.
- De novo synthesis is activated when recycling is insufficient, especially in growing and dividing cells.

The biosynthesis of nucleotides is energetically expensive and in normal conditions recycling is primary

3. Types of Nucleotides:

- Purine and pyrimidine nucleotides are primarily produced from simple metabolites.
- Ribonucleotides are synthesized first, and deoxynucleotides are made from them.

4. End Products of Nucleotide Catabolism:

- Purine bases are broken down into uric acid/urate.
- Pyrimidines are broken down into urea, which is eliminated in the urine.

Purine biosynthesis

Base	Nucleoside
adenine	adenosine
guanine	guanosine

The origin of the carbon and nitrogen atoms of the purine ring

from different precursors:

1.Glycine: C4, C5, and N7.

2.Glutamine: N3 and N9.

3.Aspartate: N1.

4.Formate (via tetrahydrofolate): C2 and C8.

5.Carbon Dioxide (Bicarbonate): C6.

*of N10-formyl-tetrahydrofolate

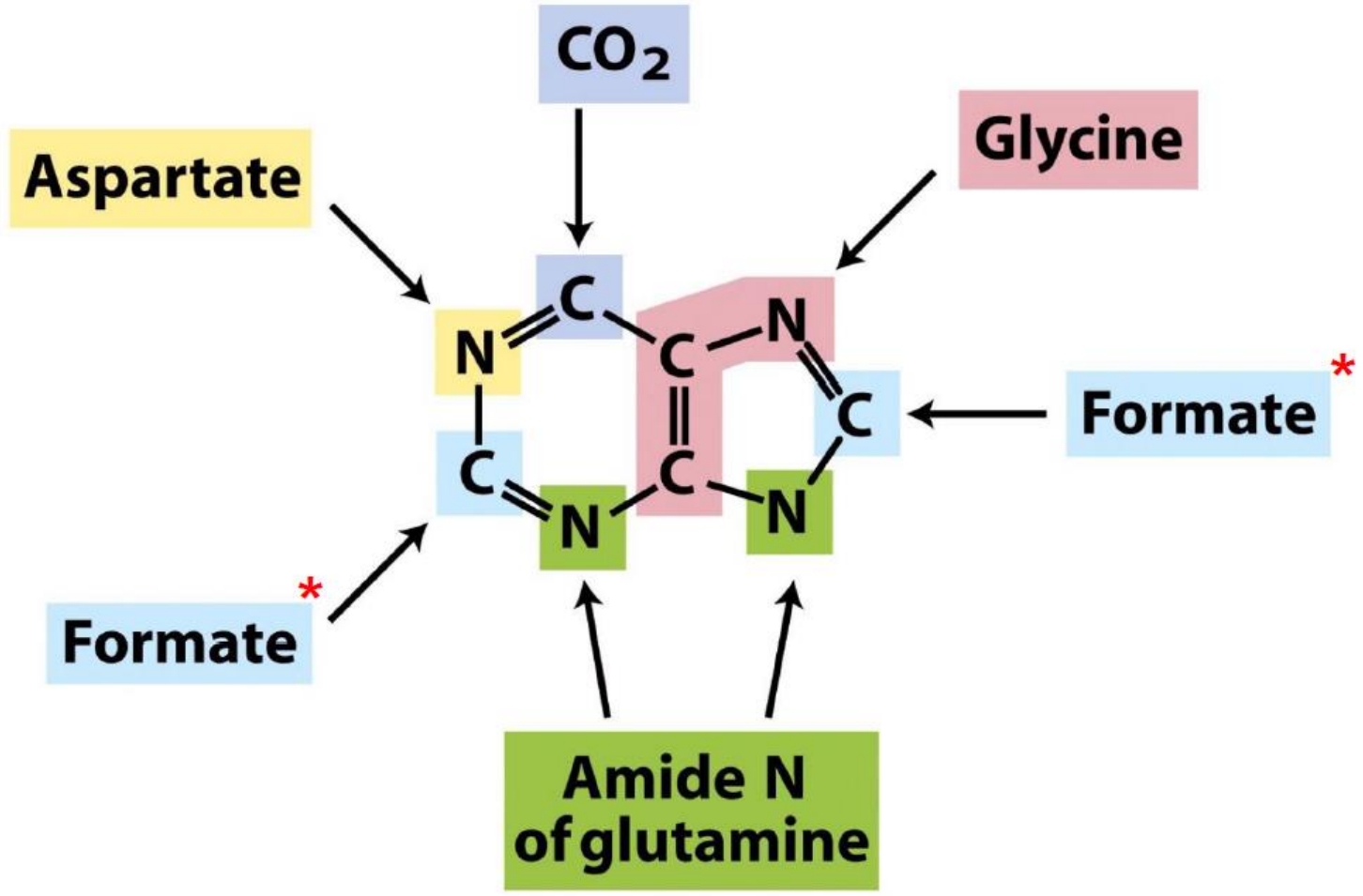

Figure 22-32
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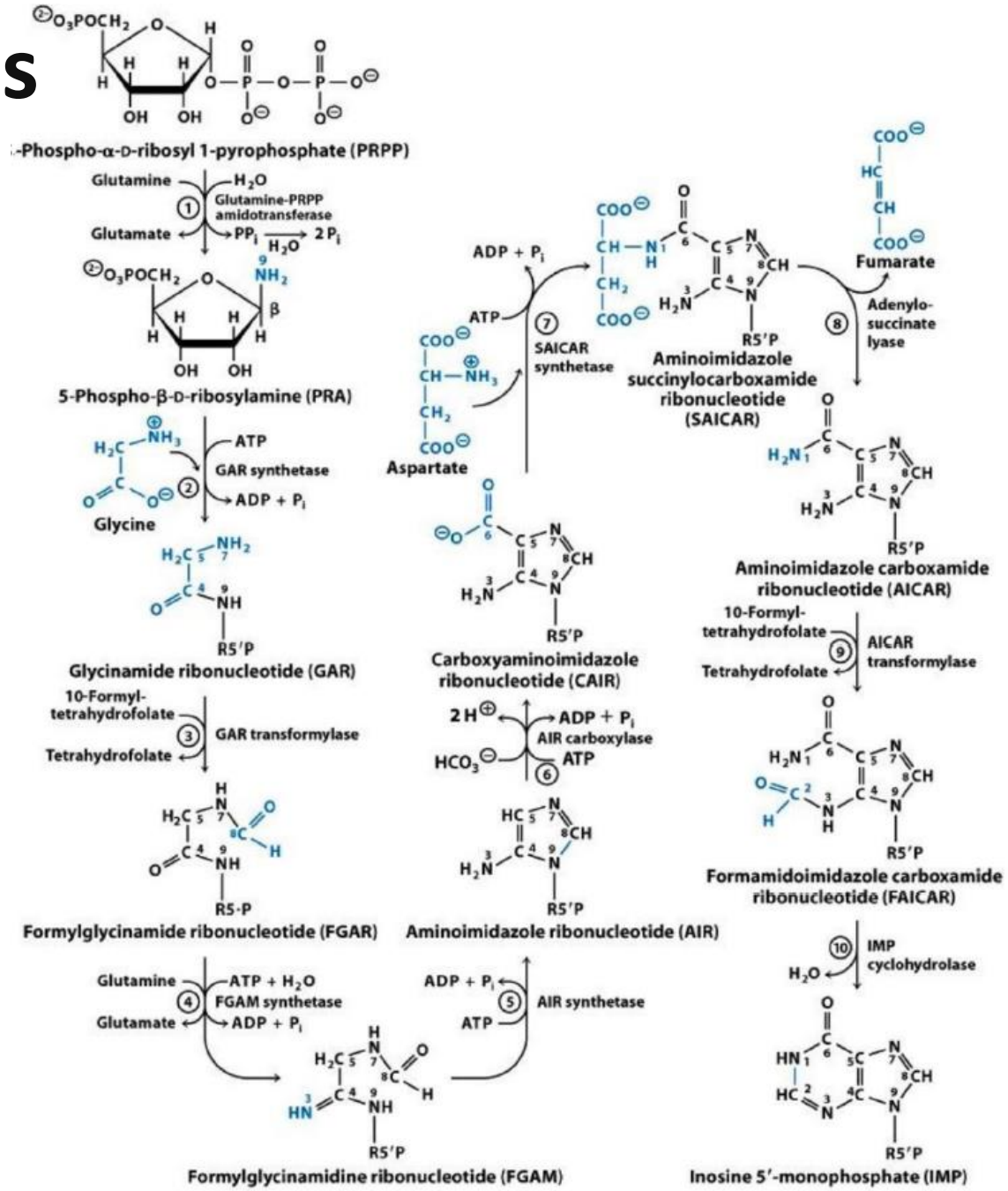
Purine nucleotide biosynthesis

1.The process starts with the phosphorylation of ribose to form 5-phosphoribosyl-1-pyrophosphate (PRPP).
The purine base is constructed step-by-step on the ribose phosphate backbone.

2.Intermediate Product: Inosine monophosphate (IMP) is formed as an intermediate. From IMP, the pathway diverges to produce guanylate (GMP) and adenylate (AMP).

3.Energy Consumption: The initial stage of synthesizing IMP requires 5 ATP molecules.



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Purine nucleotide biosynthesis

- 1 ATP is consumed in further reactions from inosine monophosphate to adenylate or guanylate

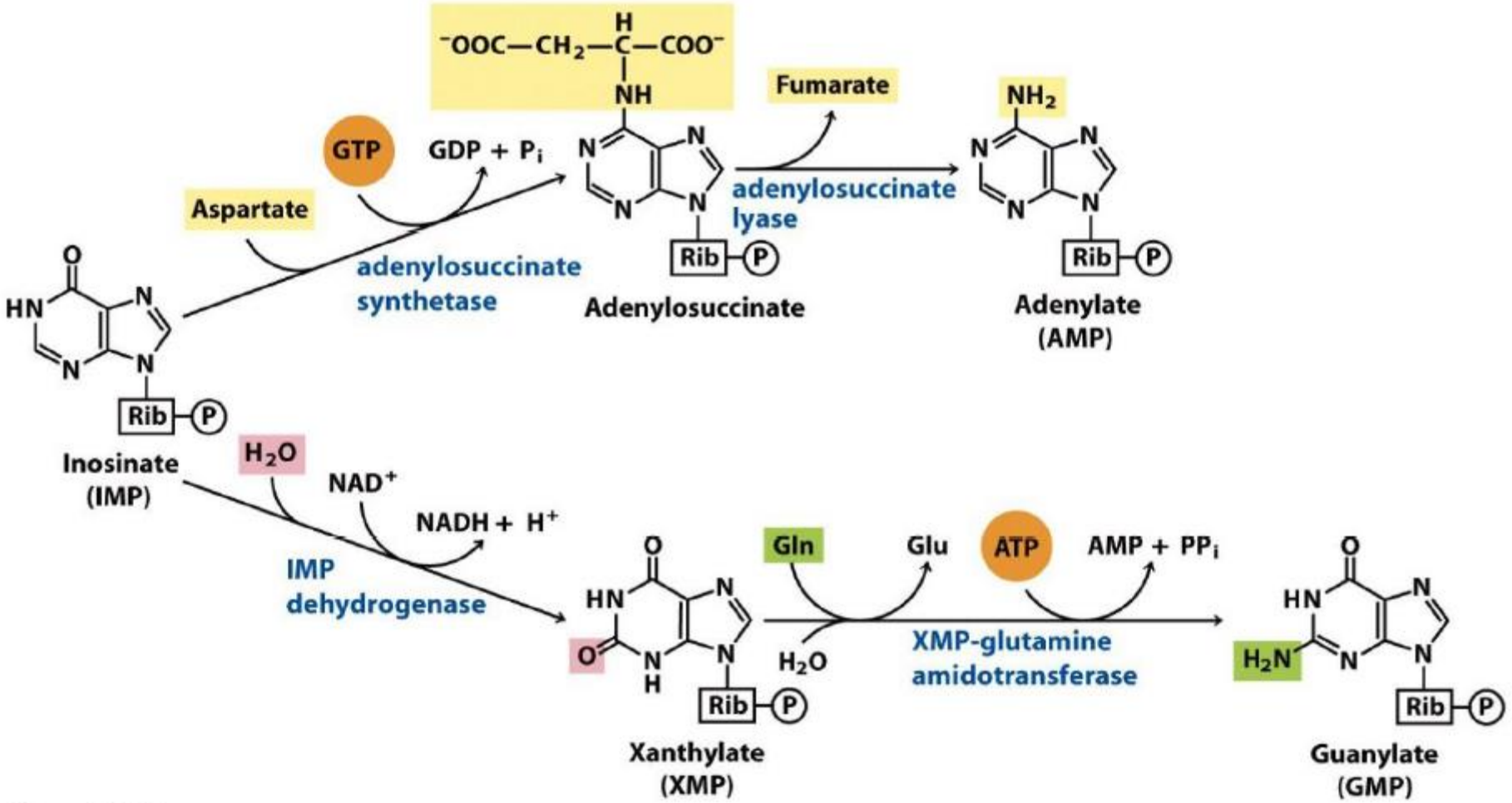

Figure 22-34
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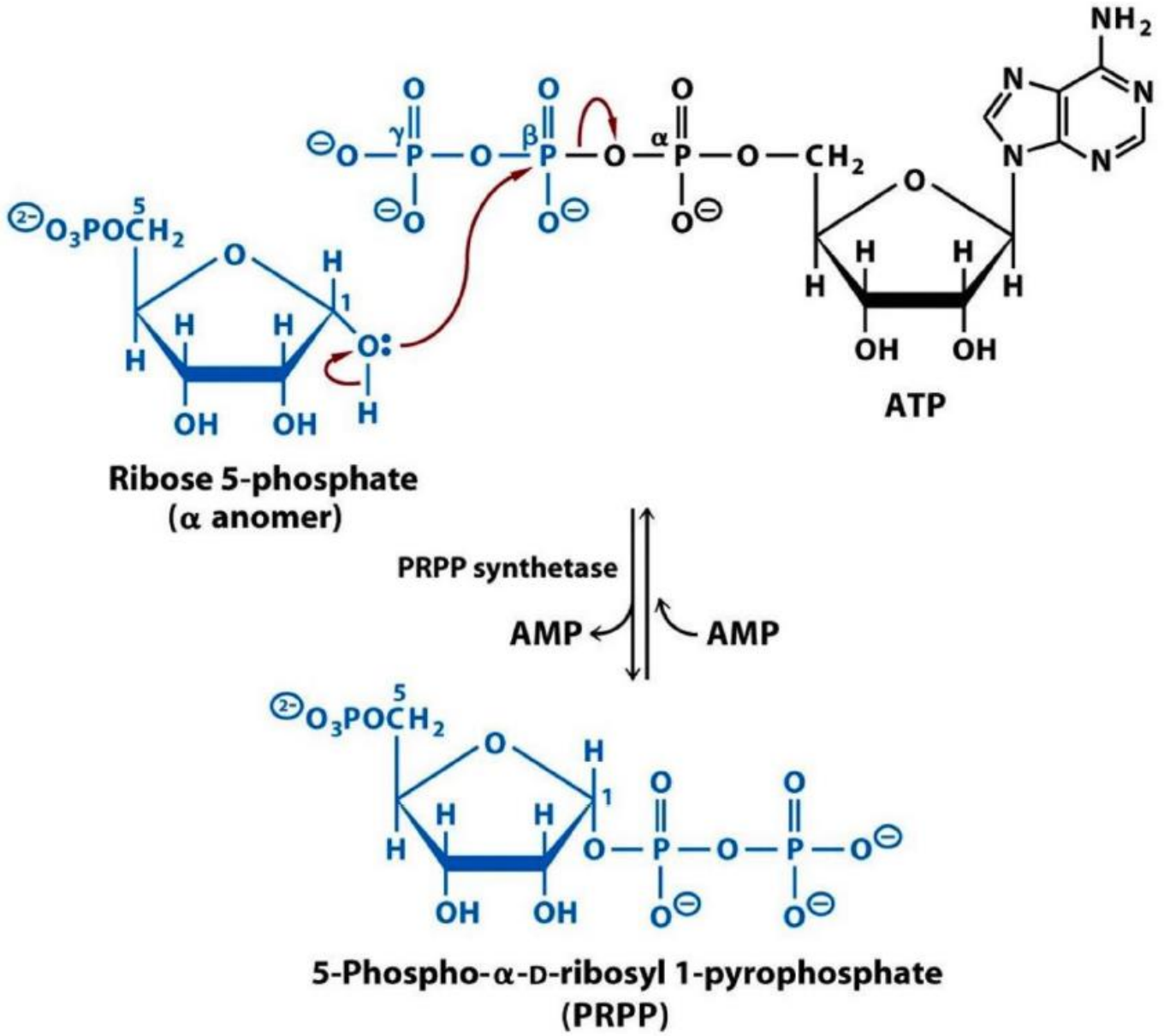
Synthesis of PRPP

- Phosphoribosylpyrophosphate synthetase catalyzes reactions beyond purine biosynthesis.
- Crucial for the de novo synthesis and recycling of both purines and pyrimidines.
- Plays a role in the biosynthesis of amino acids histidine and tryptophan (though humans do not synthesize His and Trp).
- Mutations in the PRPP gene can lead to enzyme overactivity.
- can cause an excess production of purine nucleotides.
- The breakdown of purines results in uric acid, which can crystallize in the urinary tract, leading to kidney stones and gout.



