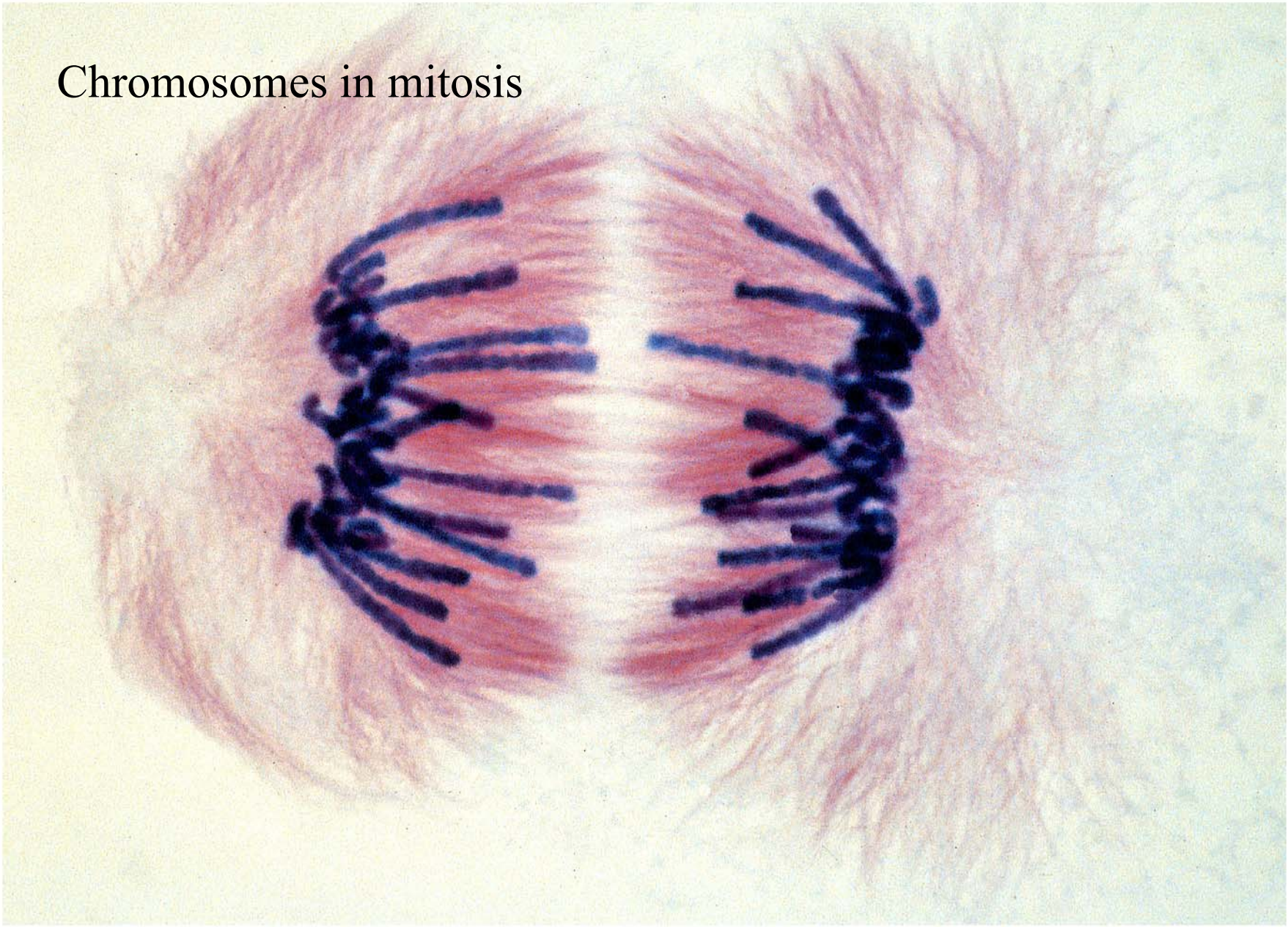
Chapter 10:
Nucleotide Metabolism

Chromosomes in mitosis



Synthesis of ribonucleotide
Purine
Pyrimidine

De novo synthesis
Salvage pathway

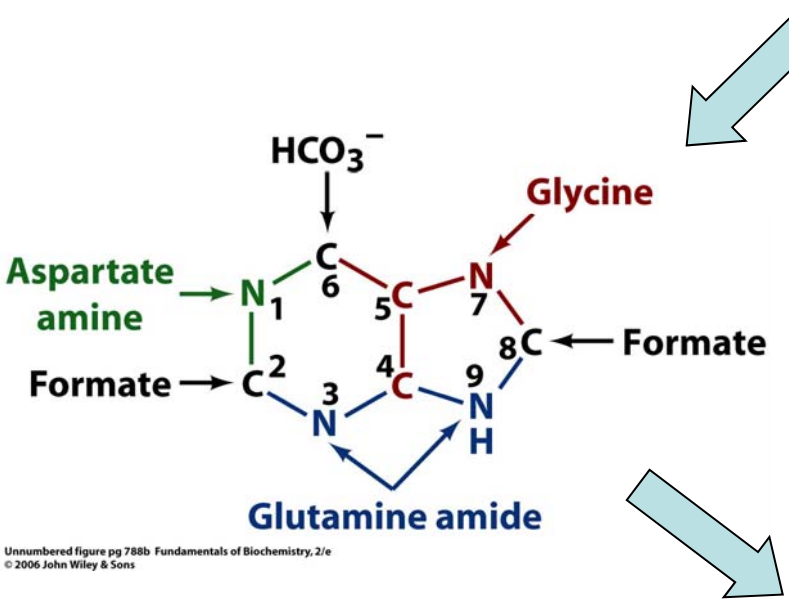
Synthesis of deoxyribonucleotide

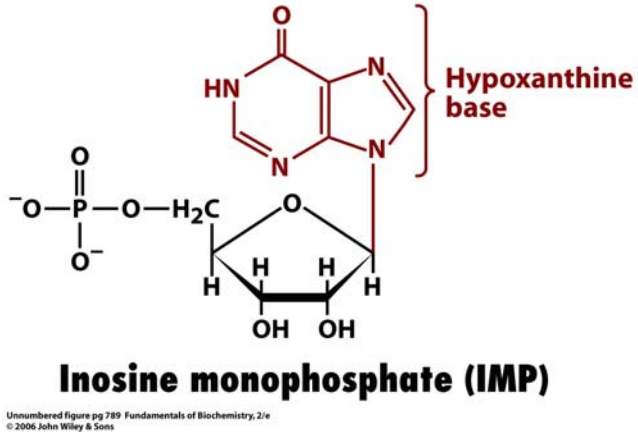
http://web.indstate.edu/thcme/mwking/nucleotide-metabolism.html

