Nucleotide Metabolism (Chapter 23)

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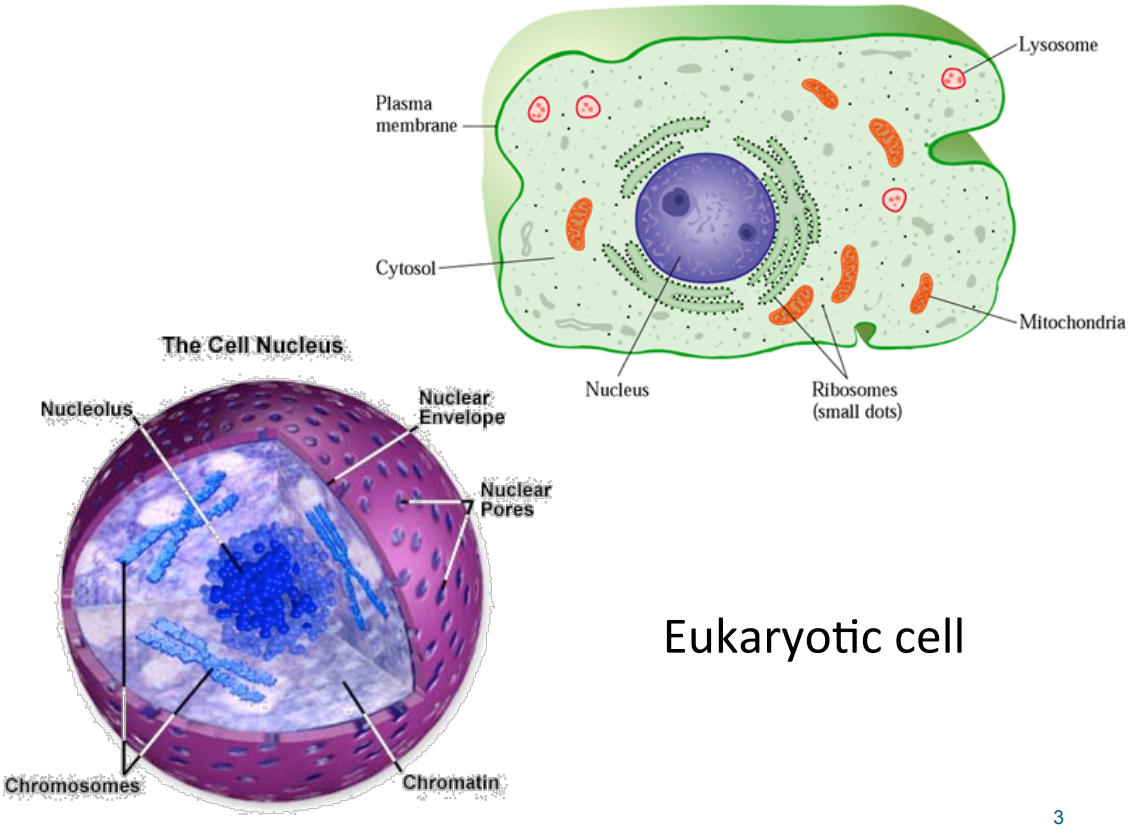
INTRODUCTION TO NUCLEIC ACIDS