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Regulation of purine synthesis

- De novo biosynthesis slows down when there are enough purines available, favoring recycling instead.
- The availability of PRPP can limit the synthesis process.
- Inosine monophosphate (IMP) acts as an allosteric inhibitor for enzymes at the early stages and branch points of the synthesis pathway.
- End products like AMP and GMP play a regulatory role.
- IMP, AMP, and GMP inhibit the enzyme glutamine PRPP-amidotransferase, effectively reducing the synthesis of new purines.

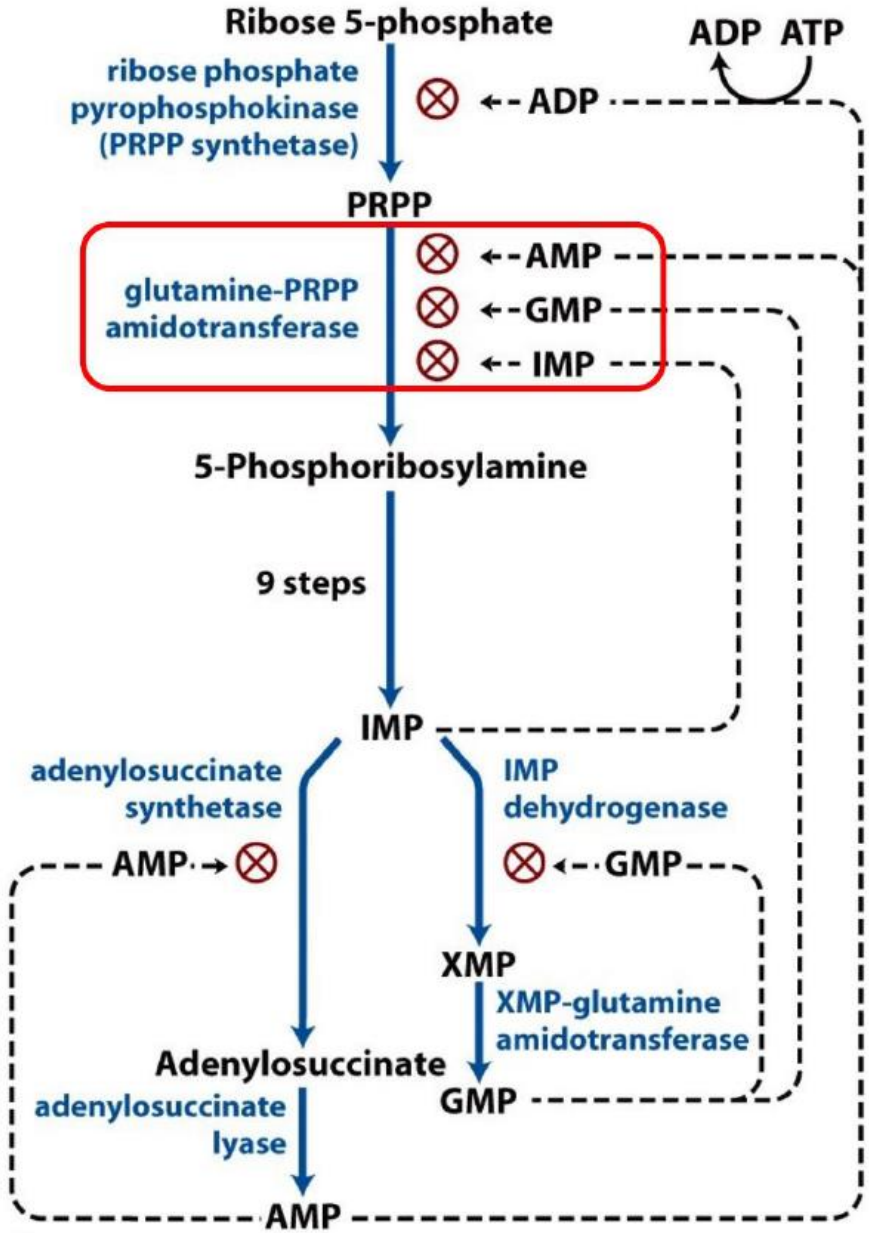

Figure 22-35
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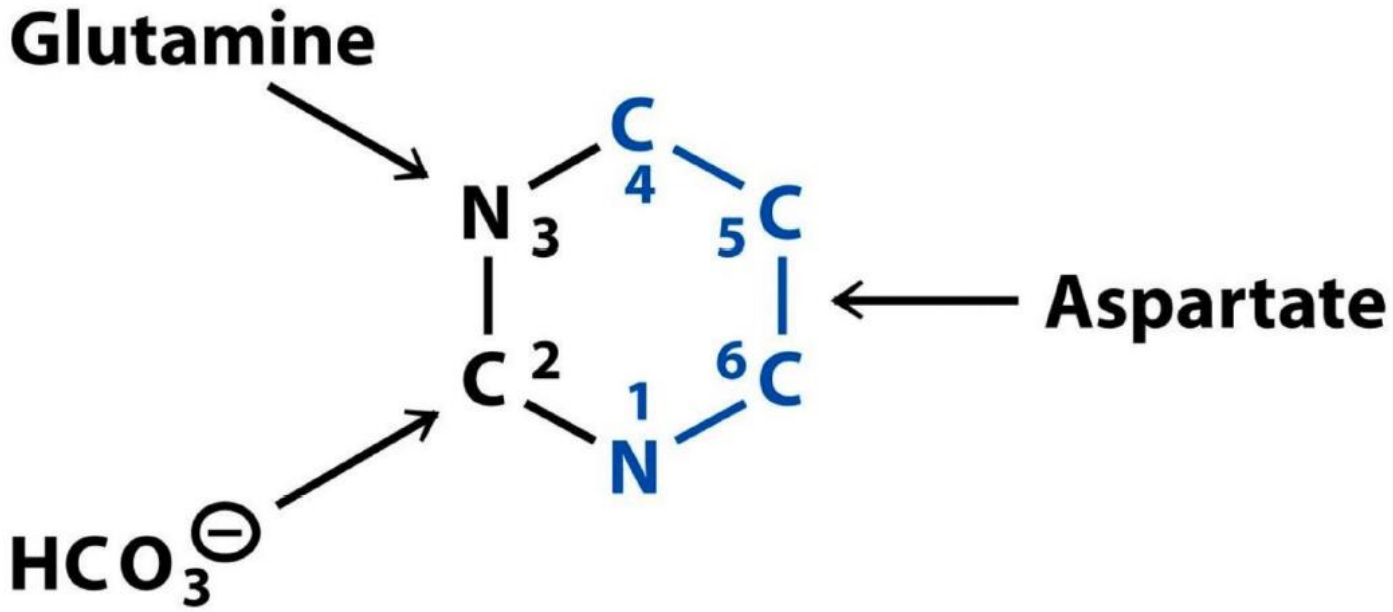
Biosynthesis of pyrimidines

Base	Nucleoside
cytosine	cytidine
uracil	Uridine
thymine	thymidine

The origin of the carbon and nitrogen atoms of the pyrimidine ring

1. **Aspartate**: N1, C4, C5, and C6.
2. **Glutamine**: N3.
3. **Bicarbonate (HCO_3^-)**: C2.



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Biosynthesis of pyrimidine nucleotides

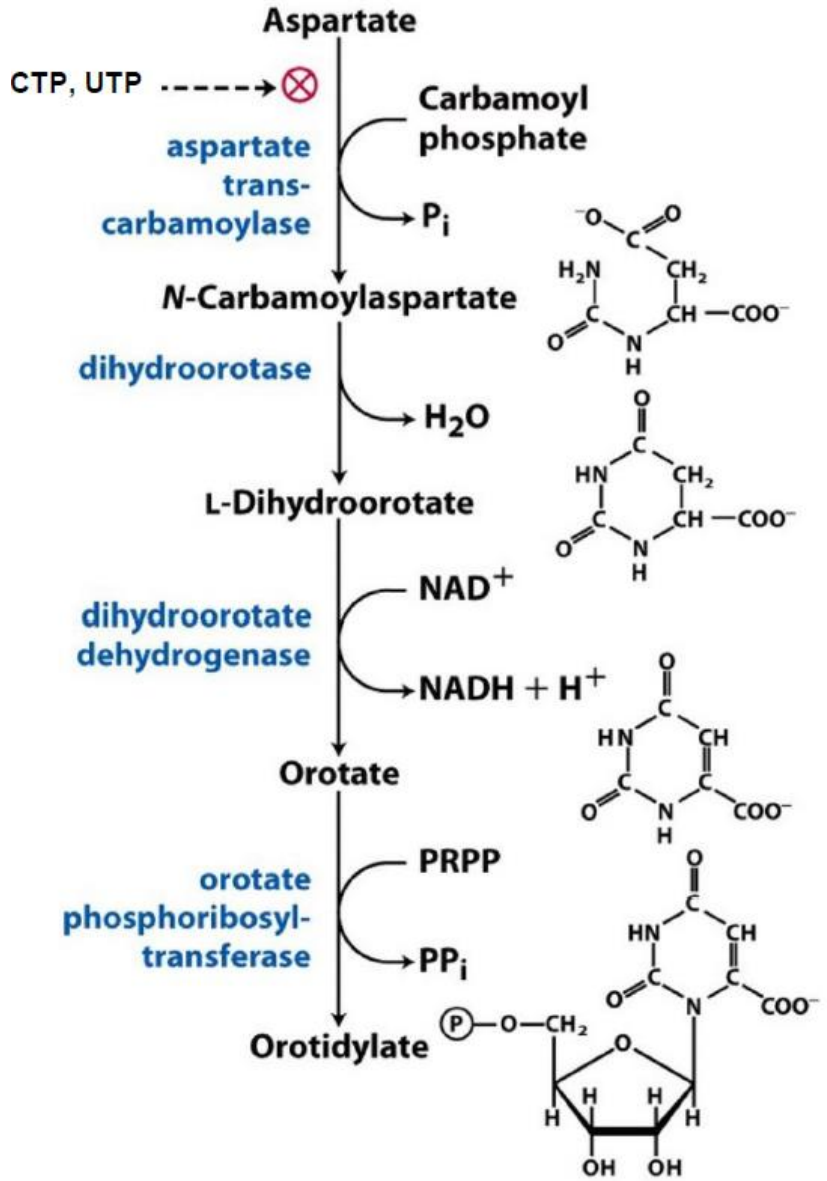
•**Catalysis**: the biosynthesis of pyrimidine nucleotides (CTP, UTP) is catalyzed by aspartate transcarbamylase (ATCase).

•**Formation of Orotate**: the first intermediate with a pyrimidine ring, orotate, is attached to ribose phosphate (PRPP) to form orotidylate (OMP).
Carbamyl phosphate combines with aspartate to form carbamyl aspartate.

The amino acid **aspartate** is a key starting material.

•**Synthesis**: unlike purine synthesis, where the base is built on a pre-formed ribose-phosphate framework, pyrimidine bases are constructed first and then attached to ribose phosphate (PRPP).

•**Regulation**: the first reaction step is the rate-limiting phase of the synthesis pathway and is regulated by feedback inhibition from CTP and UTP.



Biosynthesis of pyrimidine nucleotides

- Decarboxylation of OMP base => UMP (uridylate)
- UMP is phosphorylated to the triphosphate form => UTP
- Modification of the base of UTP => CTP

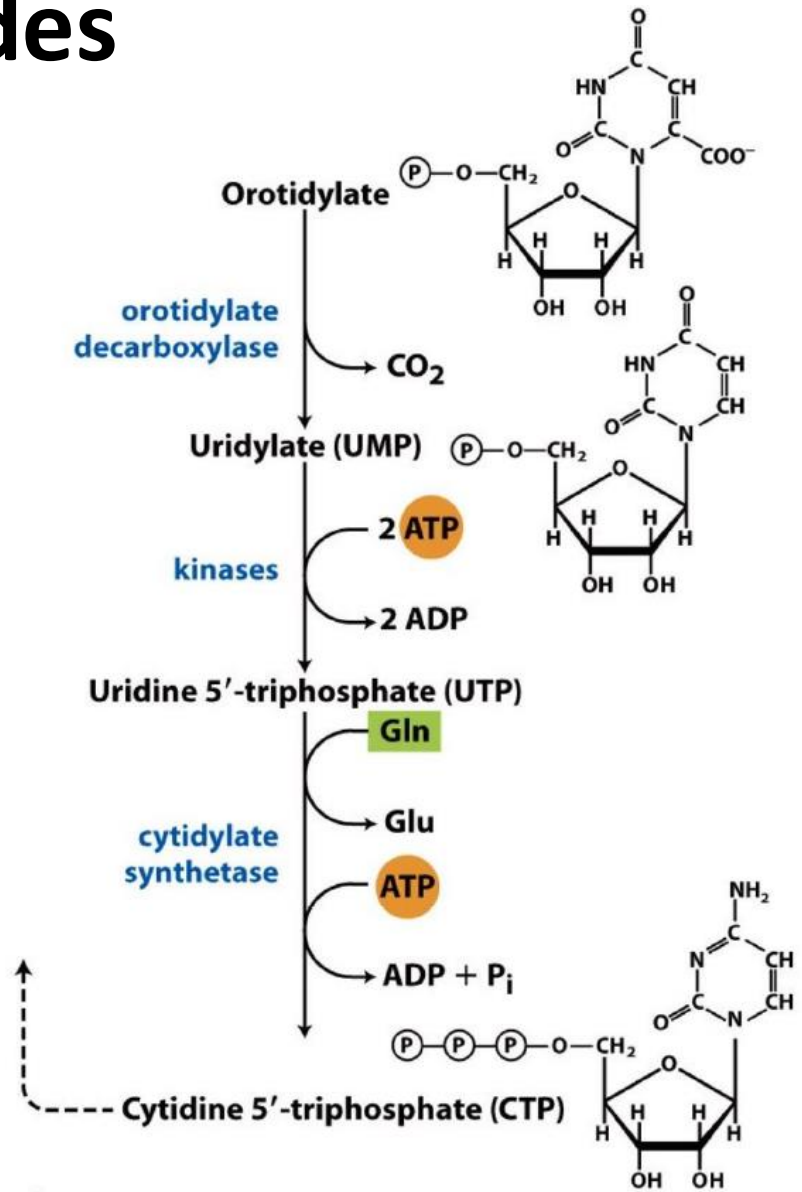


Figure 22-36 part 2
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Carbamyl phosphate synthetase

- **Isoenzymes**:
CPS I and CPS II
CPS II uses nitrogen from glutamine to form carbamyl phosphate in the cytosol.
CPS I uses free ammonia (NH4+) and is involved in the urea cycle in mitochondria.