De novo synthesis of purine ribonucleotides

Isotopically labeled compounds →

→





The precursor of AMP & GMP

de novo synthesis of IMP

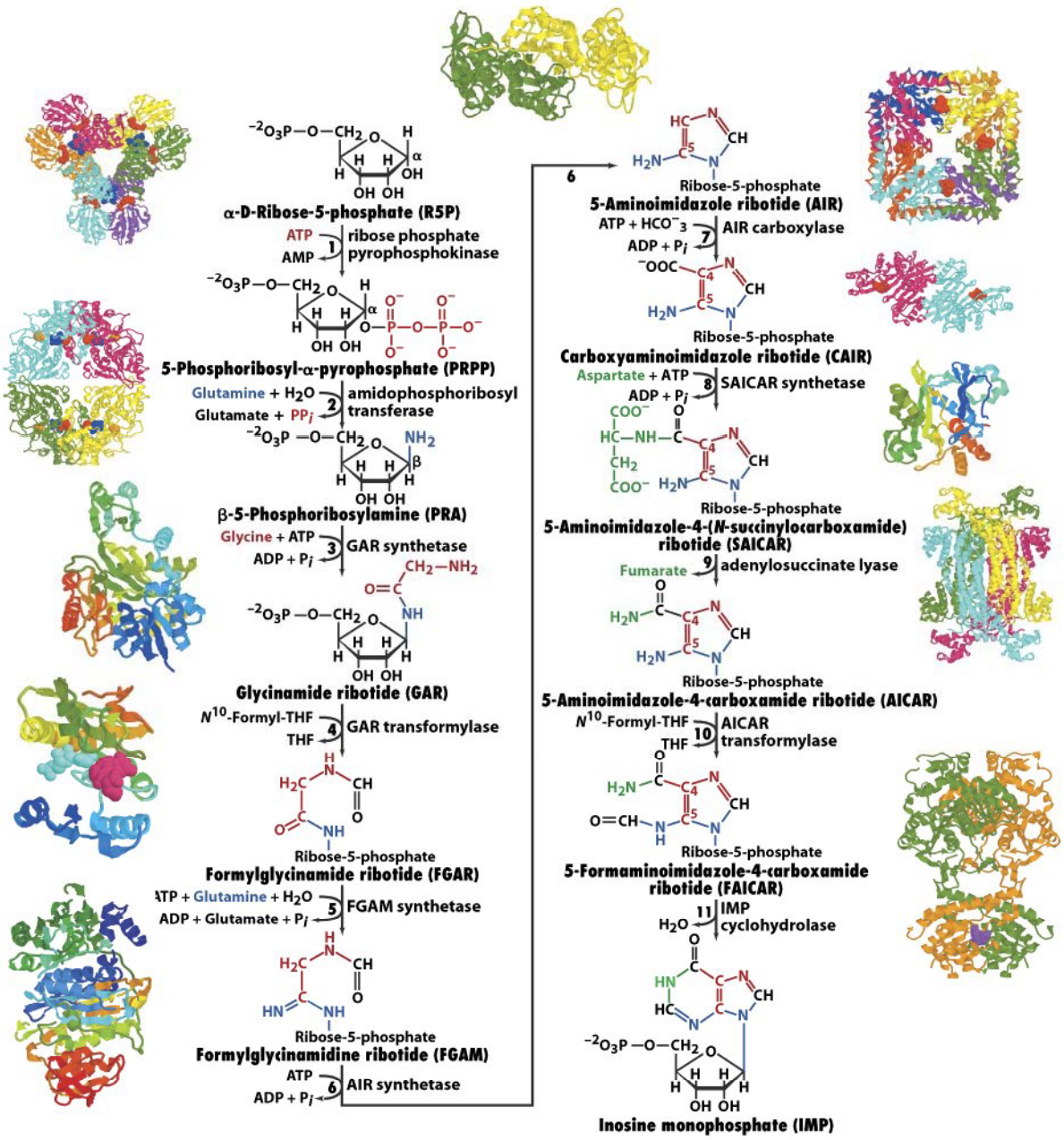


Figure 22-1 Fundamentals of Biochemistry, 2/e

Synthesis of adenine and guanine ribonucleotides

Rapid conversion of IMP to AMP & GMP in two-step reactions

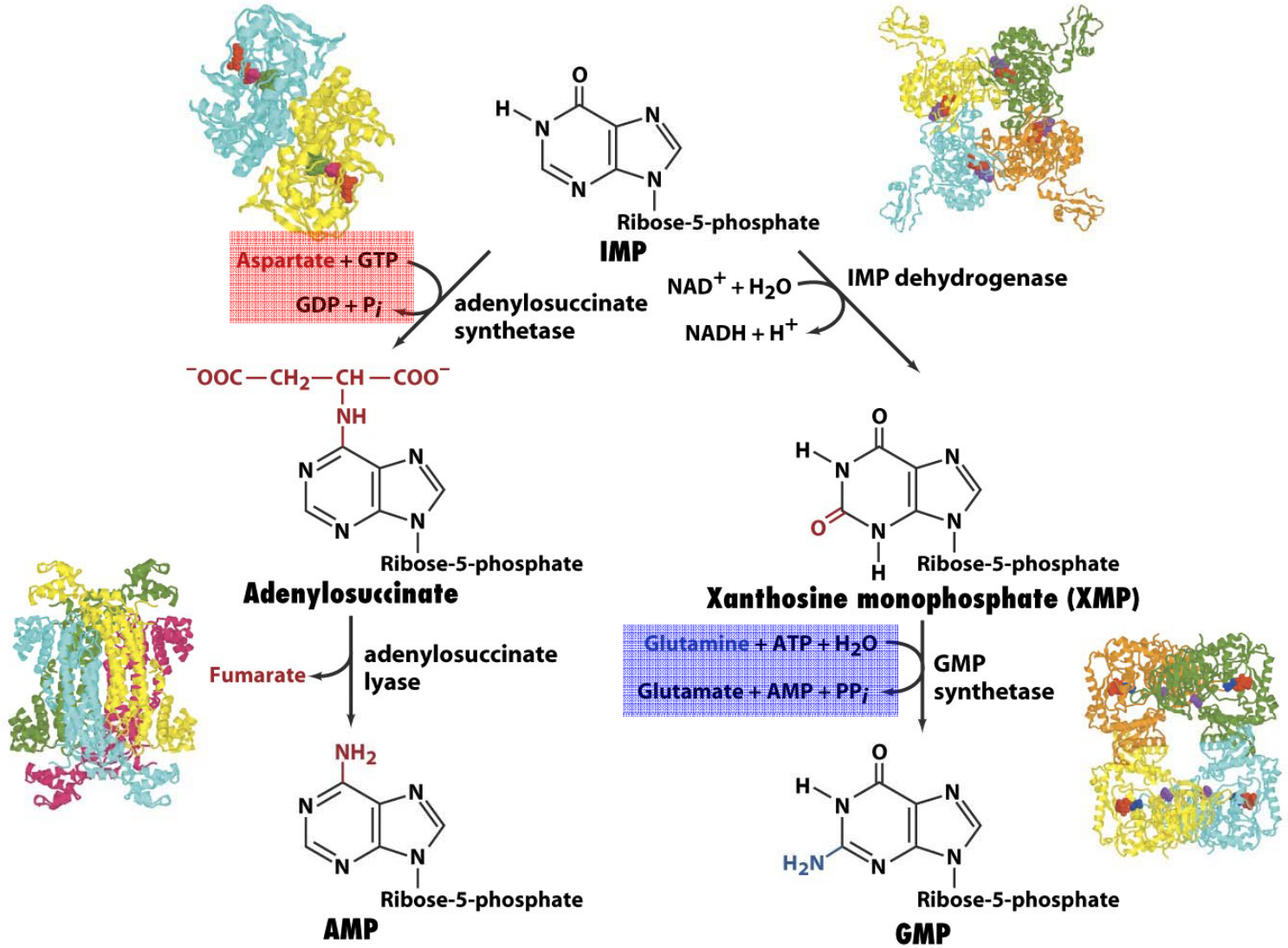
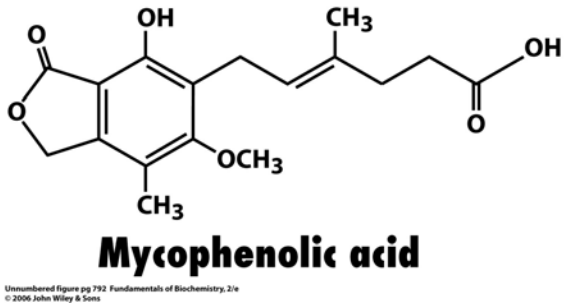


Figure 22-3 Fundamentals of Biochemistry, 2/e

Mycophenolic acid: fungal product

Inhibitor of IMP dehydrogenase in B & T cells

Immune suppressor



Synthesis of di- and triphosphates

Nucleoside monophosphate kinases (don't discriminate between ribose and deoxyribose)

Adenylate kinase: AMP + ATP ↔ 2 ADP

Guanylate kinase: GMP + ATP ↔ GDP + ADP

Nucleoside diphosphate kinase (no preference for bases or for ribose over deoxyribose)

GDP + ATP ↔ GTP + ADP

Regulation of purine nucleotide biosynthesis

Total amounts of purine nucleotides as well as the relative amounts of ATP and GTP

1. Before the branch point
   - Independent and synergistic control by the levels of adenine and guanine nucleotides
   - PRPP (the 1st step): inhibited by both ADP and GDP
   - 5-phosphoribosylamine (the committed step):
     - different binding sites for ATP/ADP/AMP and GTP/GDP/GMP
   - Feed forward activation by PRPP
2. Below the branch point

AMP and GMP are competitive inhibitors of IMP

Reciprocal balance of both purines: coordinated synthesis

- GMP increases with [ATP]
- AMP increases with [GTP]

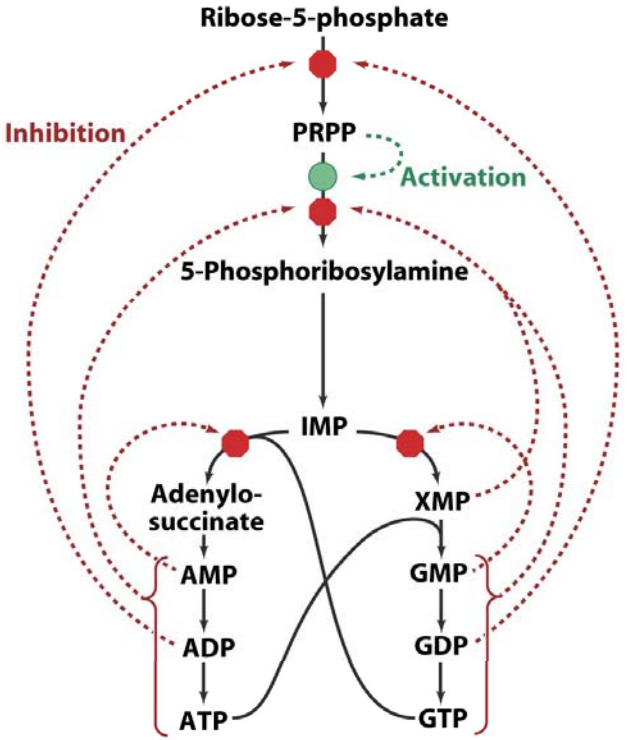


Figure 22-4 Fundamentals of Biochemistry, 2/e

Salvage pathway of purines

Adenine phosphoribosyltransferase (APRT)

Adenine + PRPP ↔ AMP + PPi

Hypoxanthine-guanine phosphoribosyltransferase (HGPRT): higher in brain

hypoxanthine + PRPP ↔ IMP + PPi

guanine + PRPP ↔ GMP + PPi

Lesch-Nyhan syndrome: HGPRT deficiency

X-linked recessive

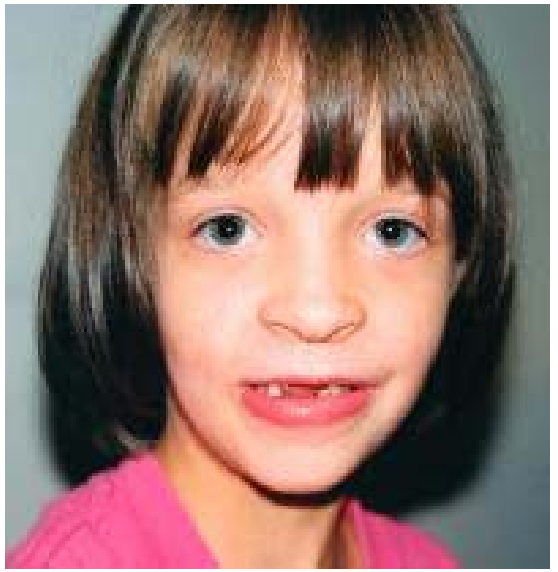
Self-mutilation

Excess uric acid production:

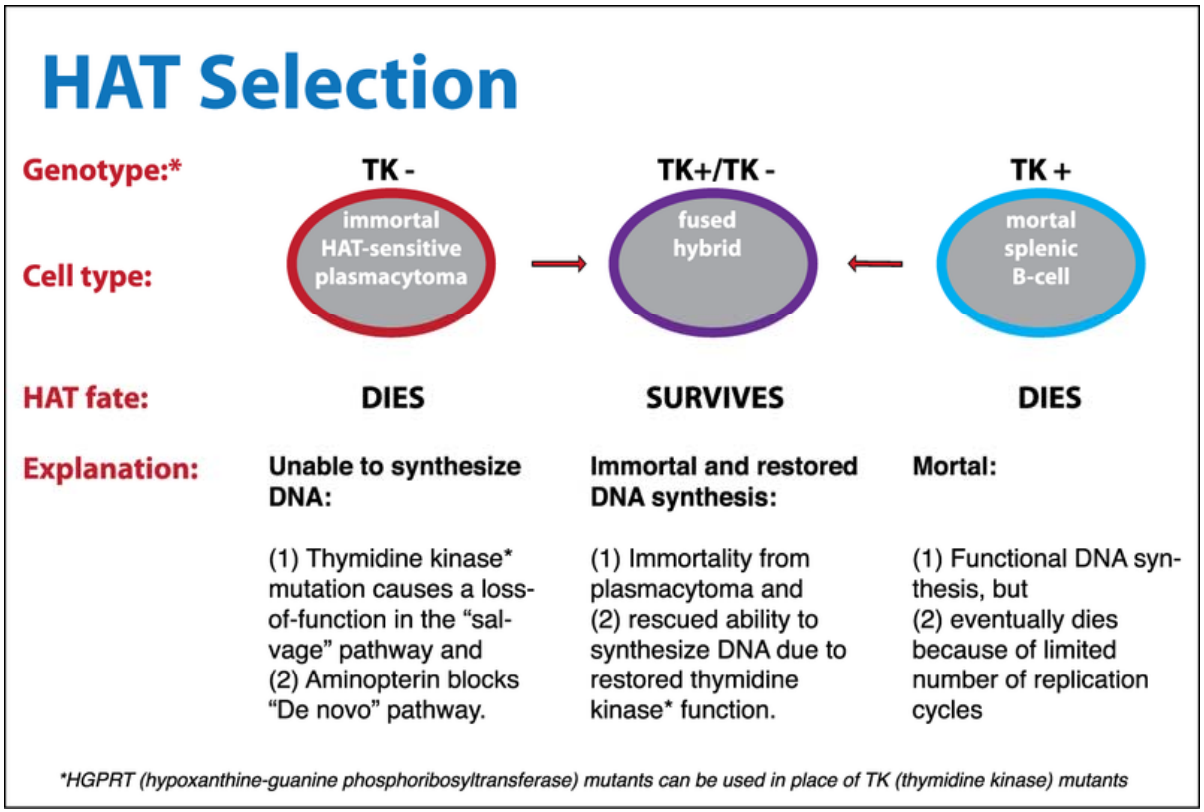
Accumulation of PRPP

Activate amidophosphoribosyl transferase

Accelerated synthesis of purine nucleotides



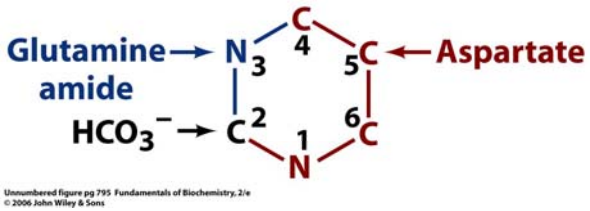
HAT Medium (**Hypoxanthine Aminopterin Thymidine medium**)
Selection of hybridoma cells producing monoclonal Ab



DHFR: H4F from H2F, inhibited by aminopterin
TK: TMP from thymidine
HGPRT: IMP from hypoxanthine

Synthesis of pyrimidine ribonucleotides

Synthesis of UMP



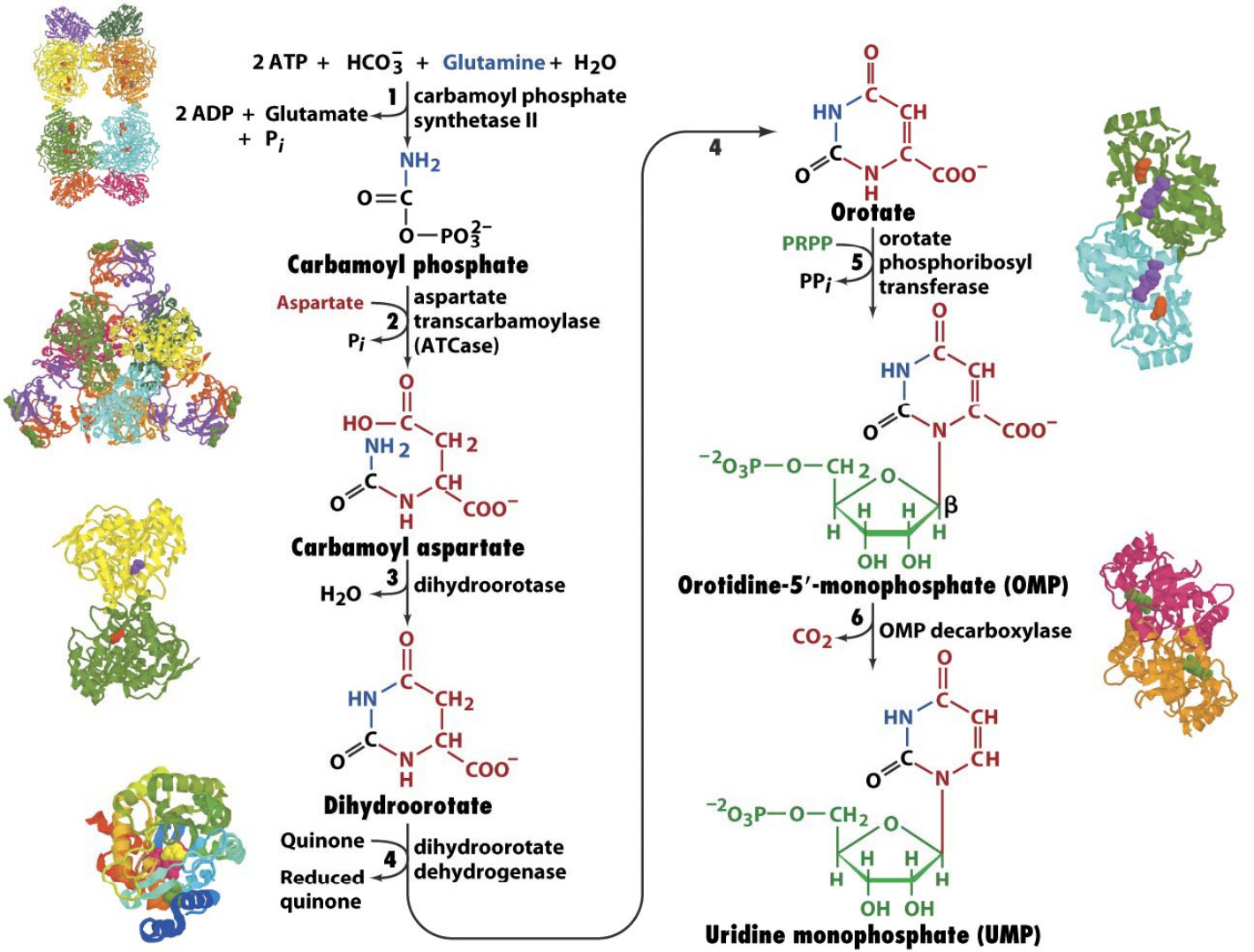


Figure 22-5 Fundamentals of Biochemistry, 2/e
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Biosynthetic enzymes

6 bacterial enzymes

Animal enzymes: a single 210-kD polypeptide for the first 3 steps
(carbamoyl phosphate synthetase, ATCase, dihydroorotase)

Target for antiparasitic drugs

- Toxoplasmosis by *Toxoplasma gondii*
- Depends on nutrients supplied by hosts but has de novo uracil synthesis
- Target parasite's carbamoyl phosphate synthetase II, which differs from animal enzymes

Toxoplasma gondii

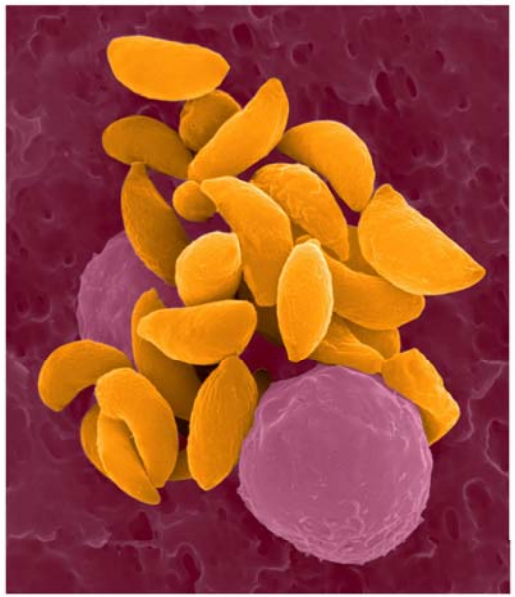


Figure 22-6 Fundamentals of Biochemistry, 2/e

Salvage synthesis

Uracil to UMP — Uridine phosphorylase: uracil + ribose-1-P → uridine + Pi
Uridine kianse: uridine (or cytidine) + ATP → UMP + ADP

Thymine to dTMP — Thymine phosphorylase: thymine + deoxyribose-1-P → thymidine + Pi
Thymidine kinase: thymidine + ATP → dTMP + ADP

Deoxycytidine kinase: deoxycytidine + ATP → dCMP + ADP

Synthesis of UTP and CTP

Same as purine nucleoside triphosphates

UMP + ATP ↔ UDP + ADP (nucleoside monophosphate kinase)
UDP + ATP ↔ UTP + ADP (nucleoside diphosphate kinase)

CTP synthetase: amination of UTP to CTP

The source of amino group (glutamine in animal, ammonia in bacteria)