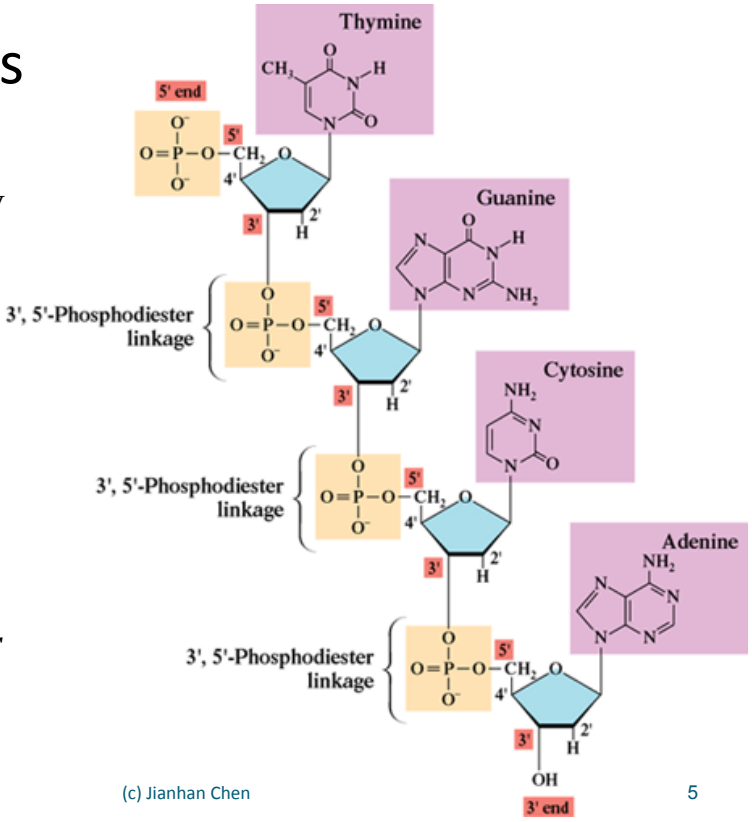


Eukaryotic cell

Genome is believed to define a species

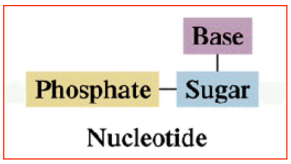
Nucleic Acids

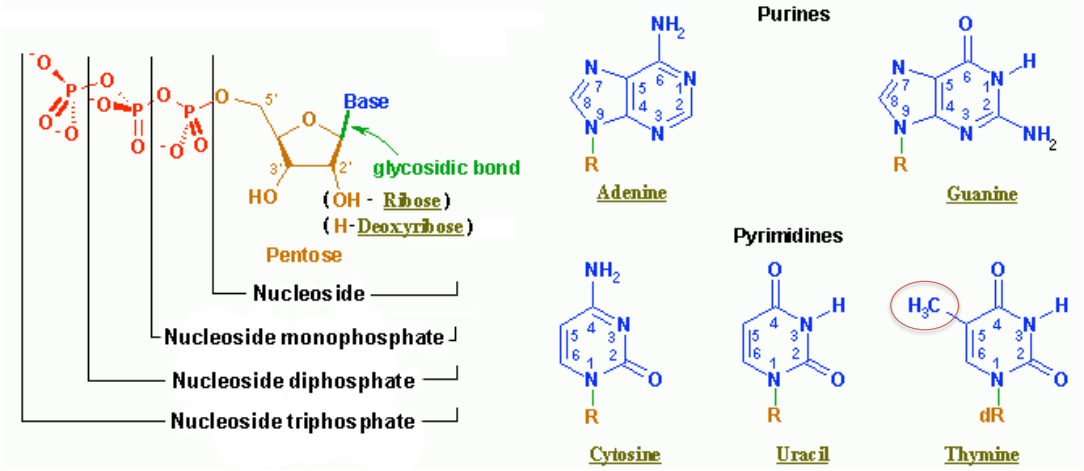
- Polymer of nucleotides: highly flexible (compared to peptides)
- Nucleic acids are universal in living things, as they are found in all cells and viruses.
- Nucleic acids were first discovered by Friedrich Miescher in 1871.



Building Blocks of Nucleotides

- Phosphate group + pentose carbohydrate + base (nitrogen-containing heterocyclic ring)
- Deoxyribonucleic acid (DNA): A/G/C/T
- Ribonuclei acid (RNA): A/G/C/U

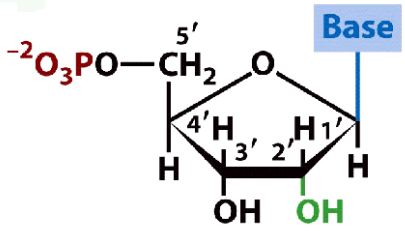


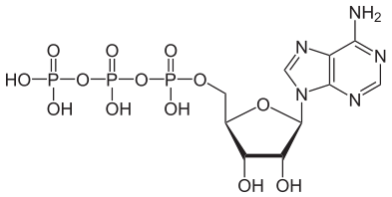


Nucleotide nucleobase

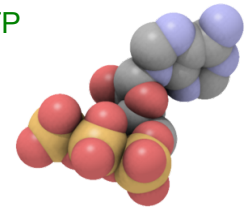
Nucleotides have many roles

- Building blocks of the nucleic acid polymers RNA and DNA.
- Energy transfer or energy coupling to drive biosynthesis and other processes (muscle contraction, transport, etc).
- Oxidation reduction reactions.
- Intracellular signaling.