- **Regulation of CPS II**:
CPS II is regulated by UTP (inhibits) and ATP (activates).
These regulators do not affect mitochondrial CPS I.

- **Role in Pyrimidine Biosynthesis**:
CPS II catalyzes the formation of carbamyl phosphate, which is a substrate for pyrimidine nucleotide biosynthesis.

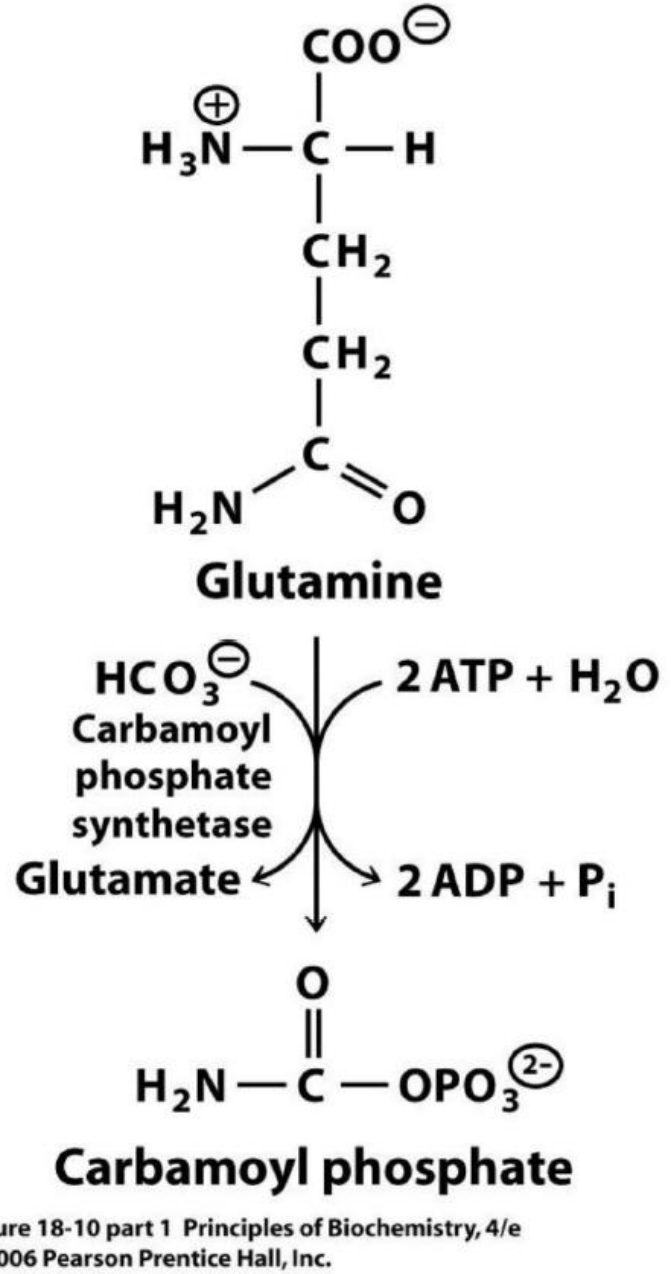

Figure 18-10 part 1 Principles of Biochemistry, 4/e

Carbamyl phosphate synthetase

Role of NH4+:

- In CPS II, NH4+ acts as a substrate, formed from glutamine during the reaction.

Structure of CPS II:

- CPS II is a dimer with three active sites located on different parts of the molecule.

Binding and Channeling:

- Glutamine binds to active site 1, releasing NH4+, which is channeled to ATP at active site 2.
- The formed carbamate is then directed to active site 3, where it is phosphorylated to form carbamyl phosphate.
- This process prevents the release of harmful ammonia (NH3/NH4+) into the cytosol.
- It exemplifies "substrate channeling," where intermediates are directly passed between active sites.

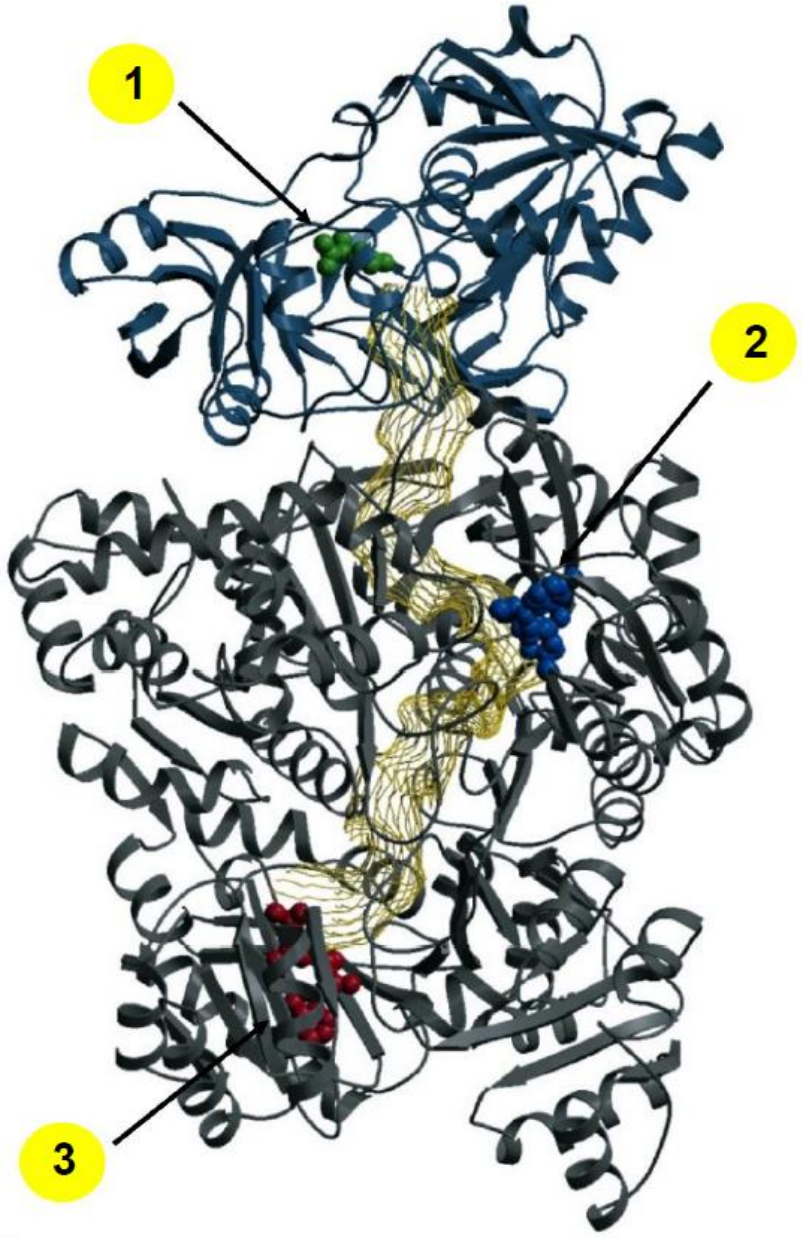

Figure 22-37
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Multienzyme complexes in pyrimidine biosynthesis

- **Enzyme Complexes**:
Carbamyl phosphate synthetase (CPS II), aspartate transcarbamylase, and dihydroorotase form a large enzyme complex called CAD, consisting of multiple polypeptide subunits.

- **UMPS Complex**:
Orotate phosphoribosyltransferase (converts orotate + PRPP to OMP) and orotidylate decarboxylase (converts OMP to UTP) also form a complex known as UMPS, with distinct subunits and active sites for each reaction.

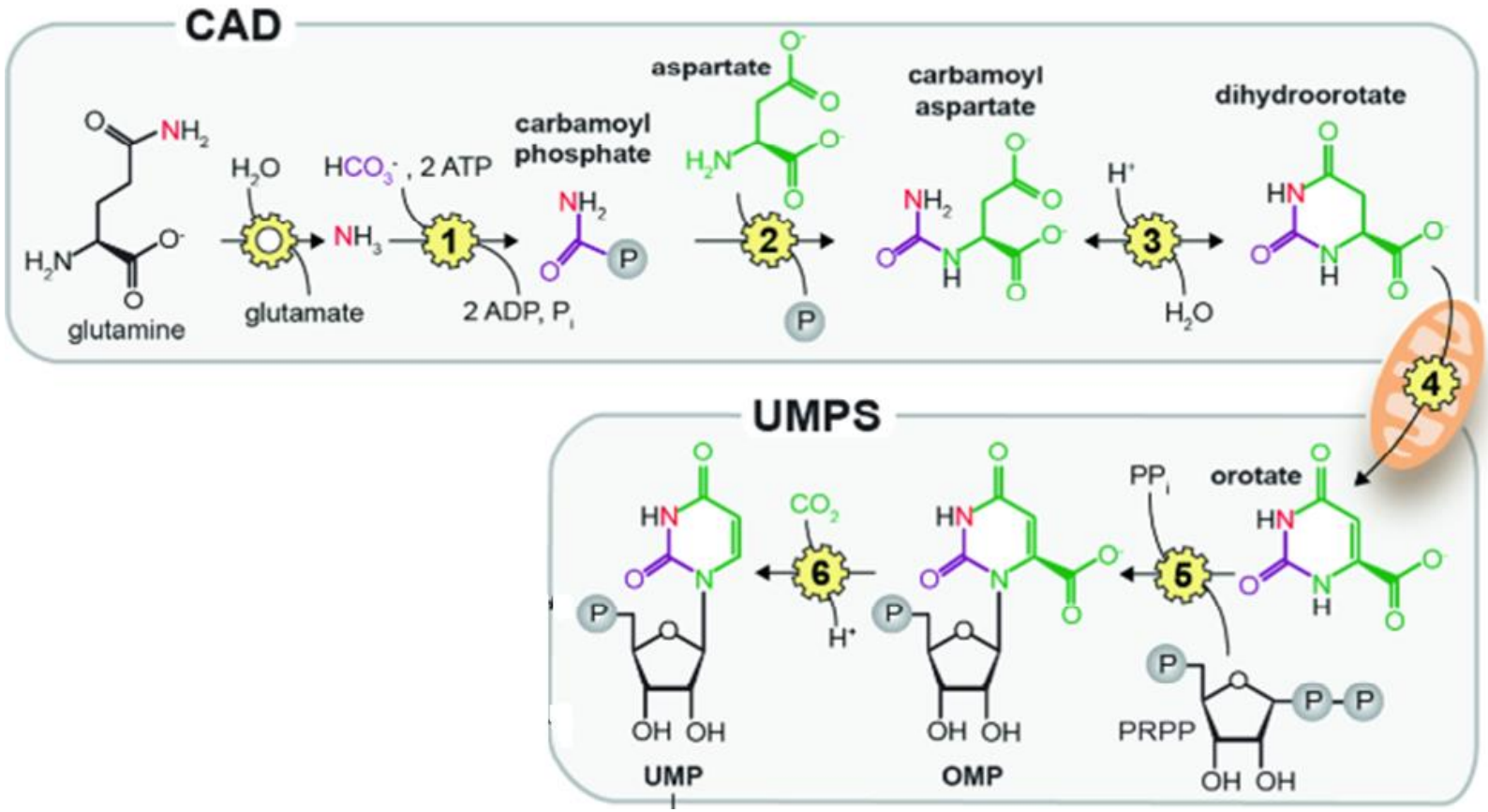
- **Catalysis in Mammals**:
In humans and other mammals, the initial stages of pyrimidine synthesis are catalyzed by these multienzyme complexes.

The complex structure enhances synthesis efficiency by preventing substrate dilution in the surrounding solution and synchronizing product concentrations.

Key enzymes and their location within the cell

1. **CPS II**: Cytosol.
2. **ATCase**: Catalyzes the reaction between carbamyl phosphate and aspartate to form carbamyl aspartate.
3. **Dihydro-orotase**: Converts carbamyl aspartate to dihydroorotate.
4. **Dihydro-orotate Dehydrogenase**: Located in the mitochondria, it converts dihydroorotate to orotate.
5. **Orotate Phosphoribosyl Transferase**: Attaches orotate to ribose phosphate (PRPP) to form orotidylate (OMP).
6. **OMP Decarboxylase**: Converts orotidylate (OMP) to uridine monophosphate (UMP).