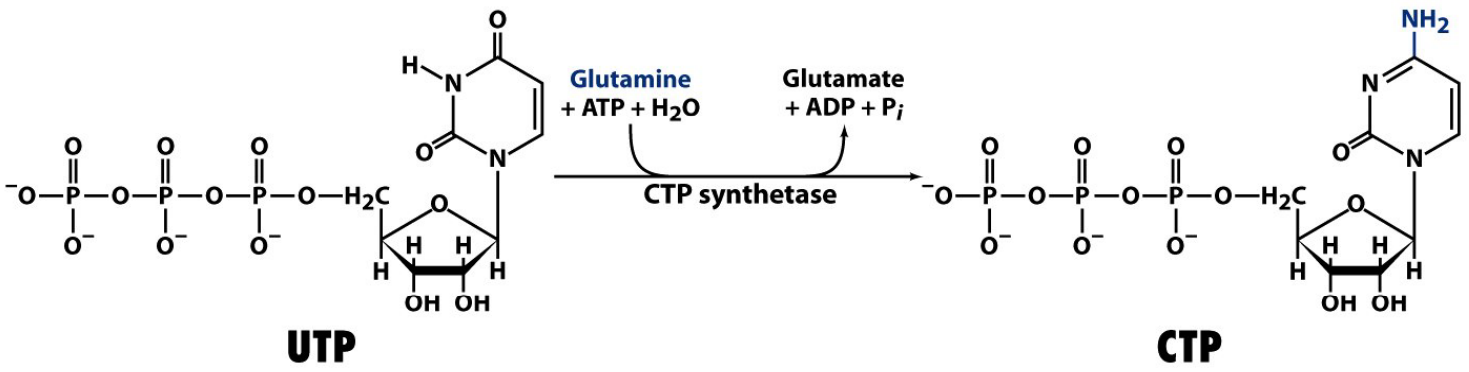


Figure 22-7 Fundamentals of Biochemistry, 2/e

Regulation of Pyrimidine nucleotide biosynthesis

Bacteria: allosteric regulation of ATCase
Animals:
  allosteric regulation of
    carbamoyl phosphate synthetase II
  OMP decarboxylase: competitive inhibition
    by UMP & CMP

<u>Orotic aciduria</u>
Deficiency of bifunctional enzyme (step 5 & 6)
Retarded growth and severe anemia
Administration of uridine/cytidine
Converted to UMP by phosphorylation
Inhibits carbamoyl phosphate synthetase II

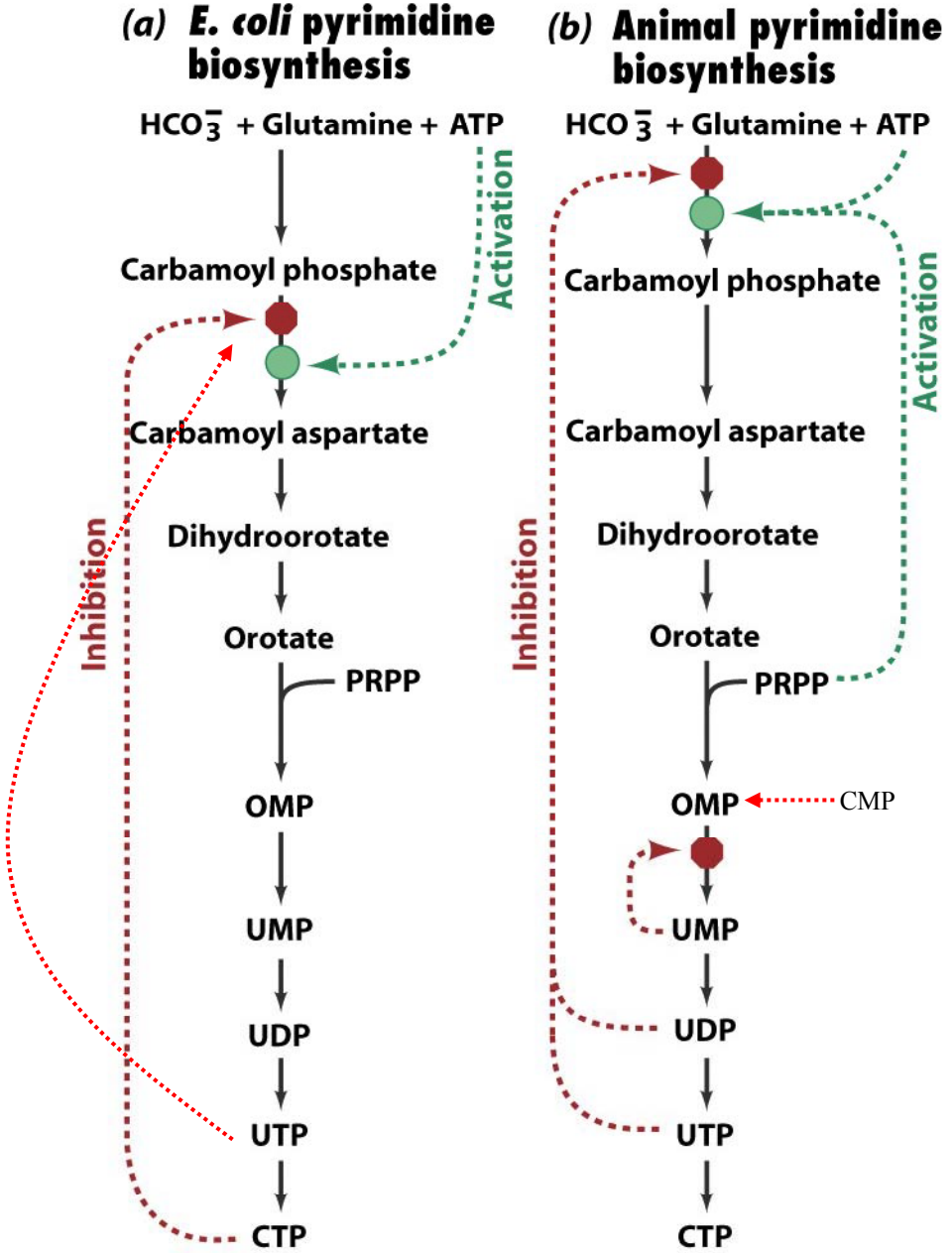


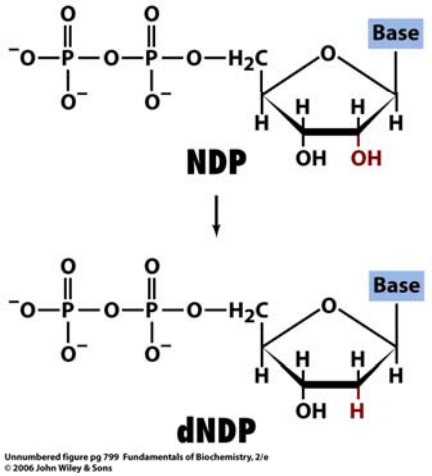
Figure 22-8 Fundamentals of Biochemistry, 2/e

Formation of deoxyribonucleotides

DNA has 2'-deoxyribose & thymine (5-methyluracil)

Production of deoxyribose residues

Ribonucleotide reductases (RNRs)
3 classes: differ in their prosthetic groups
Class I RNRs (most eukaryotes and aerobic prokaryotes)
Heterotetramer: inactive heterodimeric $R1_2$ and $R2_2$



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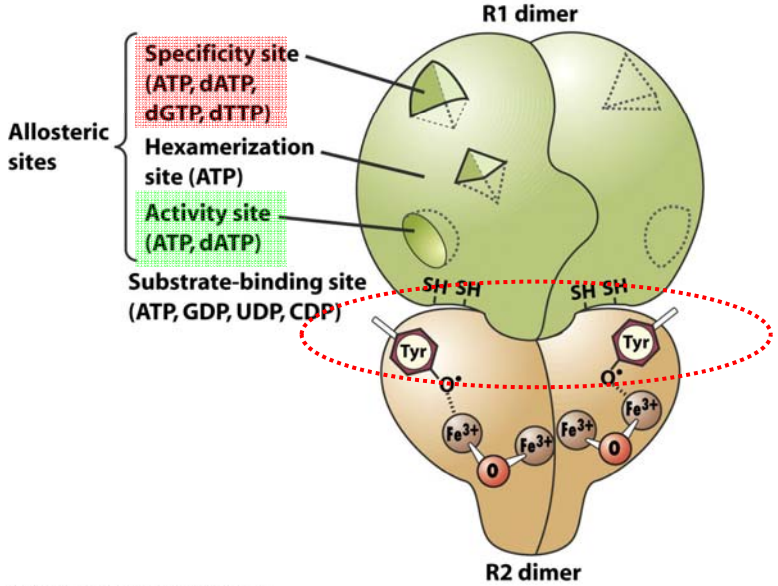

Figure 22-9a Fundamentals of Biochemistry, 2/e
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$R2_2$

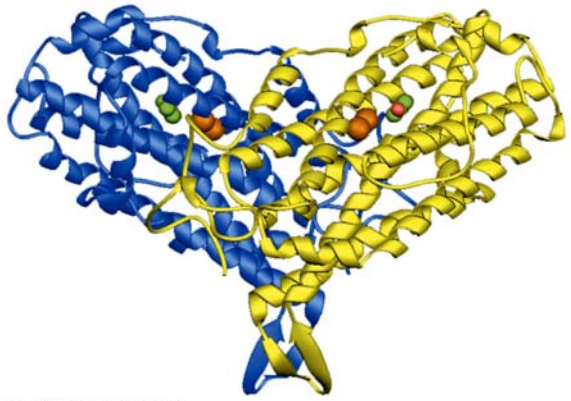
Figure 22-9b Fundamentals of Biochemistry, 2/e