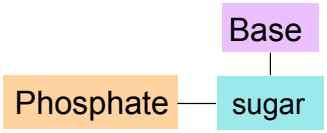




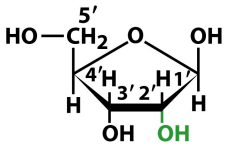
ATP



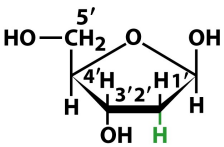
Pentose sugars



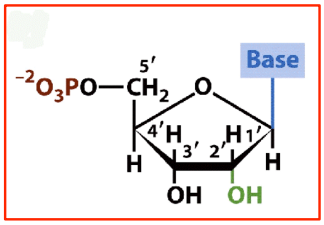
- The pentose sugar in the nucloetide is either ribose or deoxyribose.



Ribose

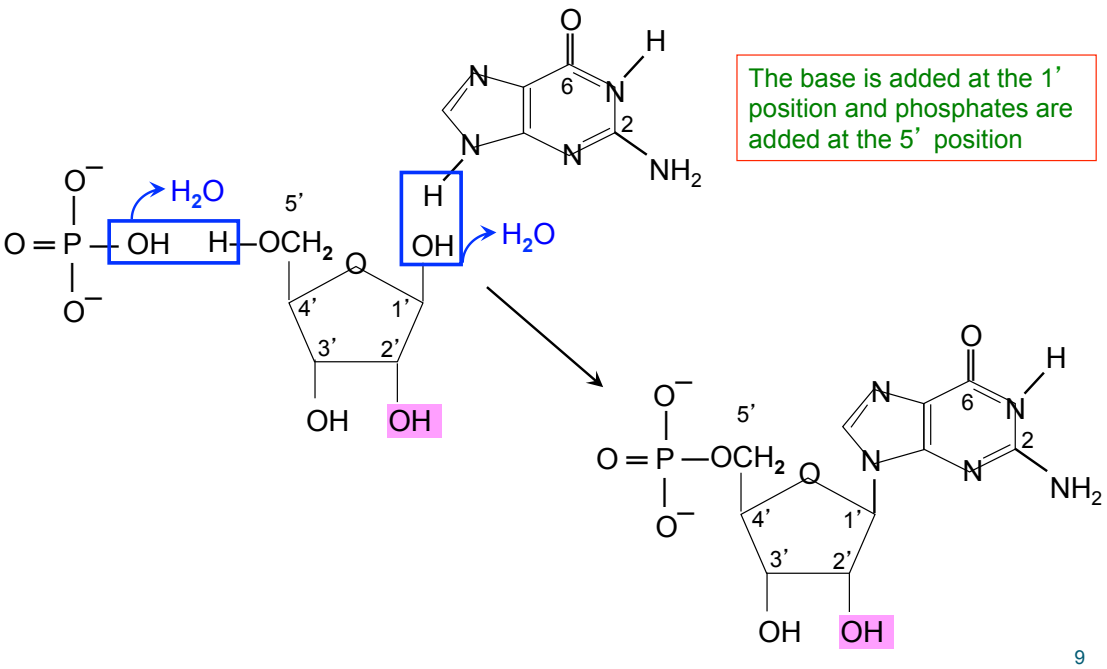


Deoxyribose



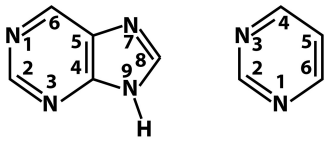
  - The base is added at the 1’ position and phosphates are added at the 5’ position.
  - Most nucleotides, including those incorporated into RNA, contain ribose.
  - 2’-OH reduced to –H in deoxynucleotides
  - 3’-OH participates in forming phosphodiester linkage
  - Deoxynucleotides are **exclusively** used for DNA synthesis.

Nucleotide formation



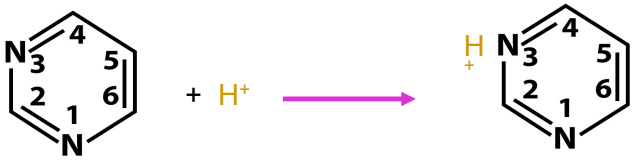
Nitrogen-Containing Heterocyclic Bases

- There are two classes of aromatic amine structures used for the bases: purines and pyrimidines.



Purine **Pyrimidine**

  - There are two purine bases and three pyrimidine bases
  - Why are they (weak) bases?