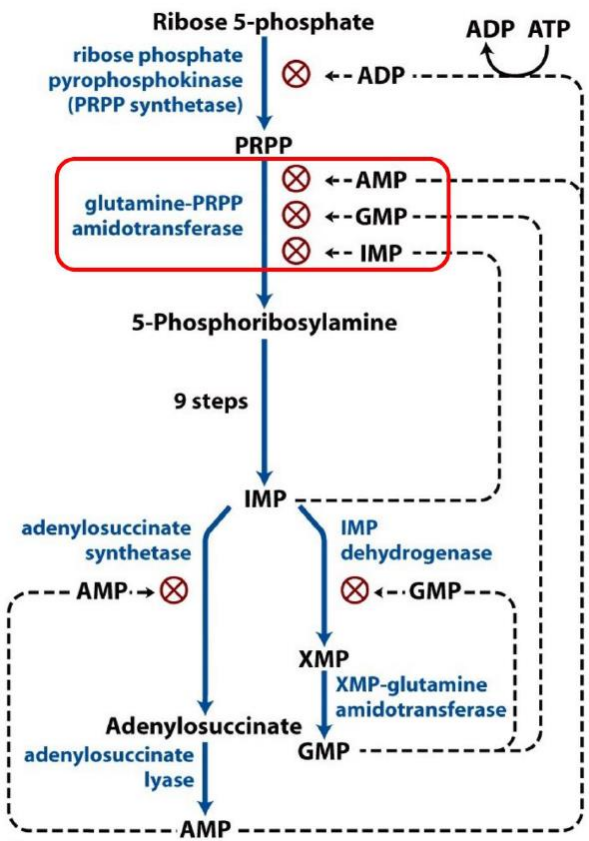
Dihydro-orotate dehydrogenase: in the mitochondria, while the other enzymes, are found in the cytosol. Spatial organization is crucial for the efficient synthesis of pyrimidine nucleotides.



del Caño-Ochoa et al. In: Macromolecular Protein Complexes II: Structure and Function, Springer, 2019_

Which molecule is the precursor for both purine and pyrimidine synthesis?

a) PRPP
b) UMP
c) Adenine
d) Cytidine



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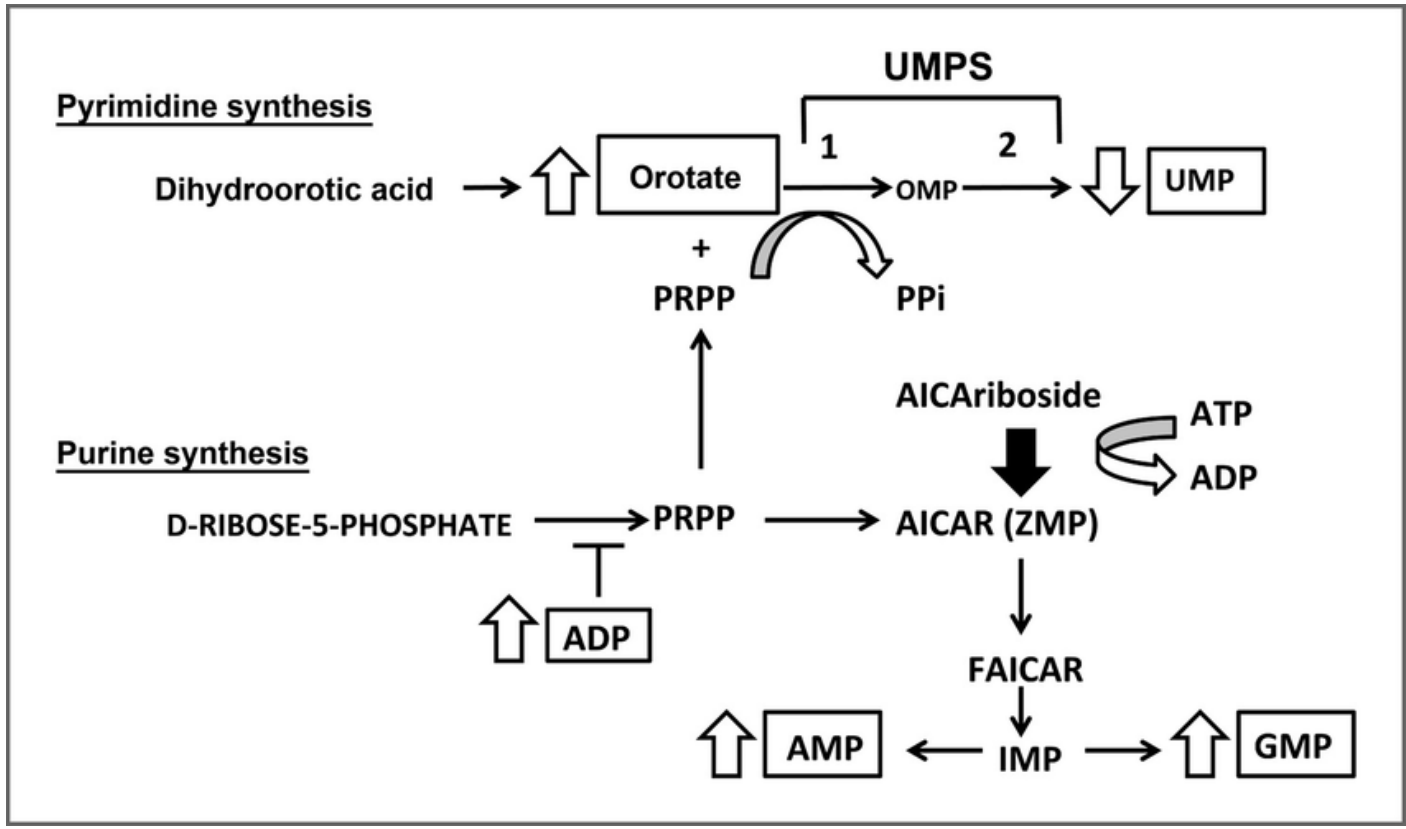
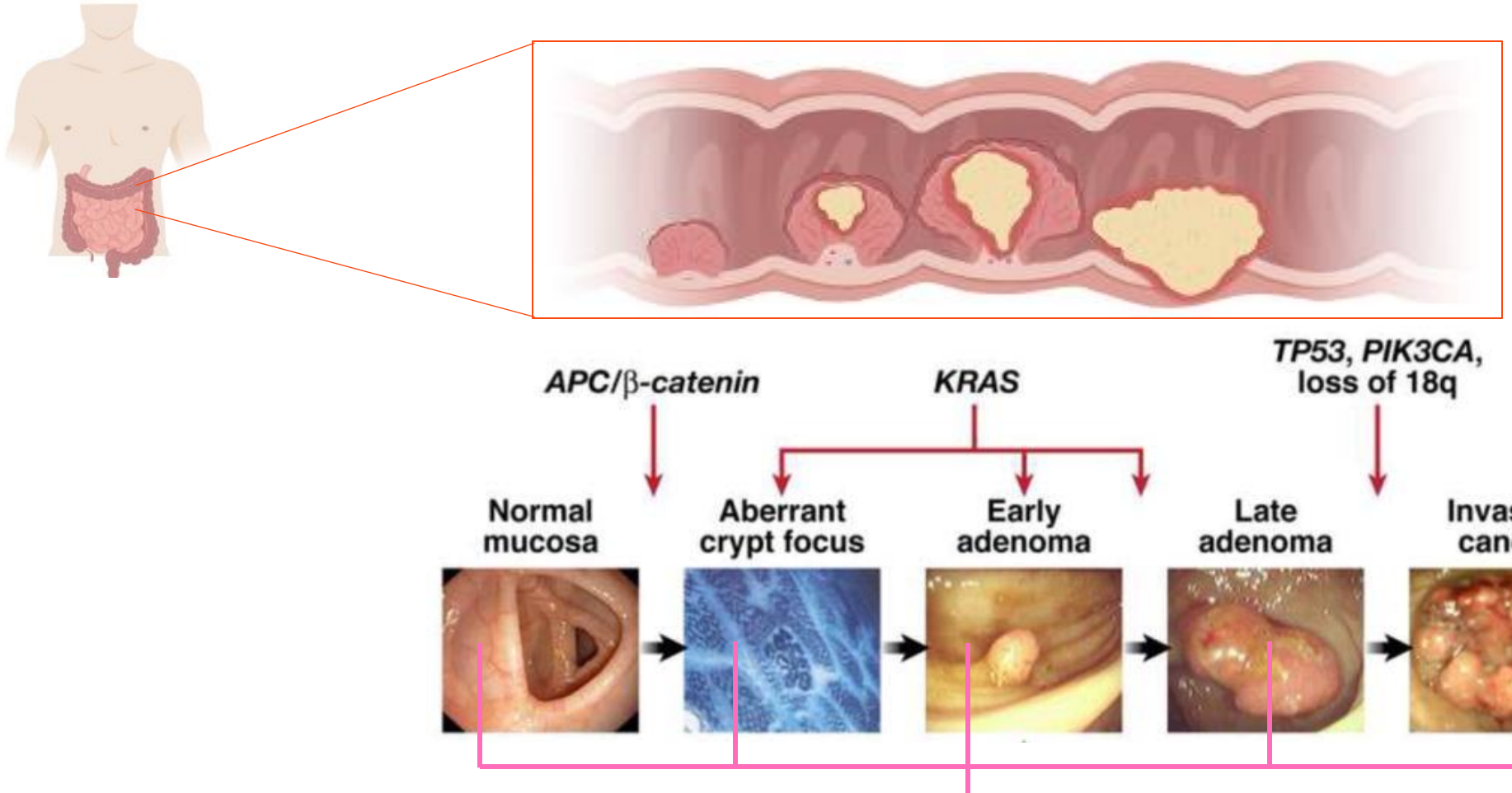


Nucleotide metabolism is exploited by cancer cells

- Hyperactive synthesis and use of nucleotide triphosphates (NTPs) and their deoxy counterparts (dNTPs) is a universal feature of cancer cells that is highly druggable.

- The supraphysiological abundance of intracellular nucleotides contributes to many aspects of cancer cell behaviour, including uncontrolled proliferation, immune evasion, metastasis and therapy resistance.

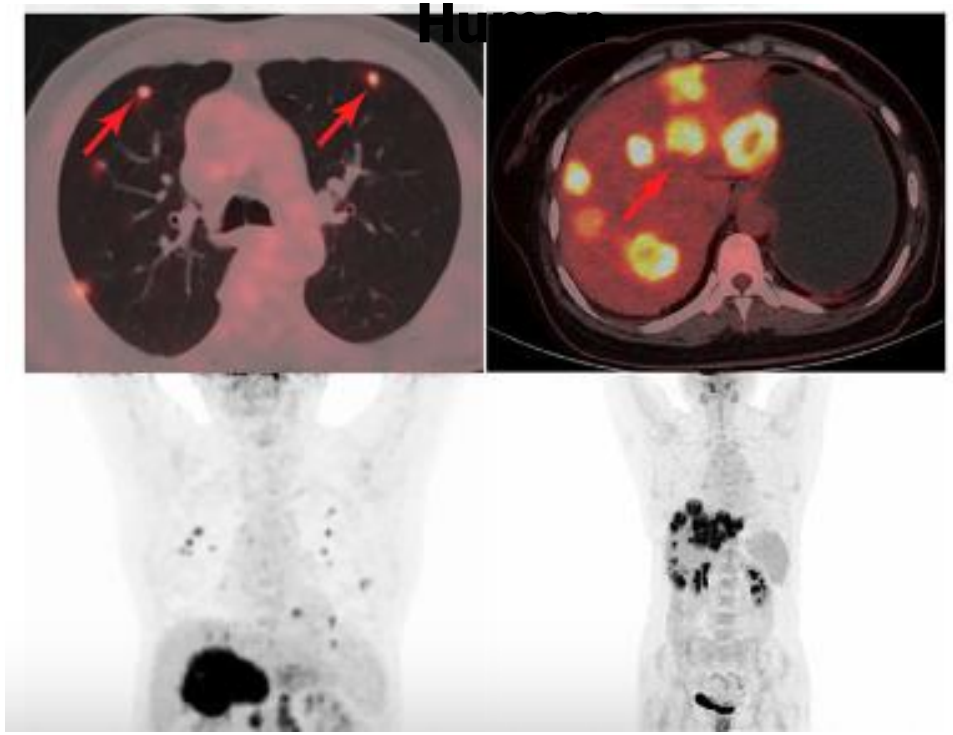
Colon cancer cells change their nucleotide metabolism



Pino & Chung, 2012

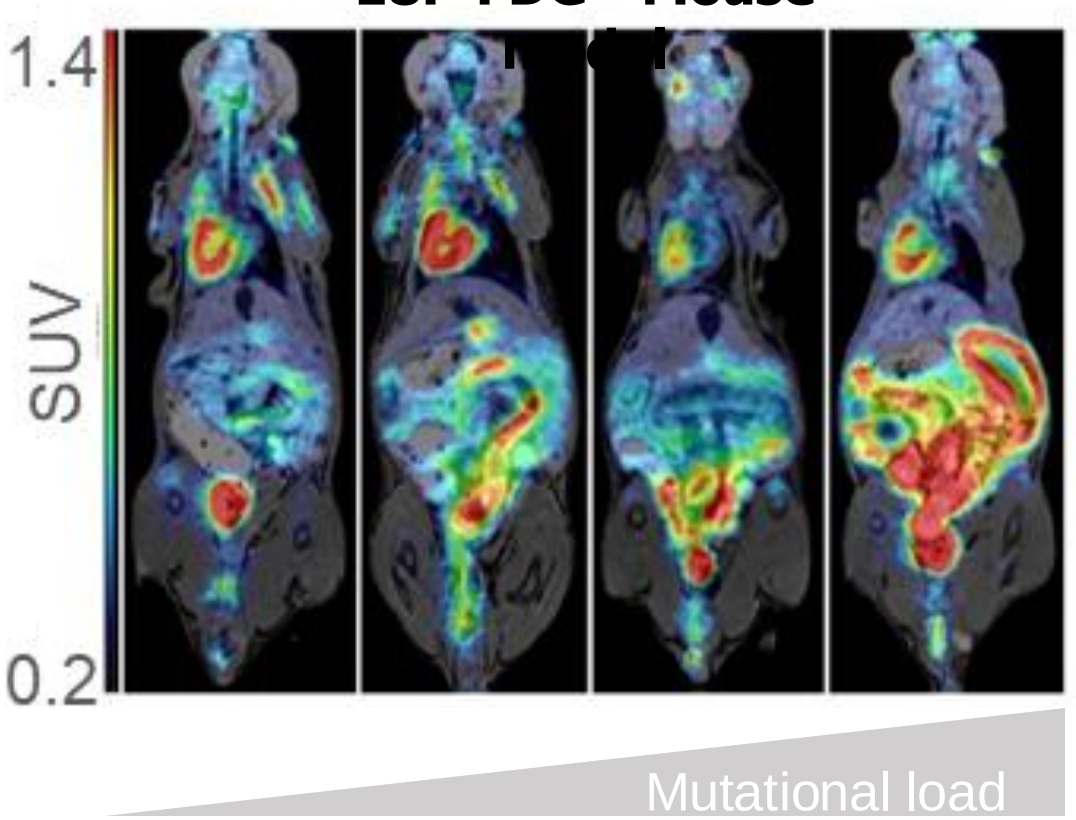
Cancer causing mutations increase metabolic activity