Enzymatic mechanism of ribonucleotide reductase

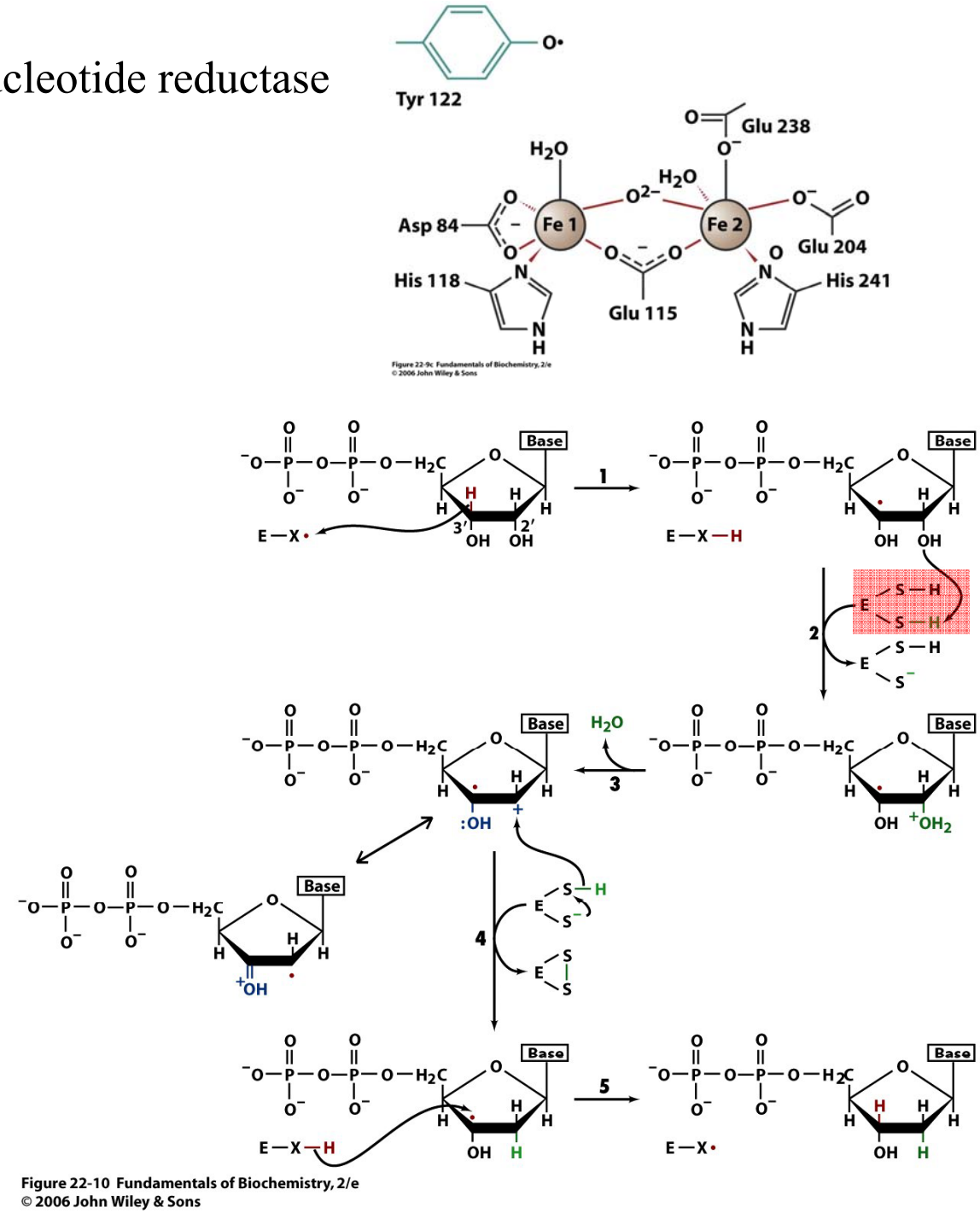

Figure 22-10 Fundamentals of Biochemistry, 2/e
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The inability of oxidized RNR can't bind substrate serves as essential protection function

Control the release of the radical's powerful oxidizing capability

By preventing the binding of substrate while the enzyme is in its oxidized form

Thioredoxin reduces RNR

The final step in RNR catalytic cycle: reform of its redox-active sulfhydryl pair

Human thioredoxin
(reduced form)

Protruded -SH

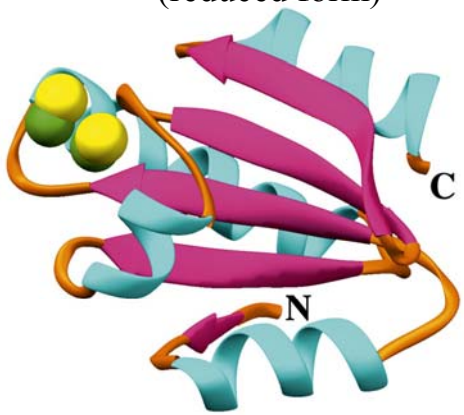
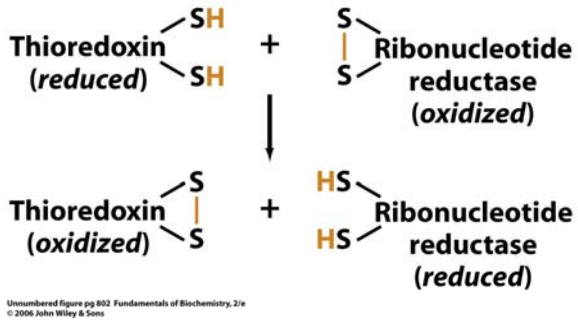


Figure 22-11 Fundamentals of Biochemistry, 2/e



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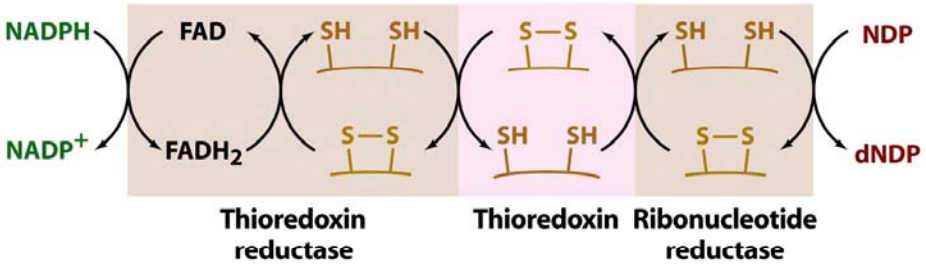

Figure 22-12 Fundamentals of Biochemistry, 2/e
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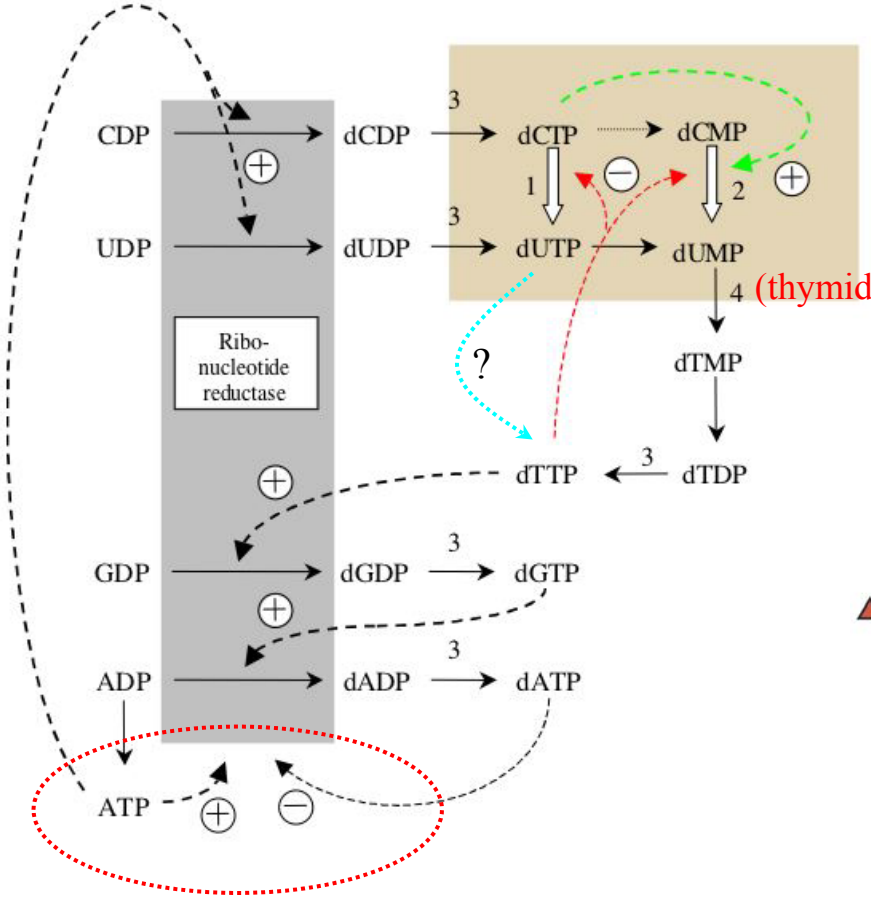
Regulation of RNR

Proper intracellular ratios of the four dNTPs by a complex feedback network

Deficiency of any of dNTPs is lethal, whereas an excess is mutagenic



overall regulation

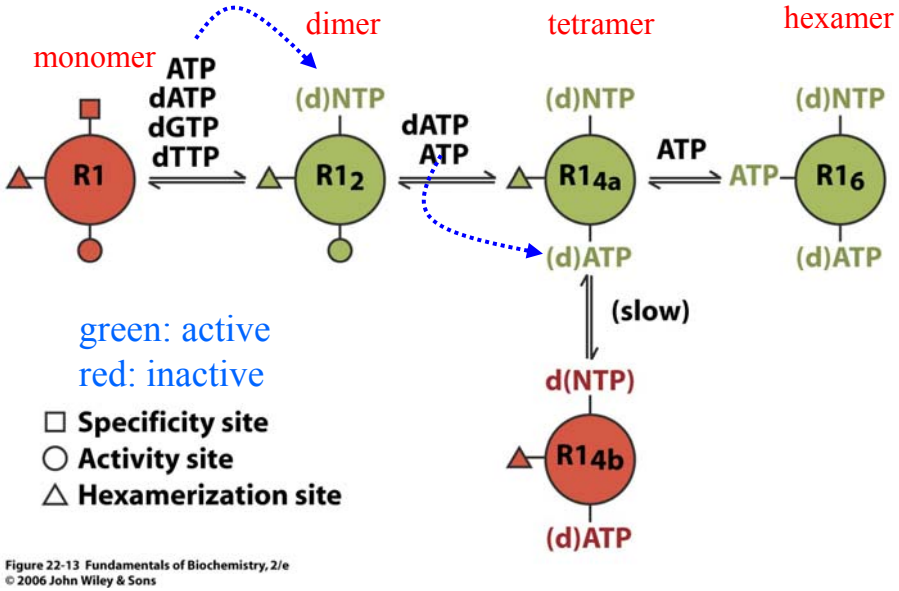


Figure 22-13 Fundamentals of Biochemistry, 2/e
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Origin of thymine