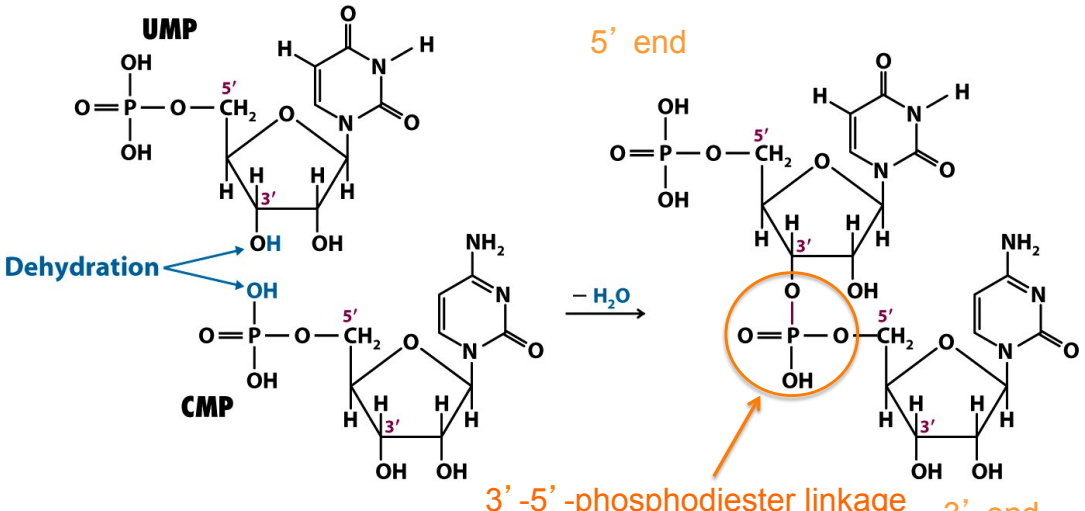
Nomenclature of nucleotides

- Suffix: "*osine*" for pur*ine* bases; "*idine*" for pyrim*idine* bases
- prefix "deoxy" for DNA

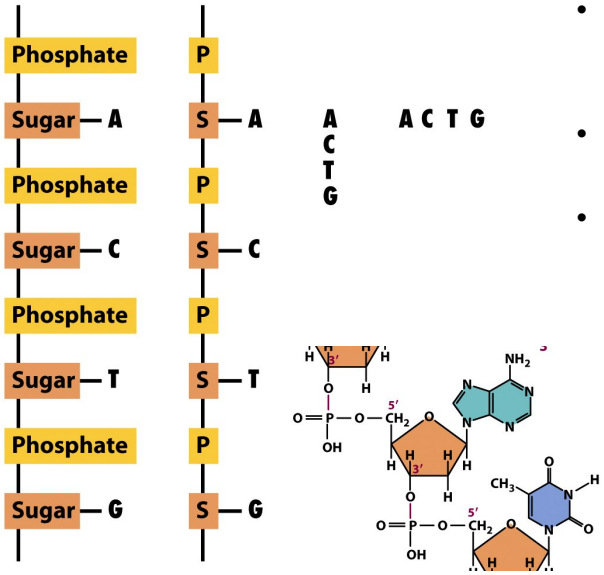
Base	Sugar	Nucleotide name	Nucleotide abbreviation
DNA Nucleotides			
adenine	deoxyribose	deoxyadenosine 5′-monophosphate	dAMP
guanine	deoxyribose	deoxyguanosine 5′-monophosphate	dGMP
cytosine	deoxyribose	deoxycytidine 5′-monophosphate	dCMP
thymine	deoxyribose	deoxythymidine 5′-monophosphate	dTMP
RNA Nucleotides			
adenine	ribose	adenosine 5′-monophosphate	AMP
guanine	ribose	guanosine 5′-monophosphate	GMP
cytosine	ribose	cytidine 5′-monophosphate	CMP
uracil	ribose	uridine 5′-monophosphate	UMP

Formation of nucleic acids

- Nucleotides are linked through phosphate groups and 3' position of the sugar (3' -5' -phosphodiester linkage)