18F-FDG - Human

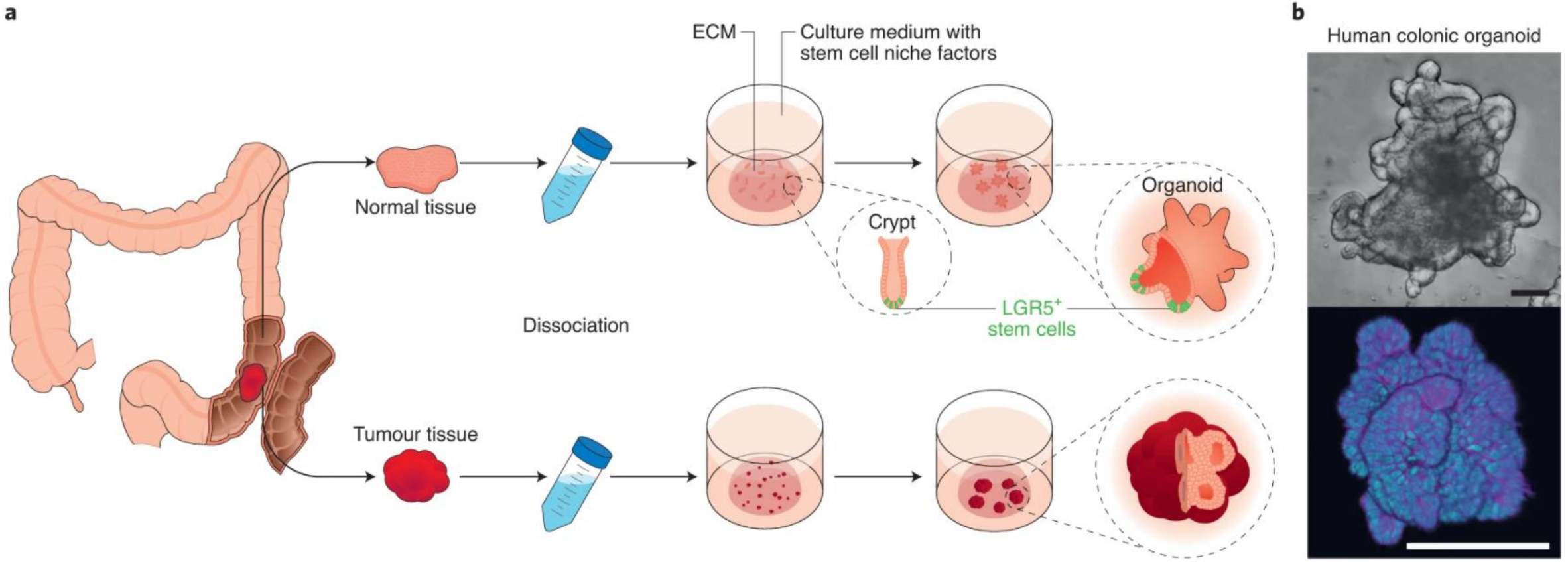


Shi *et al.,* Front. Oncol., 2022

18F-FDG - Mouse

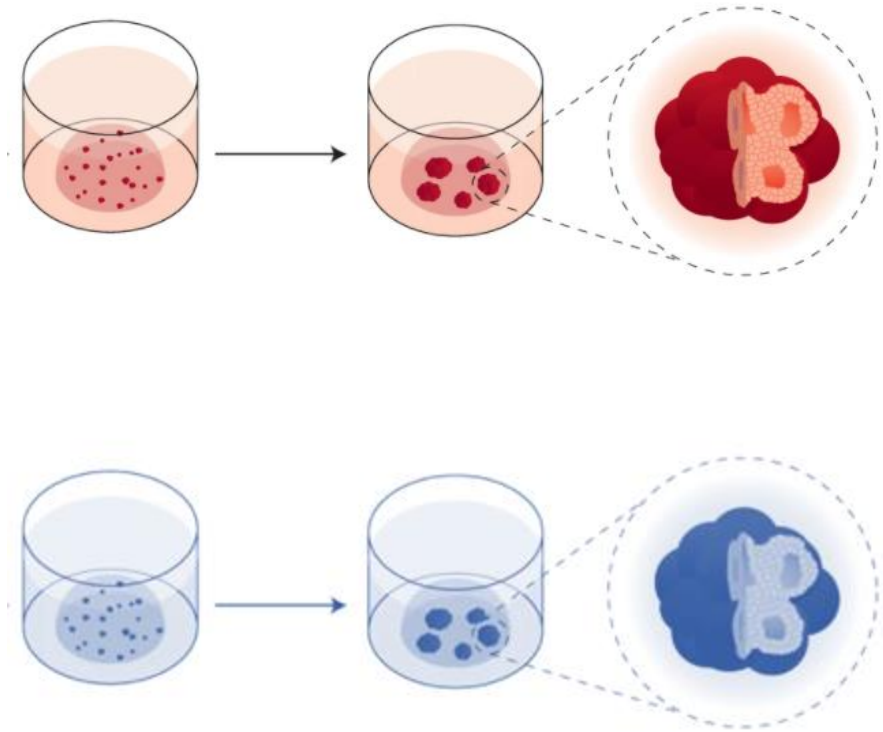


Studying metabolism in cancer cells



Nature Material, Sato, 2020

Study drug response of cancer cells



Nucleotide analogues are frequently used in the clinic

What are Nucleotide Analogues?
Synthetic molecules structurally similar to natural nucleotides.
Function: Interfere with DNA/RNA synthesis or repair.
Widely used in the treatment of **cancer**, **viral infections**, and **autoimmune diseases**.

5-Fluorouracil (5-FU)
Type: Pyrimidine analogue.
Use: Treatment of various cancers (colon, breast, stomach).
Mechanism: Inhibits thymidylate synthase, blocking DNA synthesis.

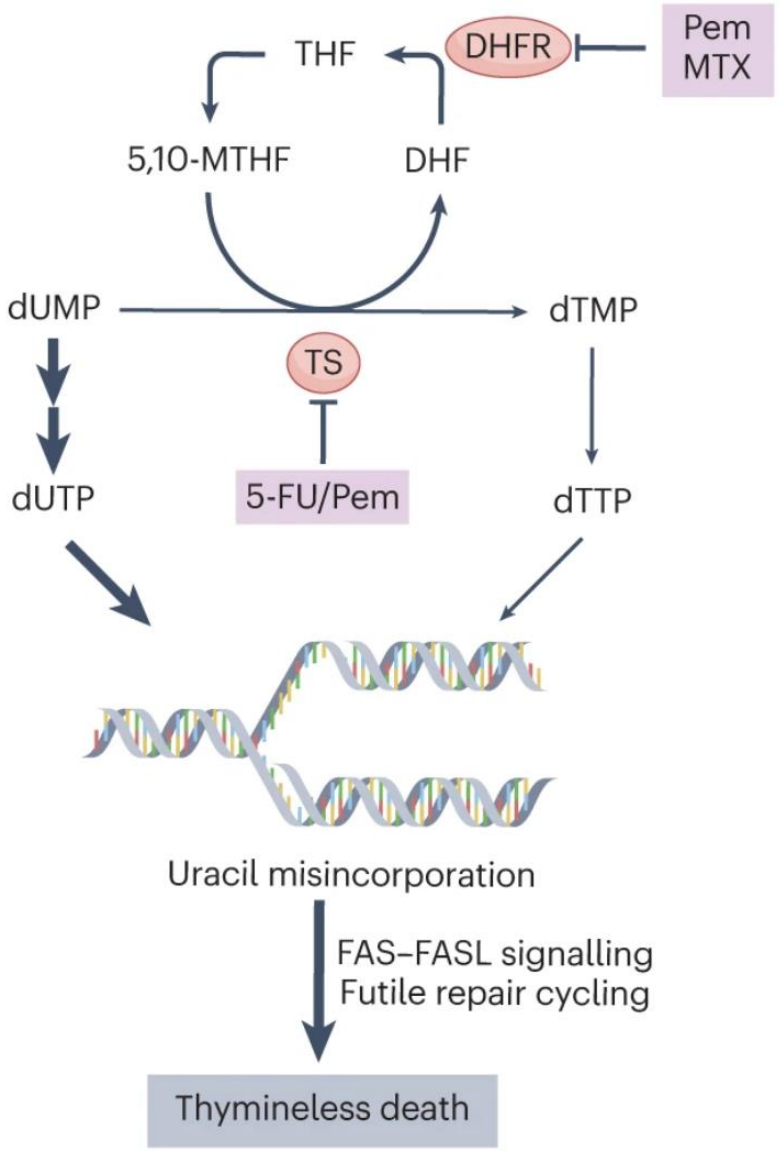
Azathioprine (Imuran)
Type: Purine analogue.
Use: Immunosuppressive therapy (organ transplantation, autoimmune diseases).
Mechanism: Incorporates into DNA and inhibits replication, reducing immune cell proliferation.

How nucleotides fuel cancer cell growth and proliferation?

Deoxythymidine triphosphate (dTTP) is required for the synthesis of DNA.

Depletion of dTTP by the inhibition of thymidine synthase (TS) results in the accumulation of deoxyuridine triphosphate (dUTP) and an increase in the dUTP-to-dTTP ratio.

As DNA polymerases cannot distinguish dUTP from dTTP, this leads to widespread misincorporation of uracil and a massive DNA damage response, ultimately resulting in thymineless death.



Formation of pyrimidine deoxyribonucleotide triphosphates

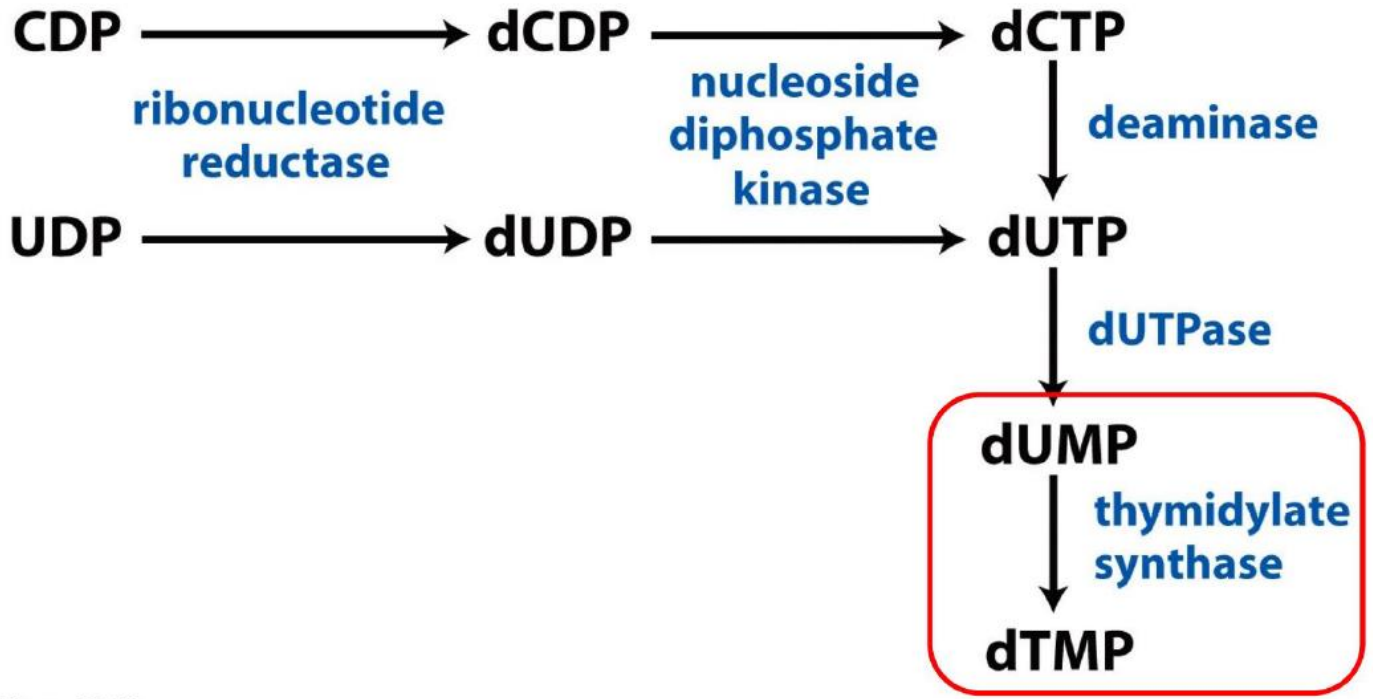
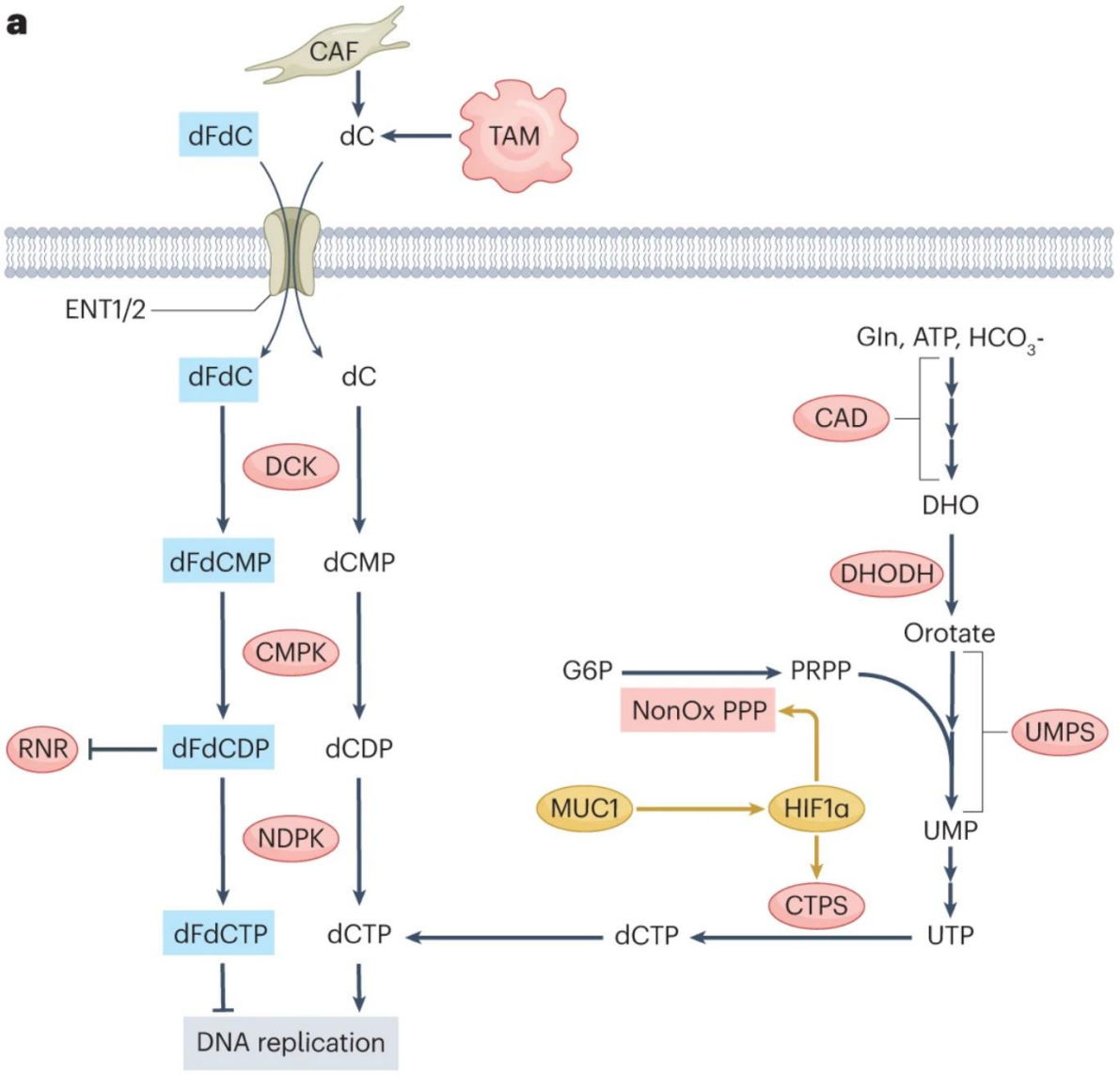


Figure 22-43
Lehninger Principles of Biochemistry, Fifth Edition

1. Synthesis of Pyrimidine Ring
2. Attachment to Ribose Phosphate
3. Phosphorylation

Hyperactive nucleotide synthesis confers resistance to a range of therapeutic interventions

Gemcitabine, or 2′,2′-difluorodeoxycytidine (dFdC), is in molecular competition with endogenous deoxycytidylate species at every stage of its metabolism, from uptake to phosphorylation to incorporation into elongating nascent DNA.