dUTP → dUMP → dTMP → dTDP → dTTP : wasteful dephosphorylation & rephosphorylation process

why? minimize [dUTP] to prevent its misincorporation into DNA

dUTP diphosphohydrolase (dUTPase)
Thymidylate synthase

Human dUTPase

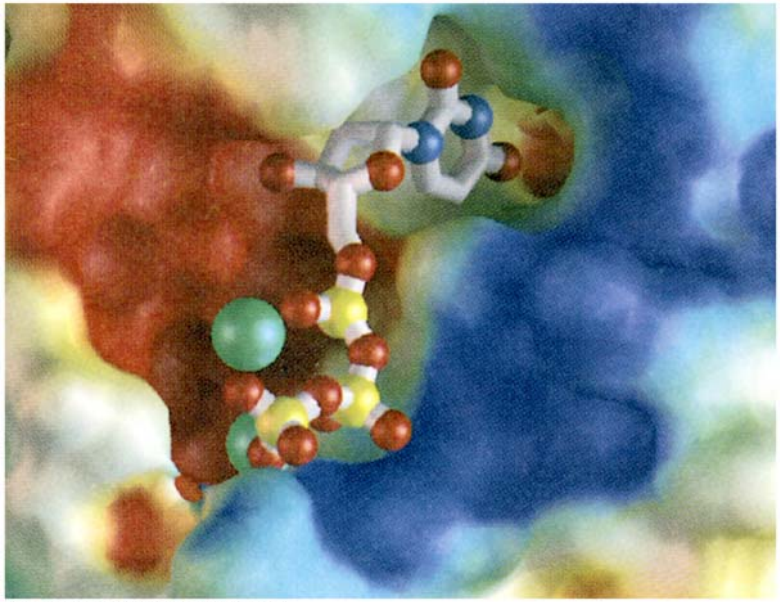


Figure 22-14a Fundamentals of Biochemistry, 2/e

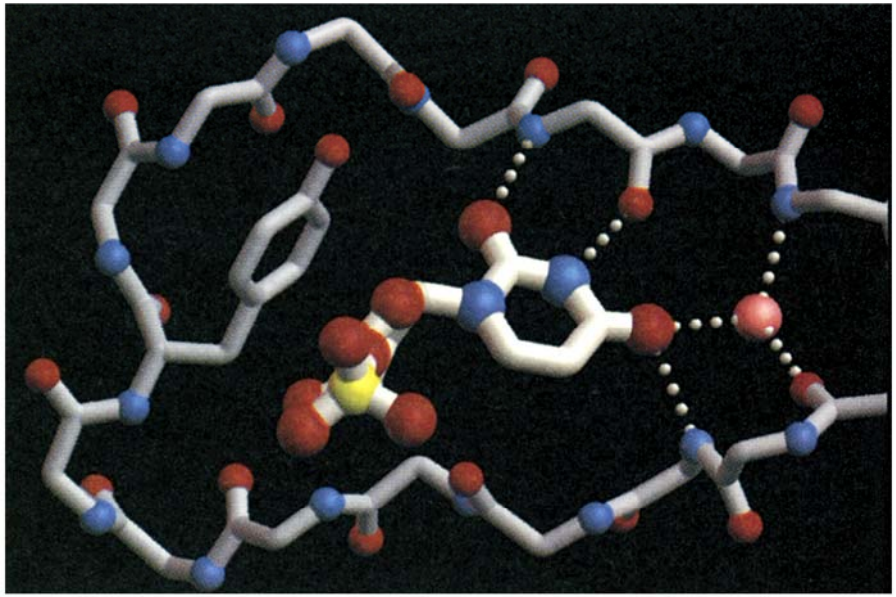
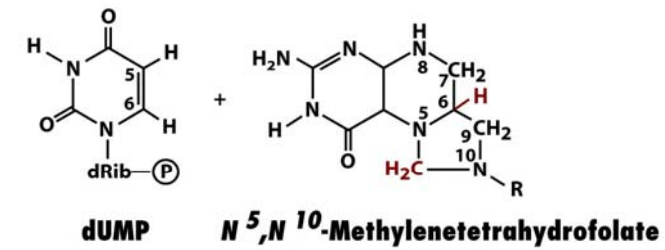
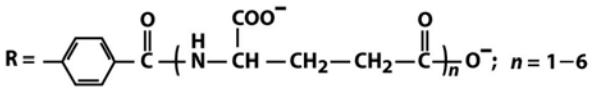


Figure 22-14b Fundamentals of Biochemistry, 2/e

Thymidylate synthase





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Reduction of methylene to methyl at the expense of the oxidation of THF to DHF

Anticancer targets

DHFR

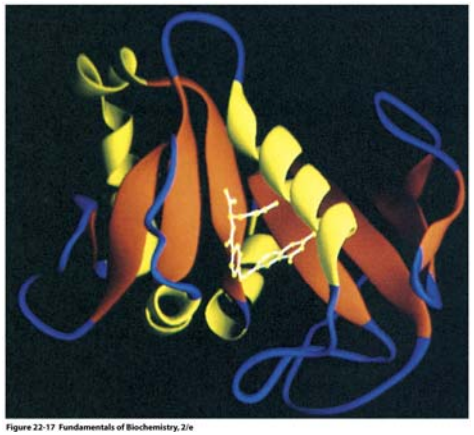


Figure 22-17 Fundamentals of Biochemistry, 2/e

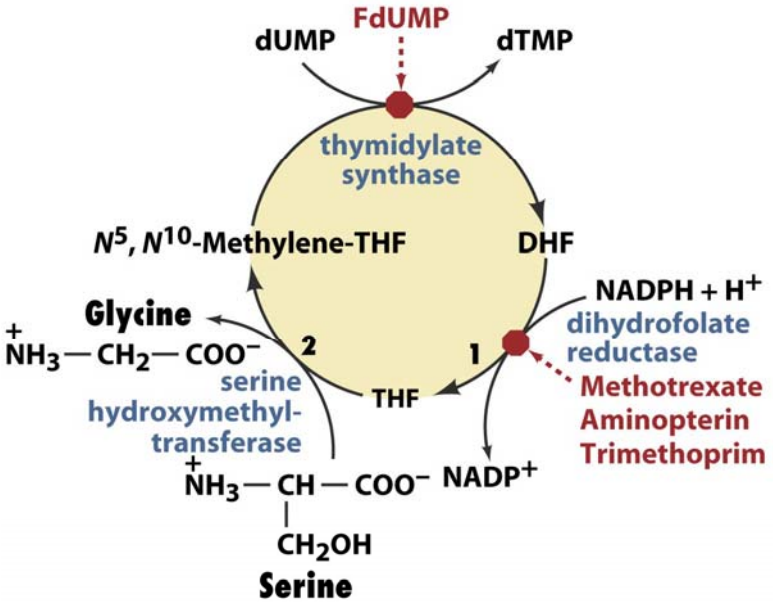


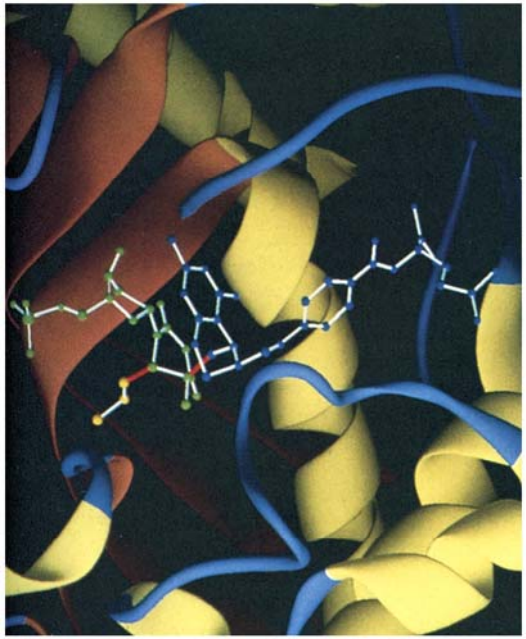
Figure 22-16 Fundamentals of Biochemistry, 2/e

Thymidylate synthase inhibitor: anticancer

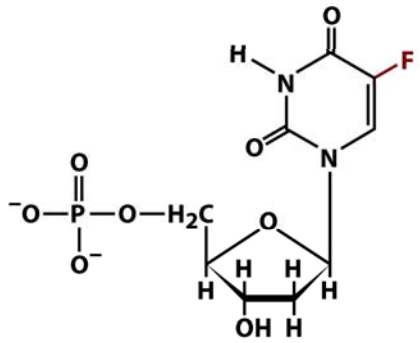
FdUMP: suicide substrate (mechanism-based inhibitor)

DHFR inhibitors: anticancer & antibacterial

antifolates



Box 22-1 figure 2 Fundamentals of Biochemistry, 2/e



5-Fluorodeoxyuridylate (FdUMP)

Box 22-1 figure 1 Fundamentals of Biochemistry, 2/e
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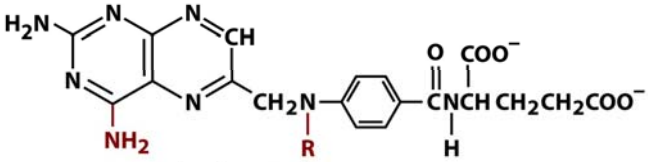
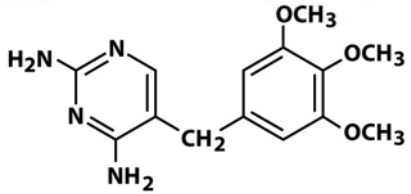


R = H **Aminopterin**

R = CH_3 **Methotrexate (amethopterin)**



Trimethoprim

Box 22-1 figure 3 Fundamentals of Biochemistry, 2/e
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Catalytic mechanism of thymidylate synthase