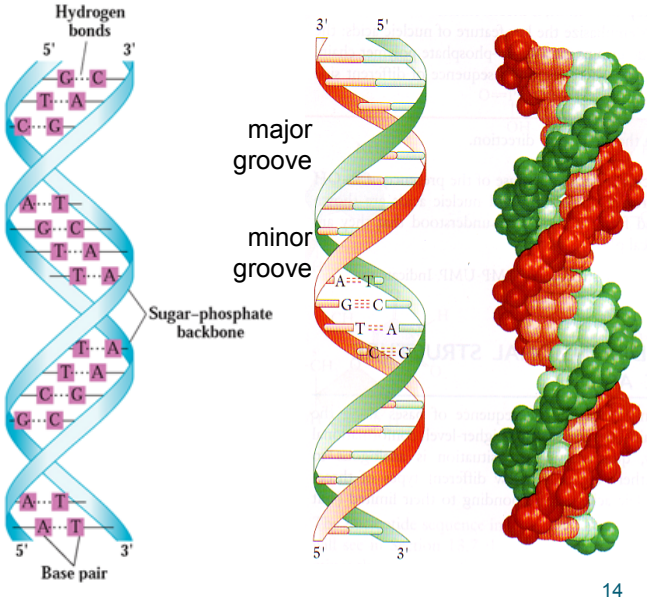
Primary structure of nucleic acid



- So-called (genome) "sequence": order of nucleotides
- Always from 5' to 3' (top to bottom or left to right)
- Nucleotides and nucleic acids are acidic because of the presence of P-OH groups
  - At pH 7, all of these groups are ionized: P-O^-, although they are usually drawn in their protonated forms for the purposes of structure illustration.

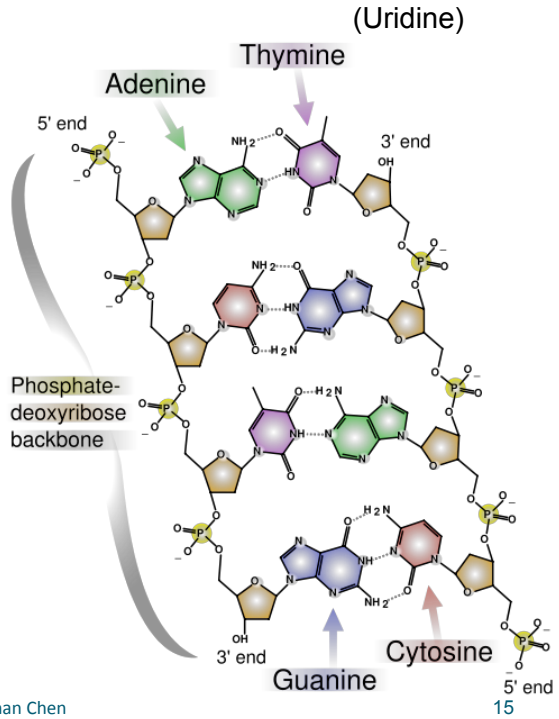
Secondary structure of DNA

- Double helix (first defined by Watson and Crick, 1953)
  - Right-hand helix
  - Antiparallel pair of strands
  - The deoxy-sugars and phosphate groups are on the outside and the heterocyclic bases are stacked on top of each other on the inside
  - Stabilized by hydrogen-bonds



Watson-Crick Base Pairs

- G-C and A-T(U)
- DNA almost exclusively exist as duplex held together by forming Watson-Crick base pairs
- A robust mechanism for faithful replication and translation
- Other pairings possible but mostly in RNA to give rise to non-trivial structures

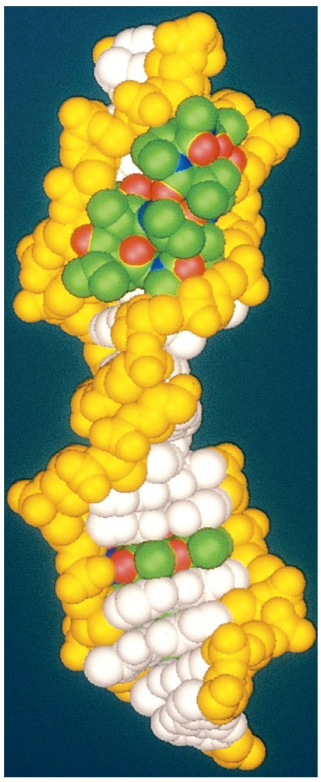


Base-pair stacking

While the phosphodiester bonds form the backbone, the H-bonds of the base pairing interactions contribute to holding the DNA strands together.

The planar aromatic bases form a stack that runs parallel to the fiber axis. The stacked bases have interbase hydrophobic and dipole interactions. These interactions contributes to the stability of DNA structure.

Therefore, a combination of the H-bonding between complementary base pairs and the stacking interactions act cooperatively to stabilize the folded double helix structure of DNA.