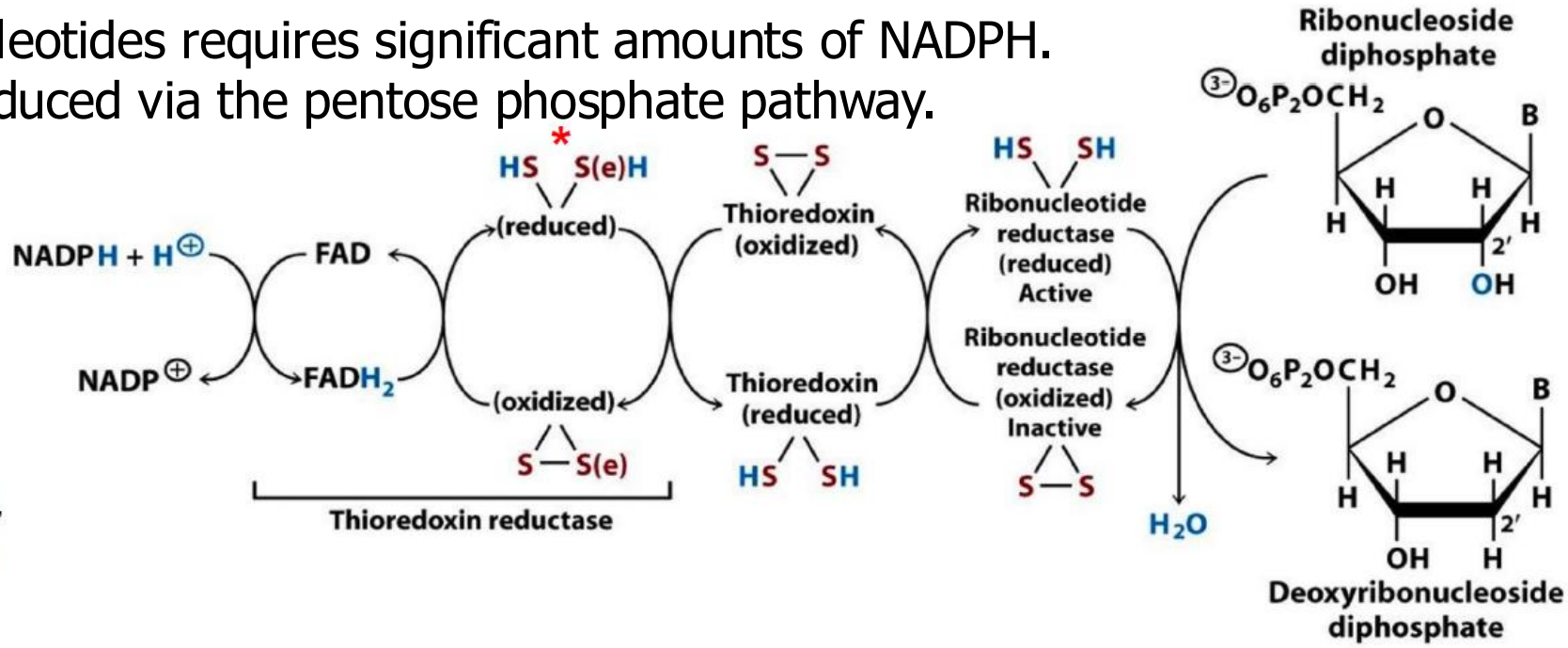


Biosynthesis of deoxynucleotides

The synthesis of deoxyribonucleotides (= reduction of the 2´-OH group of ribose) takes place at the level of nucleoside diphosphates

The synthesis of deoxyribonucleotides involves the reduction of the 2’-OH group of ribose at the nucleoside diphosphate level (NDP to dNDP).

- **Electron Transfer**:
  - Reducing electrons are sourced from **NADPH (*consumes a lot of bound NADPH*).**
  - These electrons are transferred through thioredoxin reductase and thioredoxin to ribonucleotide reductase, which catalyzes the final reduction of the 2’-OH group.
- **NADPH Consumption**:
  - The synthesis of deoxynucleotides requires significant amounts of NADPH.
  - NADPH and ribose are produced via the pentose phosphate pathway.

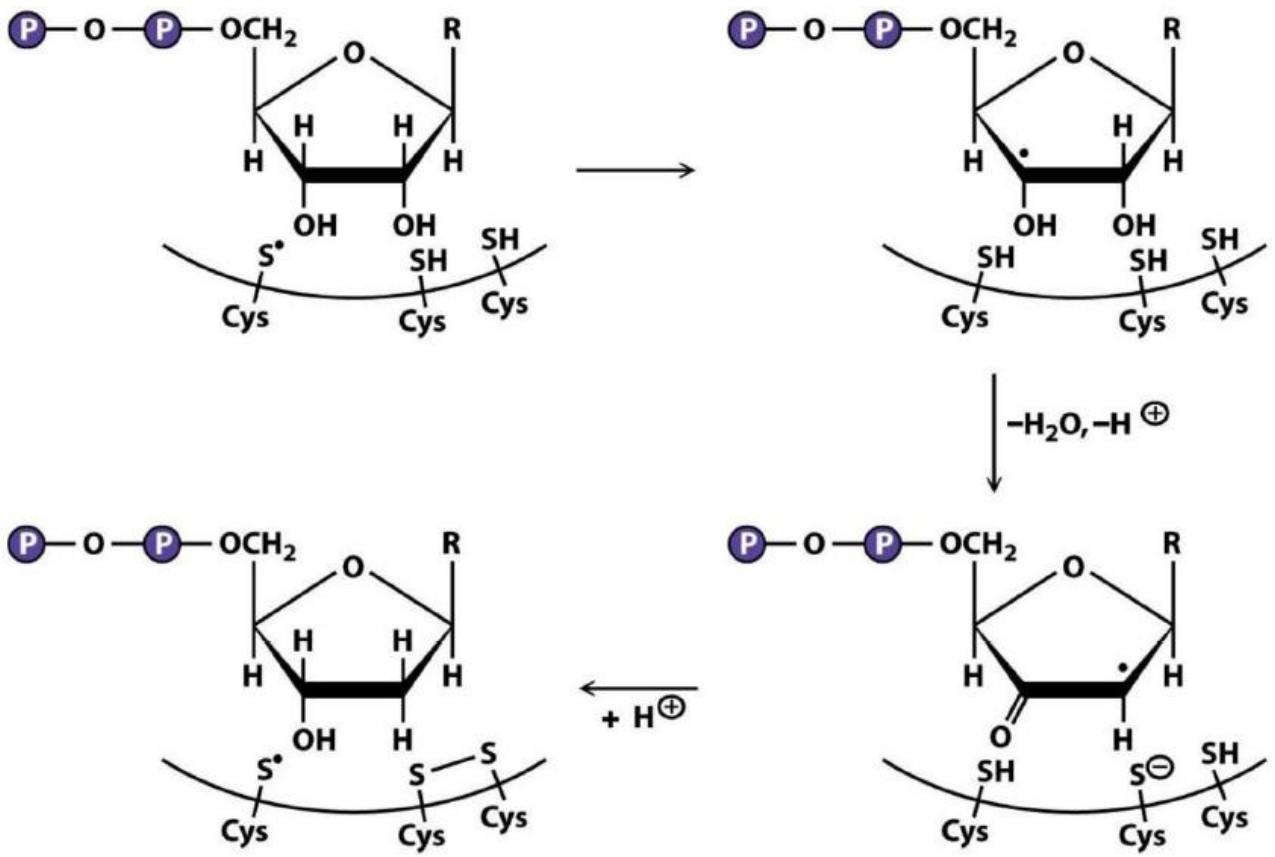


*Note: selenocysteine, “The 21st. amino acid

Figure 18-14 Principles of Biochemistry, 4/e

Ribonucleotide reductase

- The reaction mechanism involves a rare radical reaction, where a radical is a structure with an unpaired electron.
- The reactive tyrosine radical in the R2 subunit initiates the process. This induces the formation of a radical first at cysteine and then at the ribose ring.
- The 2’-OH group is released as water. Cysteines are oxidized to form cystine.
- The enzyme is regenerated via thioredoxin through disulfide exchange reactions.



Box 18-3 Principles of Biochemistry, 4/e
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Allosteric regulation of ribonucleotide reductase

- Quite sophisticated allosteric regulation
- The regulation of enzyme activity and substrate specificity is controlled at two different points, ensuring balanced concentrations of substrates and products.

TABLE 18.1 Allosteric regulation of eukaryotic ribonucleotide reductase

Ligand bound to activity site	Ligand bound to specificity site	Activity of catalytic site
dATP		Enzyme inactive
ATP	ATP or dATP	Specific for CDP or UDP
ATP	dTTP	Specific for GDP
ATP	dGTP	Specific for ADP

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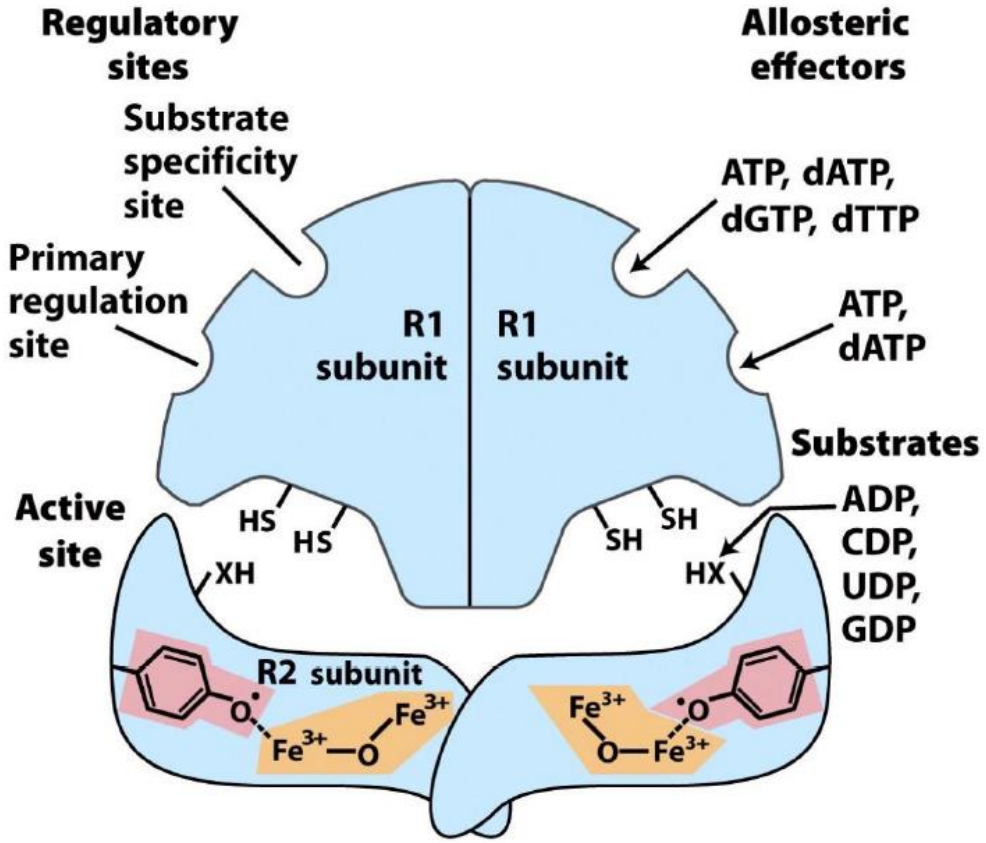

Figure 22-40a
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Thymidylate synthase

- **dUMP to dTMP**:
  - Methylation of the 5-carbon of the uracil ring.
- **Methyl Group Donor**:
  - N5,N10-methylene-THF acts as the methyl group donor and is oxidized to dihydrofolate in the reaction.
- **Coenzyme Regeneration**:
  - Catalyzed by dihydrofolate reductase (reduces dihydrofolate to tetrahydrofolate) and serine hydroxymethyltransferase (transfers methylene group to THF).
- **Thymidylate's Uniqueness**:
  - Thymidylate is unique to DNA.
  - Its synthesis is the only reaction where THF is oxidized to dihydrofolate, making it a good target for cancer drugs.

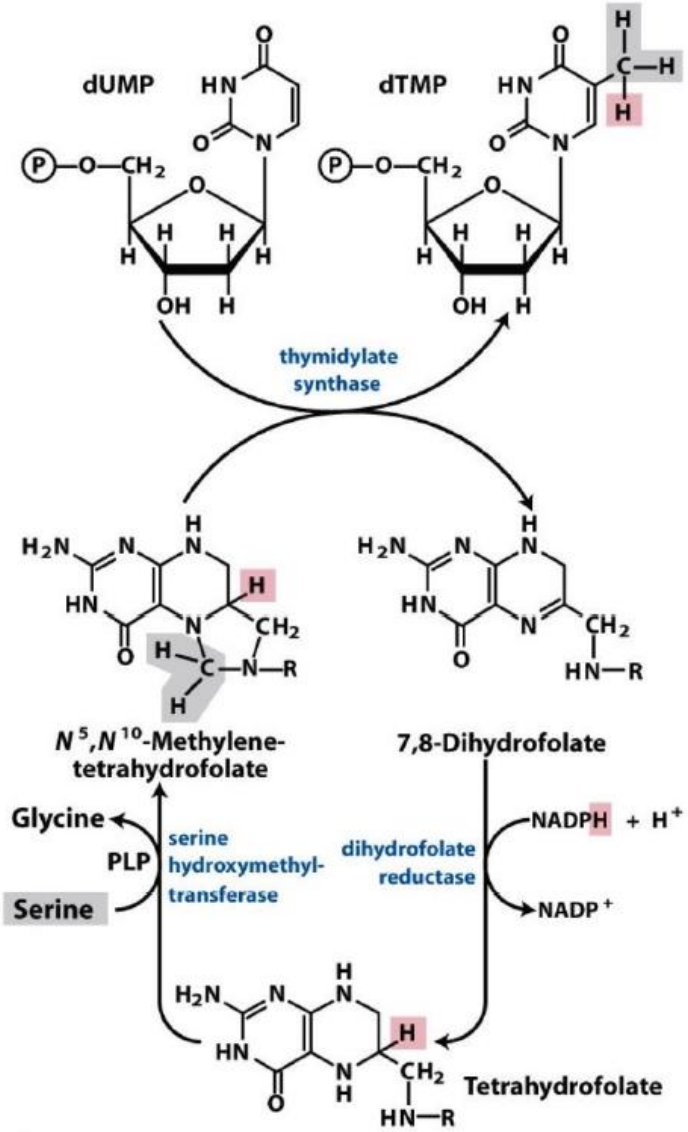

Figure 22-44
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Synthesis of thymidylate (dTMP) as a drug target