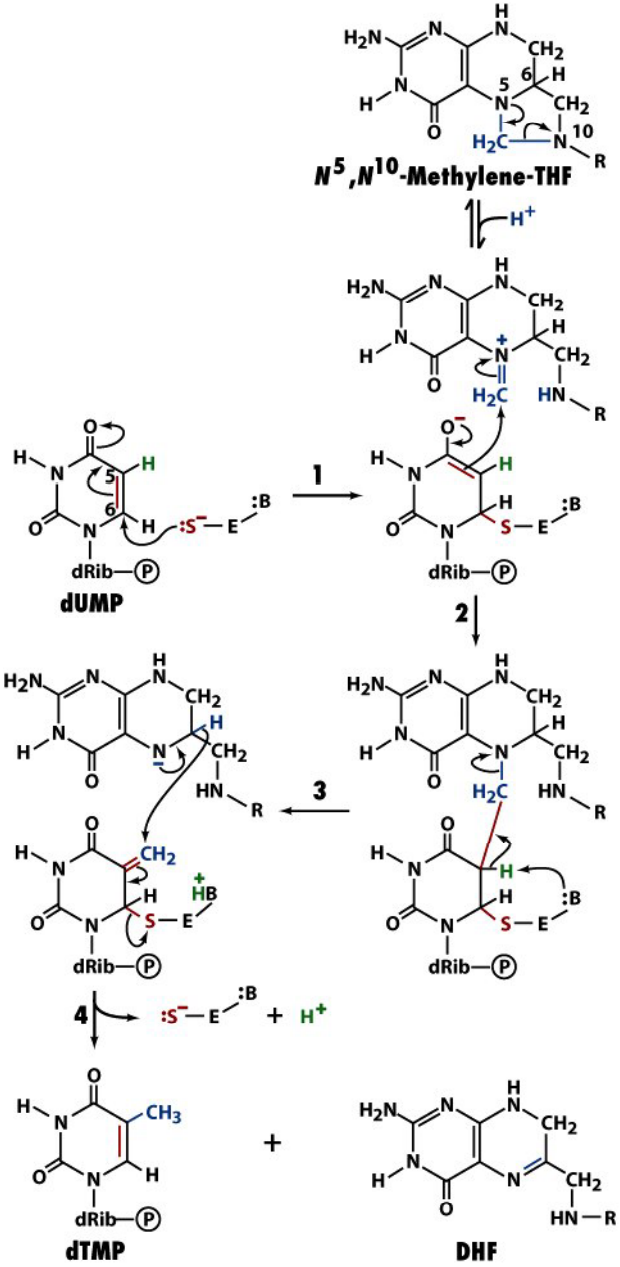


Figure 22-15 Fundamentals of Biochemistry, 2/e

Nucleotide degradation

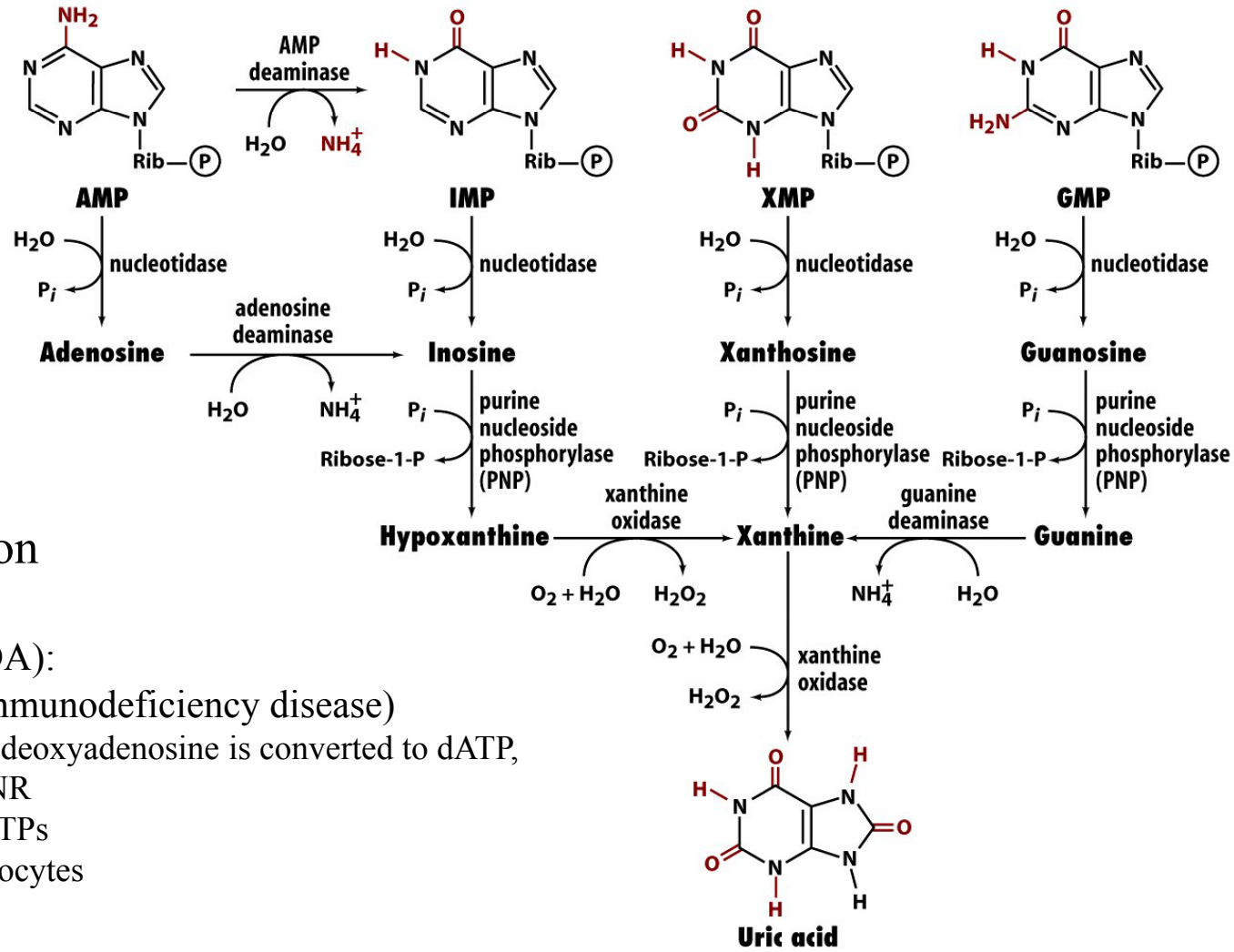


Figure 22-18 Fundamentals of Biochemistry, 2/e

Adenosine deaminase (ADA):

SCID (severe combined immunodeficiency disease)

- In the absence of ADA, deoxyadenosine is converted to dATP, which inhibits RNR
- Prevent synthesis of dNTPs
- Selective killing of leukocytes

The purine nucleotide cycle

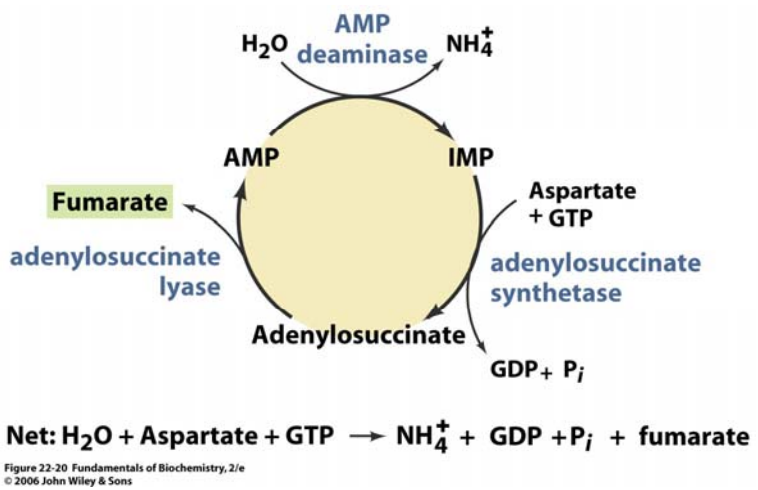


Figure 22-20 Fundamentals of Biochemistry, 2/e

Important metabolic role in skeletal muscle
(the involved enzymes are all several-fold higher in muscle)
Increased muscle activity requires increased citric acid cycle
Fumarate supplies the intermediate

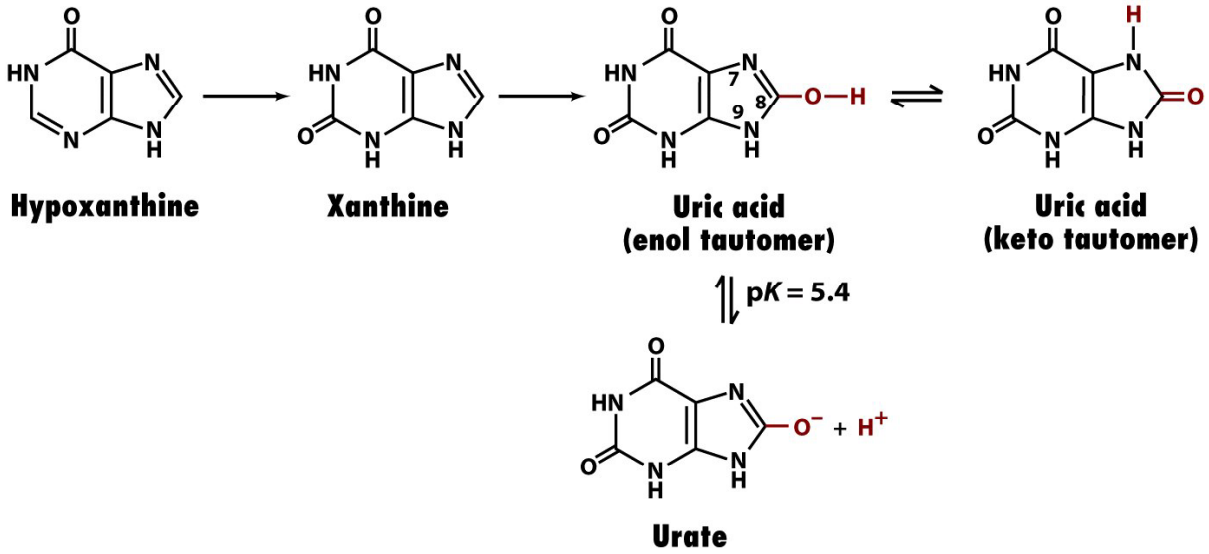
Deficiency in muscle AMP deaminase (myoadenylate deaminase deficiency)
suffers from easy fatigue and cramps after exercise