FdUMP and methotrexate cell blockers,
trimethoprim antibiotic

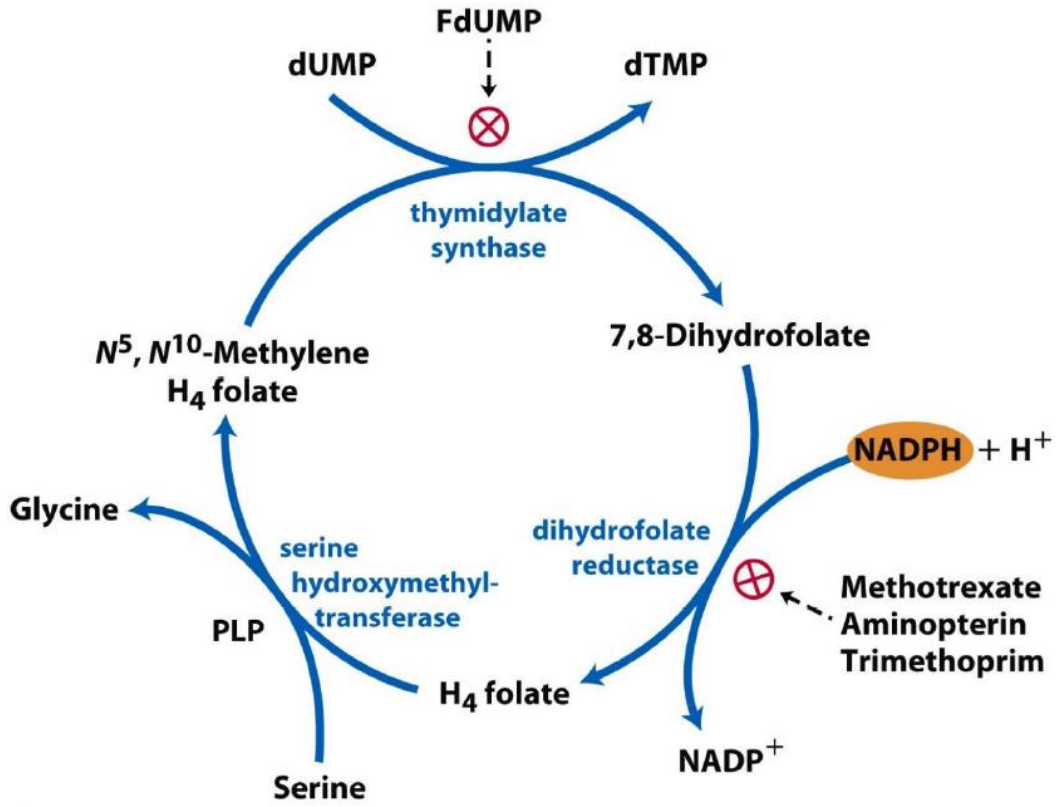

Figure 22-49a
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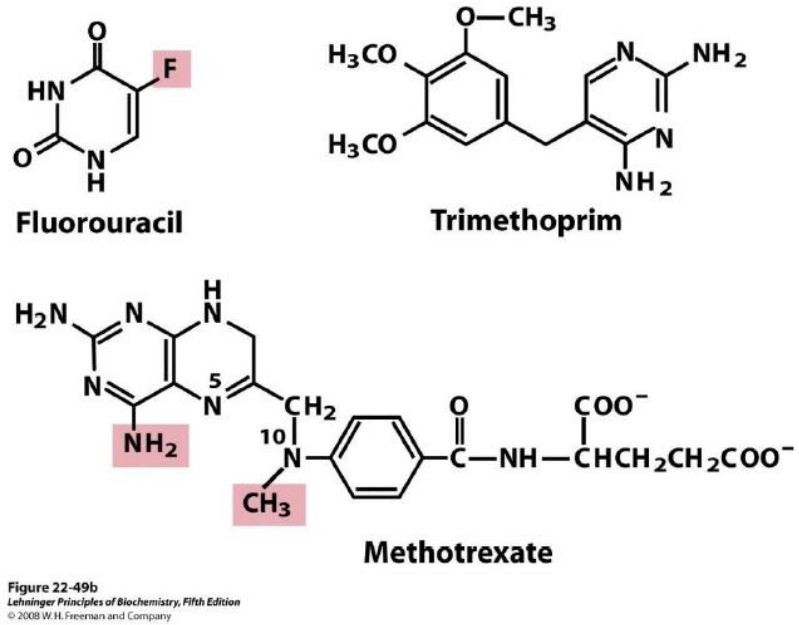

Figure 22-49b
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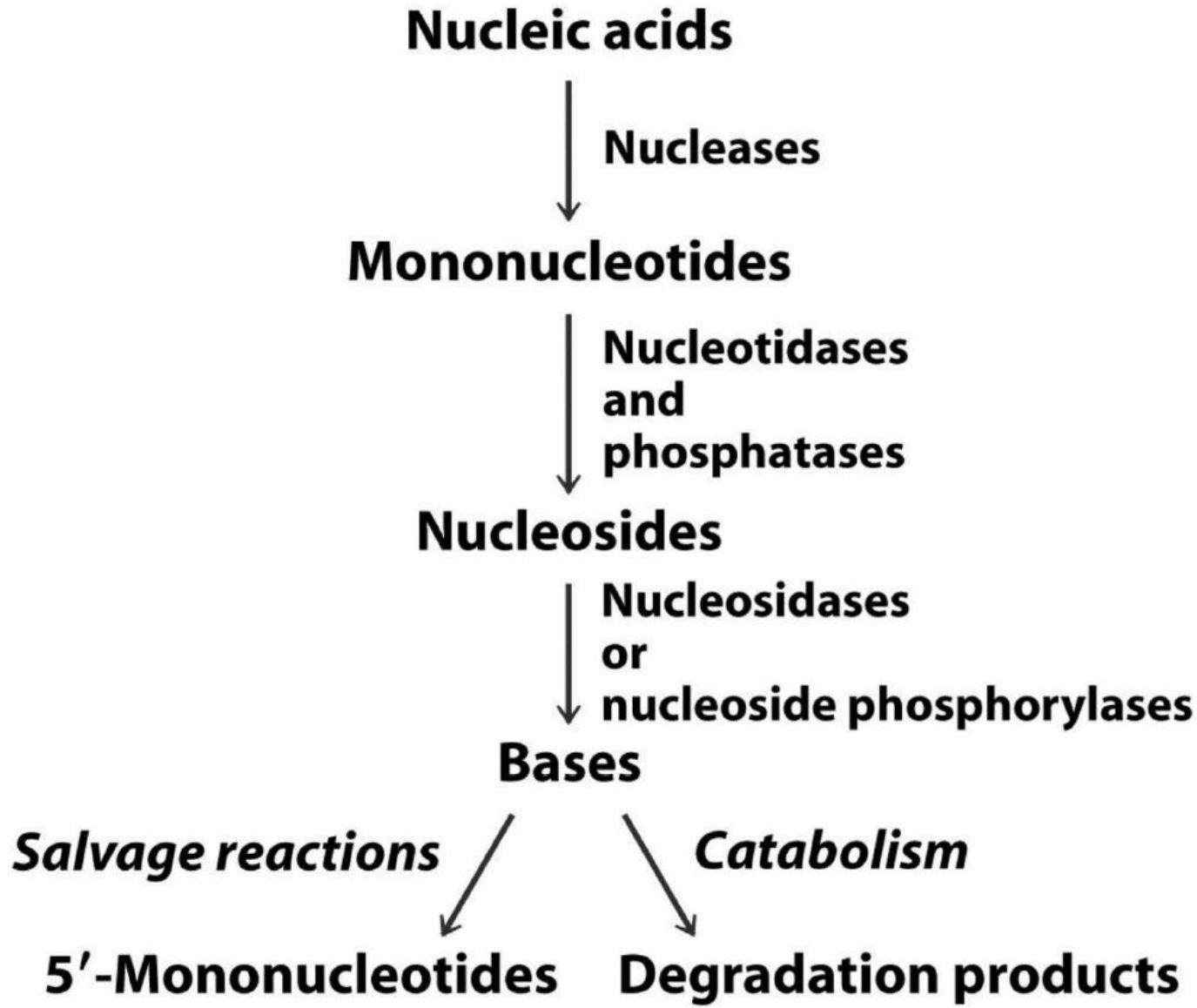
Degradation and recycling of nucleic acids and nucleotides



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Decomposition and recycling

Cells reuse the breakdown products of nucleic acids in nucleotide synthesis

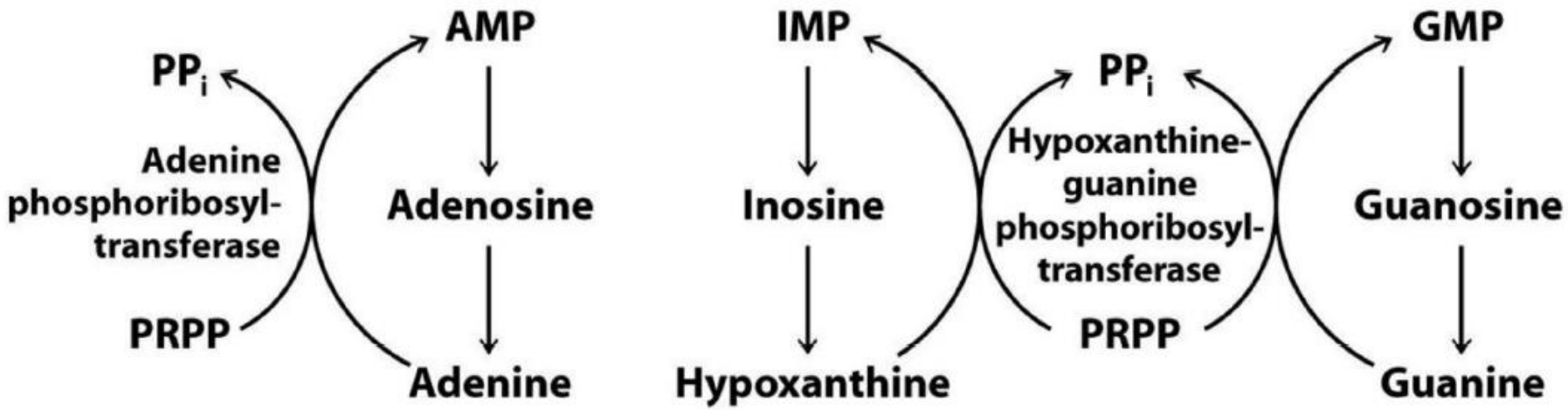
Purine Recycling:

Free purine bases are added to ribose phosphate to form mononucleotides (AMP, GMP, and IMP).

Phosphorylation:

Nucleoside kinases phosphorylate mononucleotides into di- and trinucleotides.

ATP acts as the donor of phosphoryl groups.



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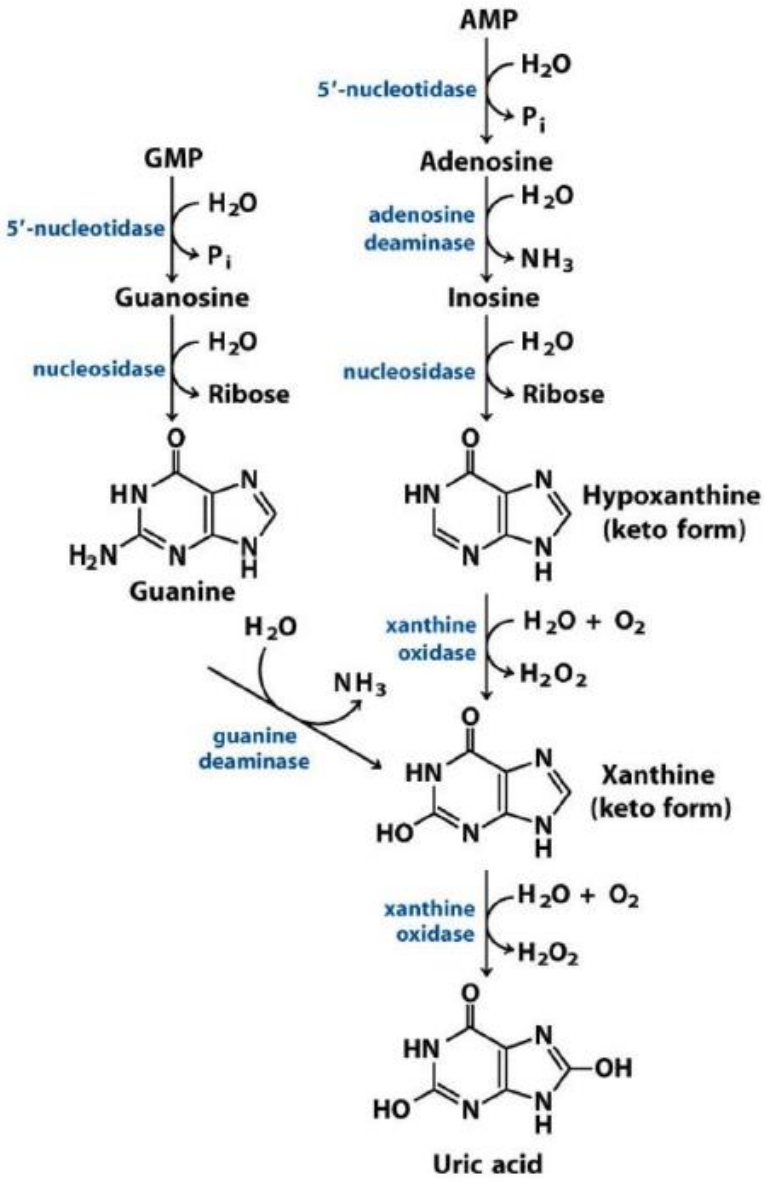
Purine catabolism

- **Uric Acid**: uric acid, as an end product in humans, is relatively sparingly soluble.
- **Crystallization**: it may crystallize when plasma urate concentration increases leading to conditions such as gout and kidney stones.