Xanthine oxidase

Hypoxanthine to xanthine
Xanthine to uric acid

Found almost exclusively in the liver and the small intestinal mucosa
Mini-electron transport system: contains entire electron transfer agents

FAD, Mo complex (cycle between VI and IV), two Fe-S clusters
Final electron transfer to O2 to generate H2O2, probably causing oxidative stress



Unnumbered figure pg 811 Fundamentals of Biochemistry, 2/e

Degradation of uric acid to ammonia

The function of conserving water
Uric acid is sparingly soluble

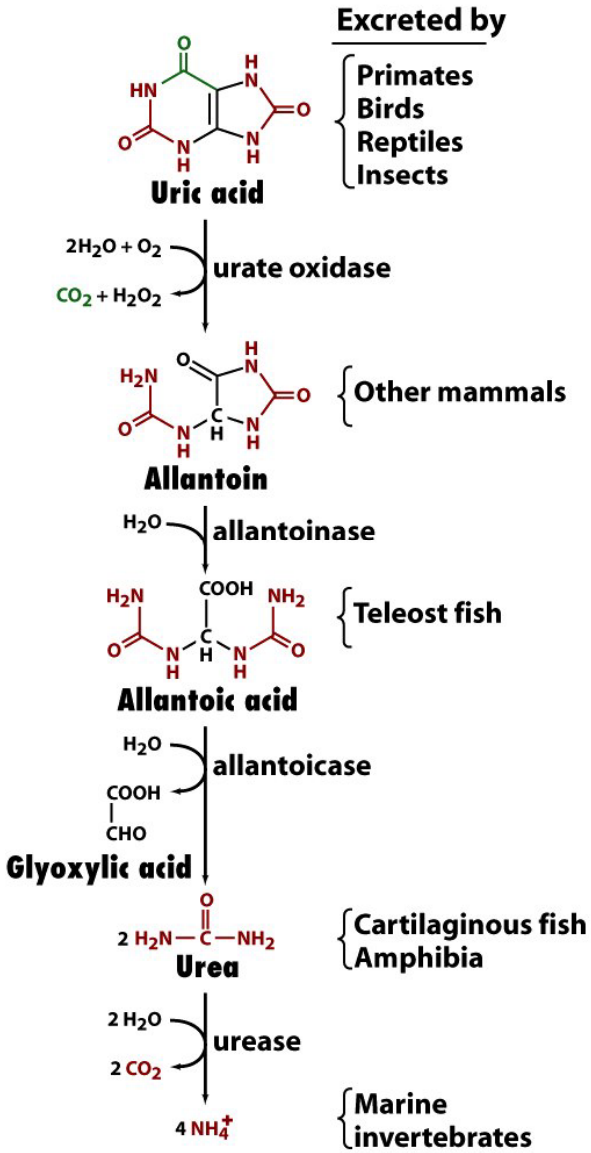


Figure 22-21 Fundamentals of Biochemistry, 2/e

Gout is caused by an excess of uric acid

Affects ~3 per 1000 persons

Impaired uric acid excretion: deposition of sodiumurate crystal

HGPRT deficiency

Treatment with allopurinol (xanthine oxidase inhibitor)

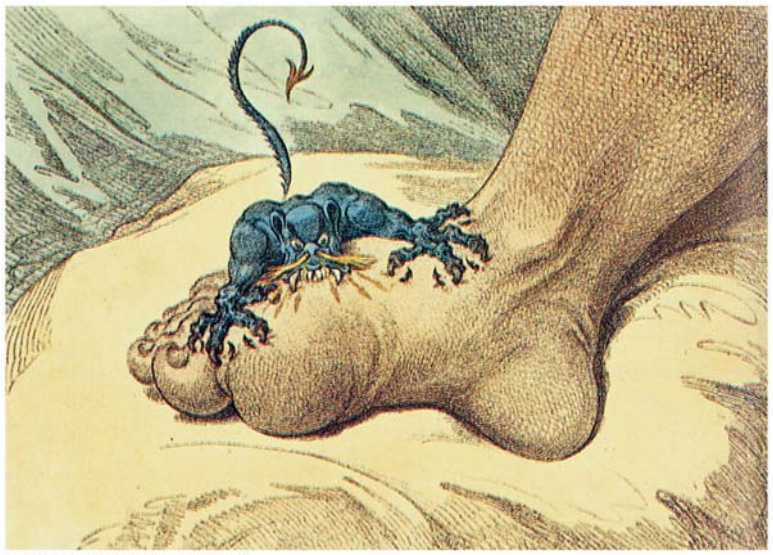
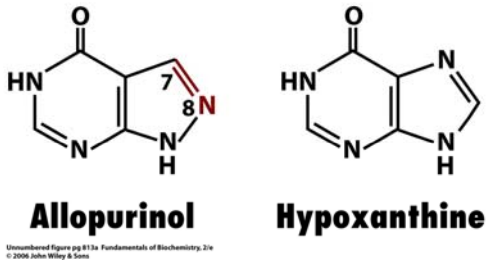
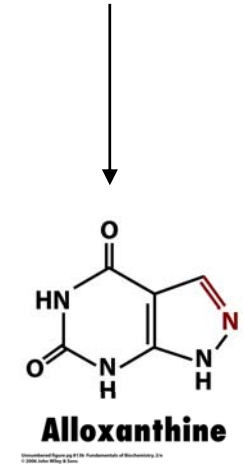


Figure 22-22 Fundamentals of Biochemistry, 2/e





Bound to XO,
thereby inactivating it

Catabolism of pyrimidines

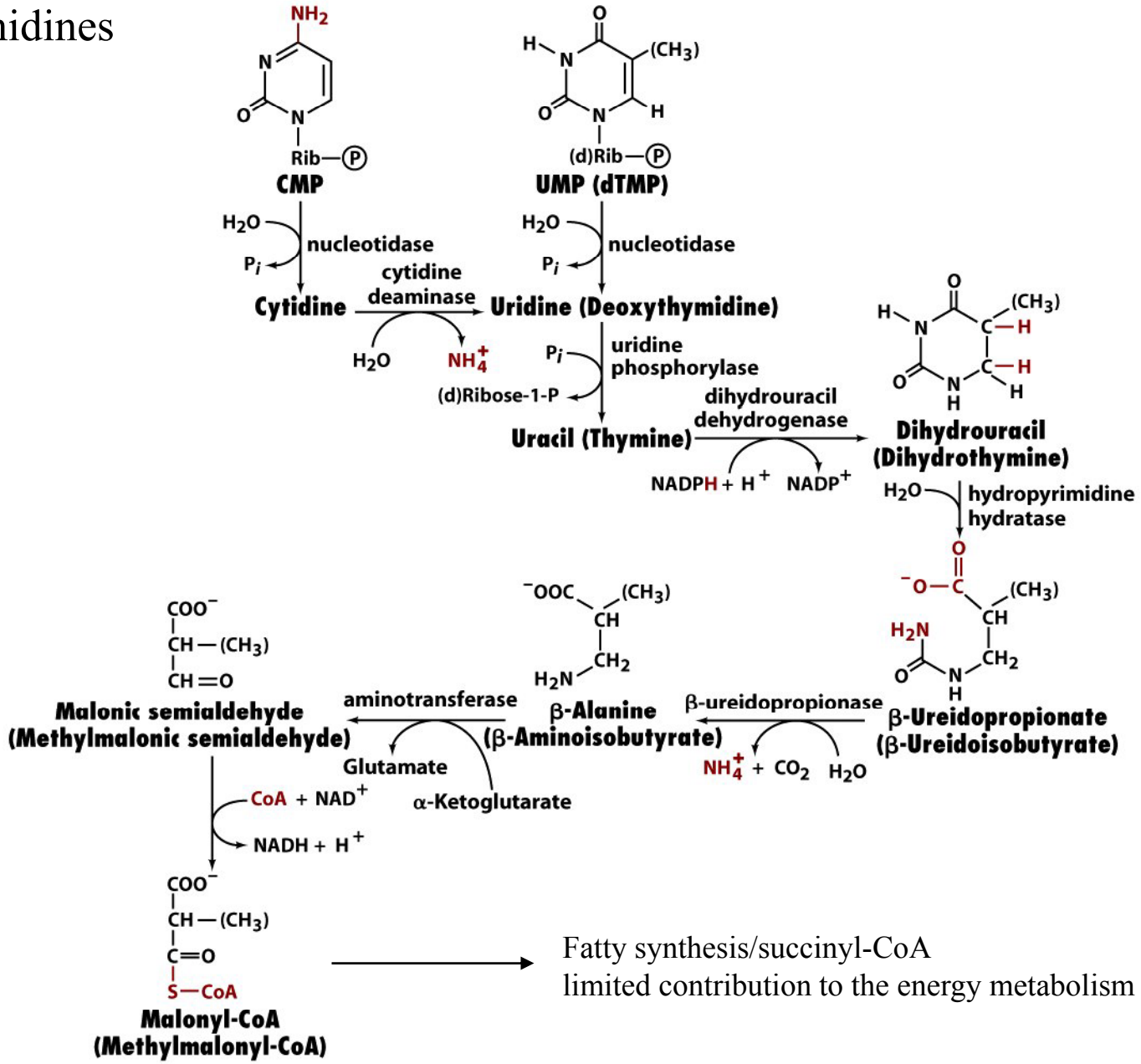


Figure 22-23 Fundamentals of Biochemistry, 2/e