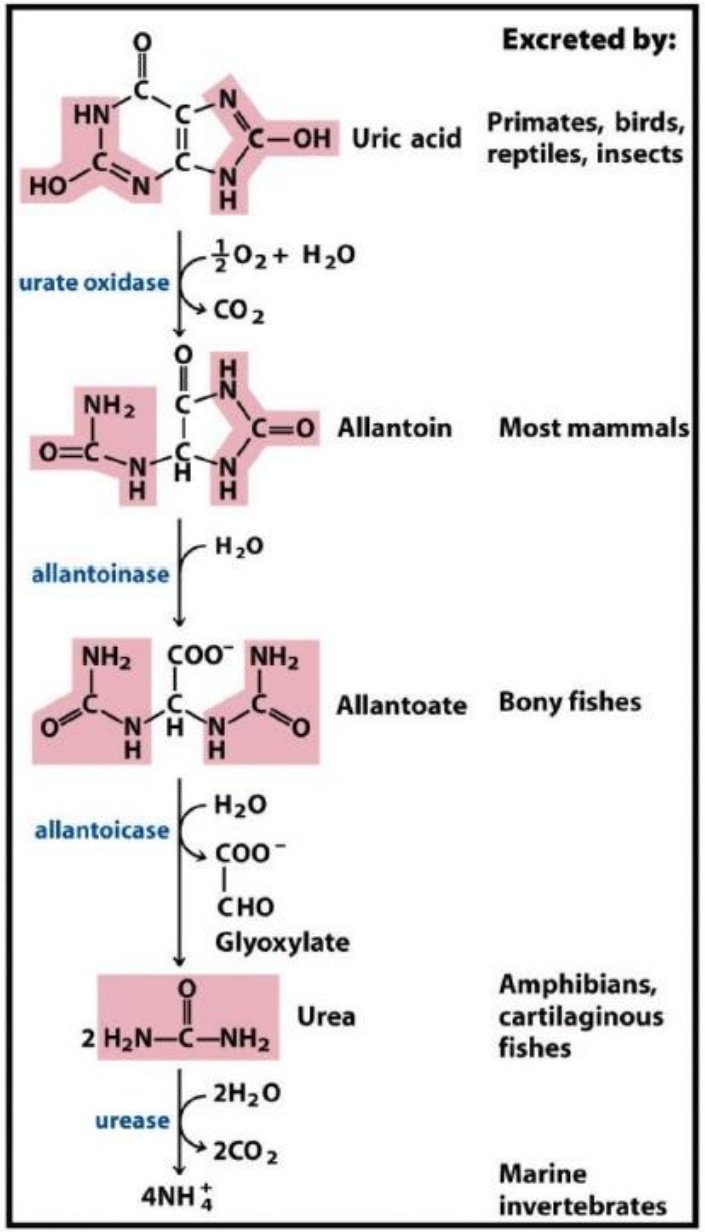


Figure 22-45
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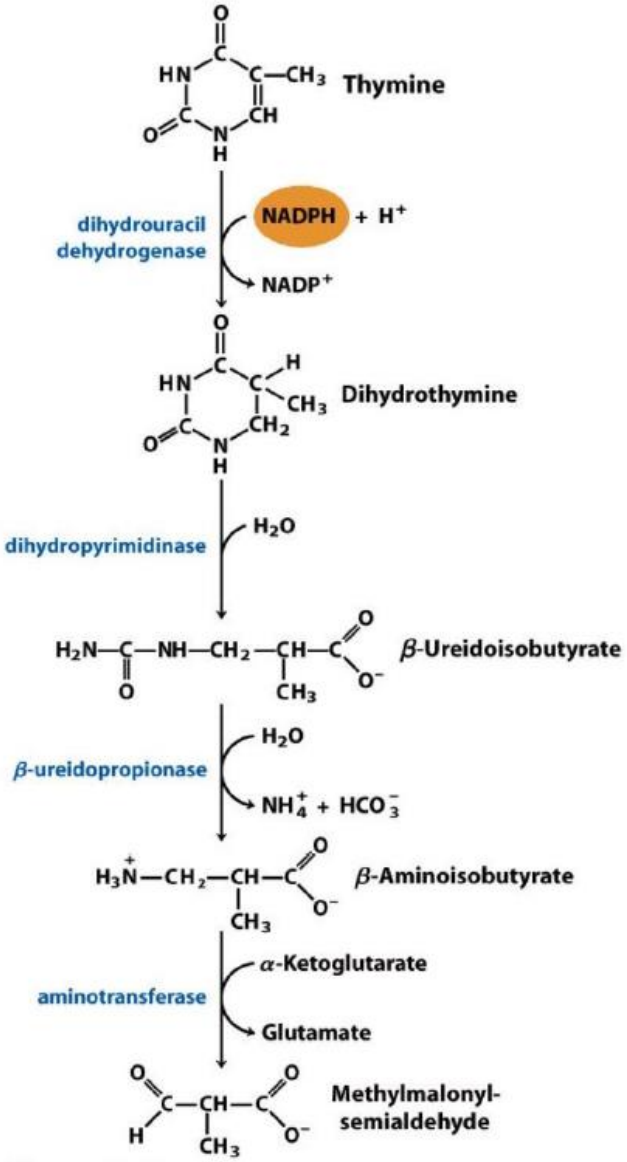
Thymine catabolism

- **Methylmalonylsemialdehyde**: Processed into succinyl-CoA, which enters the TCA cycle.
- **CMP Catabolism**: CMP is converted to cytidine, then to uridine, and finally to uracil.
- **Cytosine and Thymine Catabolism**: Cytosine is converted to cytidine, then to β-alanine, and further to malonatesemialdehyde, which enters the TCA cycle and is broken down to H2O and CO2. Thymine follows a similar pathway.
- **Nitrogen Disposal**: The nitrogens from thymine and cytosine are converted to ammonia (NH4+) and glutamate, which are then processed into urea via the urea cycle.



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Other roles of nucleotides and nucleotide derivatives

Coenzyme A

- Contains adenosine
- activation of acyl groups for synthetic reactions: e.g. acetyl-CoA, palmityl-CoA
- the acyl group is connected by a thioester bond to the thiol group of the mercaptoethylamine end

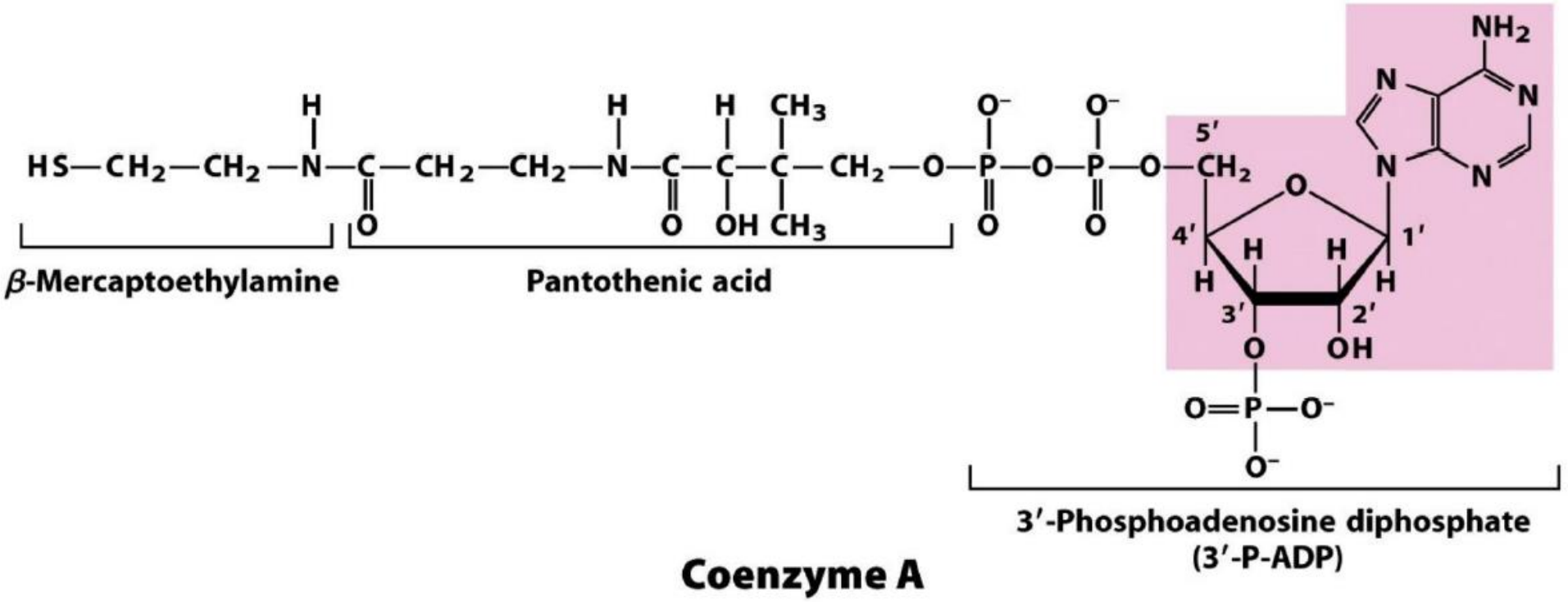

Figure 8-38 part 1
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Nicotinamide Adenosine Dinucleotide (NAD+)

- contains another nucleotide in addition to adenosine (nicotinamide nucleotide)
- as a coenzyme in oxidation/reduction reactions (reduced form NADH)
- transfers two electrons at a time (as a hydride ion)

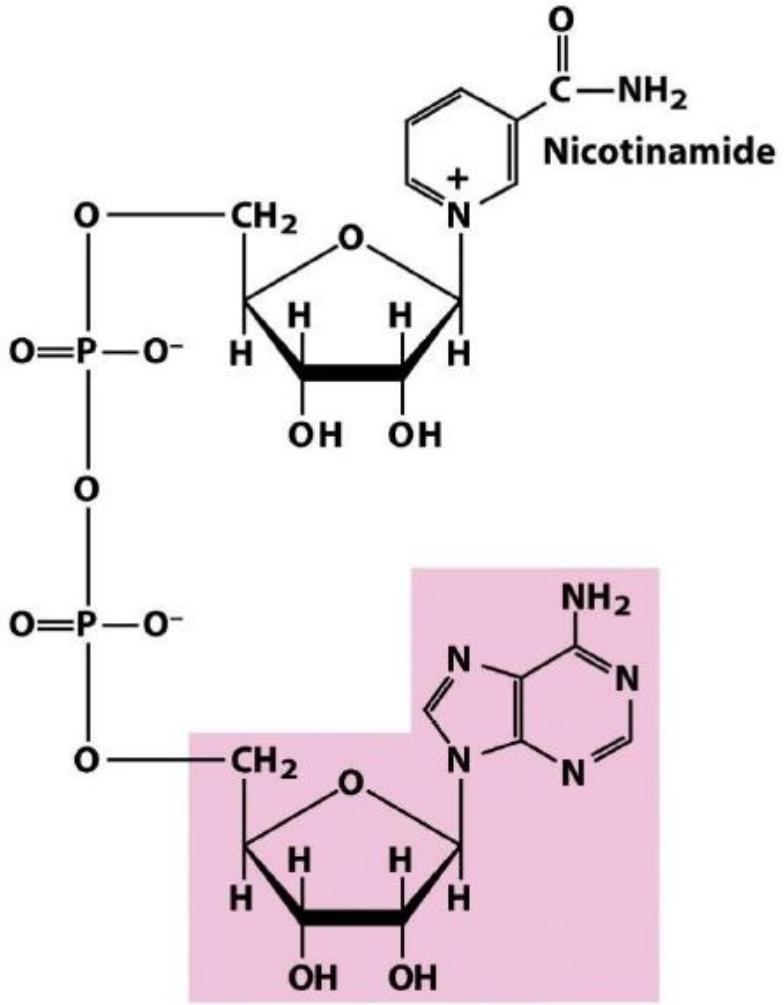

Figure 8-38 part 2
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Flavin adenine dinucleotide (FAD)

- contains, in addition to adenosine, another nucleotide (riboflavin nucleotide; instead of ribose ribitol in linear form).
- as a cofactor in oxidation/reduction reactions (reduced form FADH2).
- can transfer one or two electrons (and protons) at a time

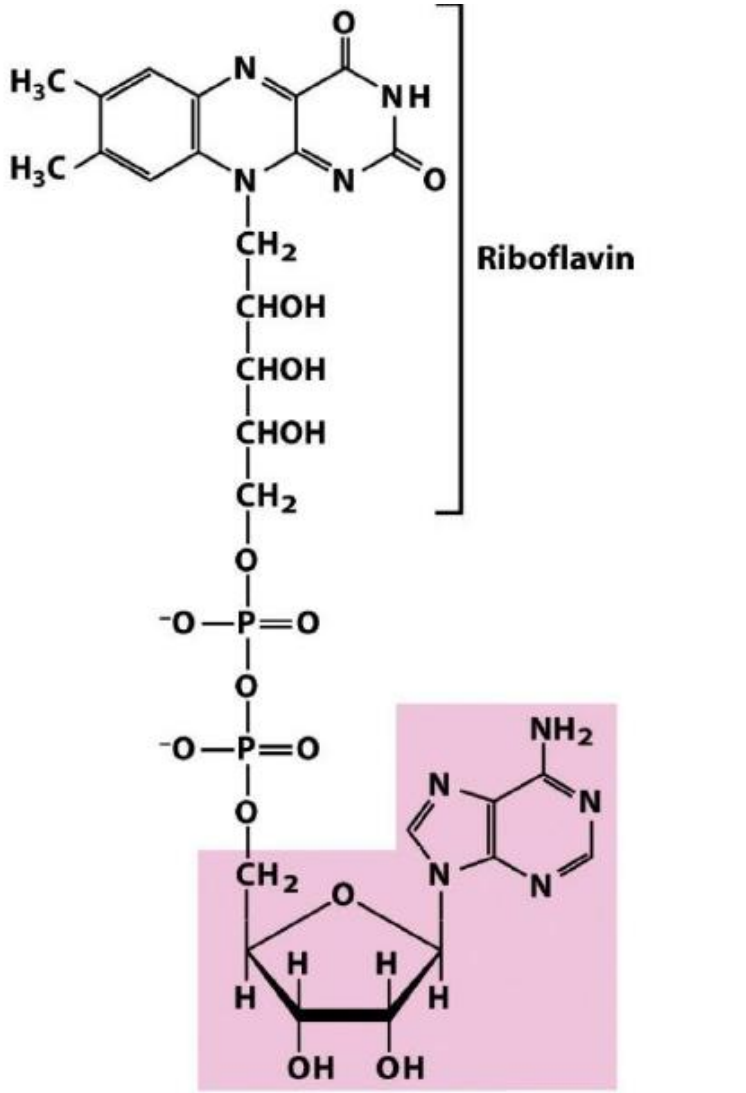

Figure 8-38 part 3
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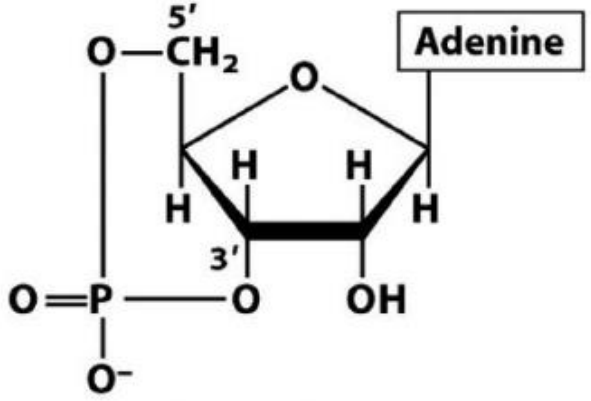
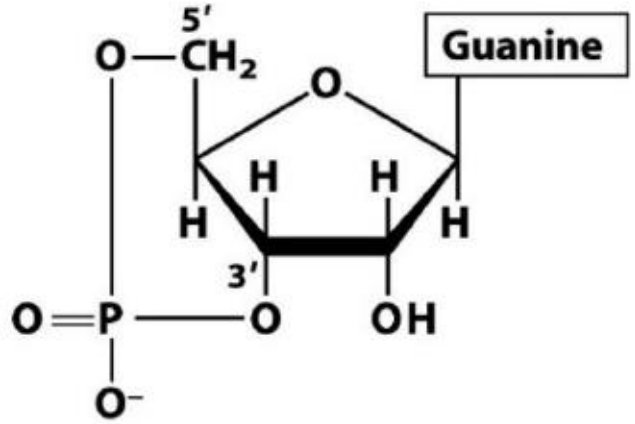
Cyclic nucleotides as secondary transmitters of cell communication



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



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

Figure 8-39
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cyclic AMP (cAMP)
synthesis from ATP (adenylate cyclase)regulated by cell membrane receptors / Gproteins activates protein kinase A-> enzymes, ionchannels, transcription factorsregulation

Cyclic GMP (cGMP). synthesis from GTP by guanylate cyclase catalyzed by; cell membrane receptor sand nitric oxide (NO) activate the enzyme. rhodopsin in retinal phototransduction activates cGMP-degrading phosphodiesterase. cGMP regulates e.g. of protein kinases and function of ion channels

Summary

•**Nucleotide Synthesis Pathways**:
Purine and pyrimidine nucleotides are synthesized either from simple metabolites (de novo biosynthesis) or by reusing nucleosides and free bases from food or endogenous nucleic acids (salvage pathway).
•**Purine Biosynthesis**:
Gross net reaction (non-stoichiometric): PRPP + glutamine + glycine + formyl-THF + aspartate + ATP → purines.
•**Pyrimidine Biosynthesis**:
Glutamine + CO2 + aspartate + PRPP + ATP → UTP and CTP.
•**Purine Recycling**:
Hypoxanthine/guanine + PRPP → IMP/GMP + PPi (catalyzed by hypoxanthine-guanine phosphoribosyl transferase).
Adenine + PRPP → AMP + PPi (catalyzed by adenine phosphoribosyltransferase).
•**Pyrimidine Recycling**:
•'Uracil + PRPP → UMP + PPi.
Uracil/cytosine/thymine + ribose-1-phosphate → UMP/CMP/TMP + Pi (catalyzed by nucleoside phosphorylase).