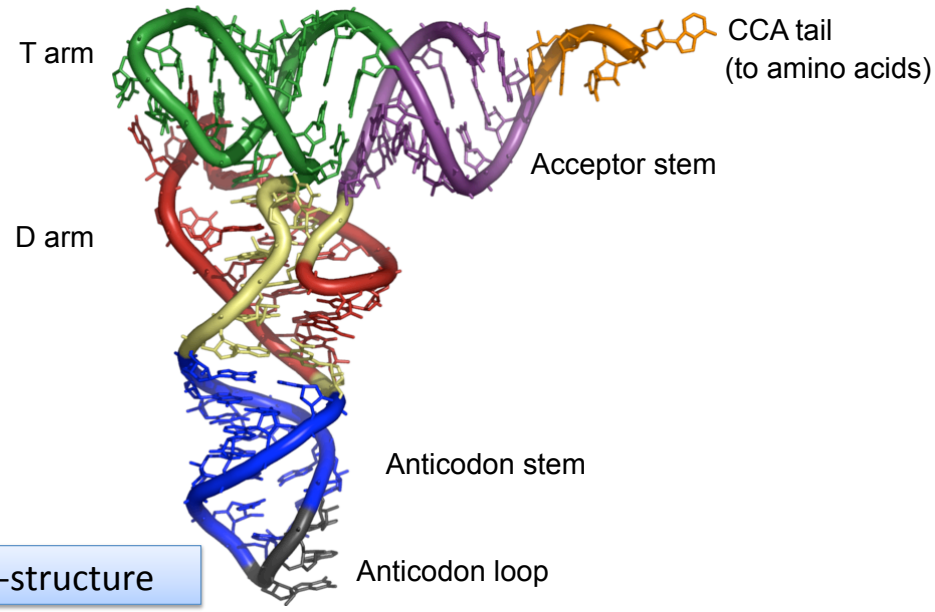


Ribonucleic acids (RNA)

- RNAs exist as single stranded molecules.
- Several types: transfer RNA (tRNA), messenger RNA (mRNA), ribosomal RNA (rRNA)
- Much shorter than DNAs
- More flexible and often lack stable secondary structures like double-helix of DNA
- Less stable with shorter life-time (DNA is stable throughout cell life cycle, while RNAs are typically not)
- Some RNAs (tRNA and rRNA) can have tertiary structures (like proteins).

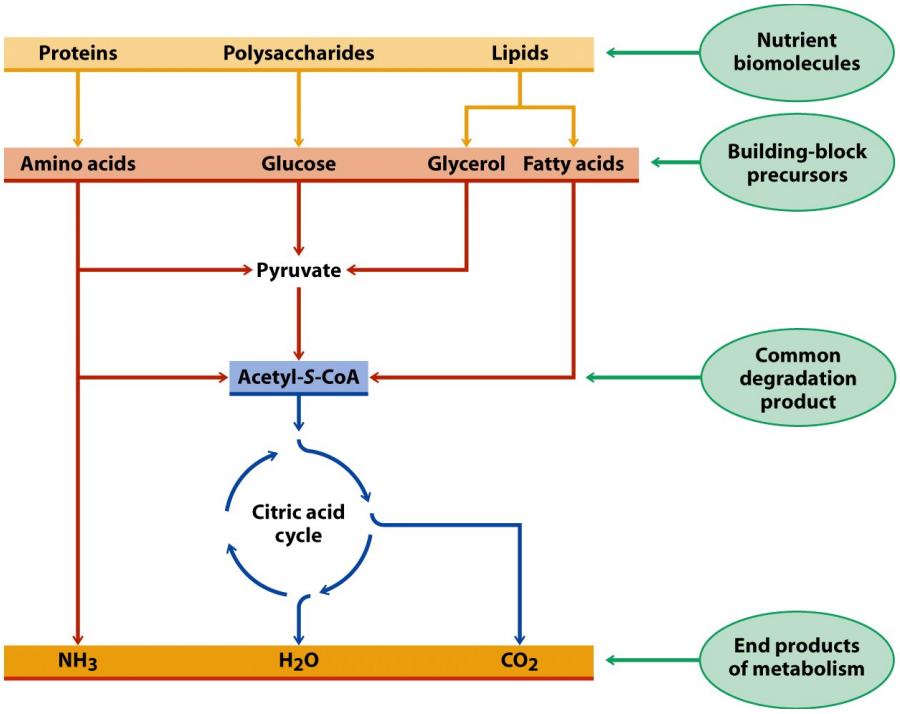
Tertiary Structure of RNA

- Some RNA fold into tertiary structures



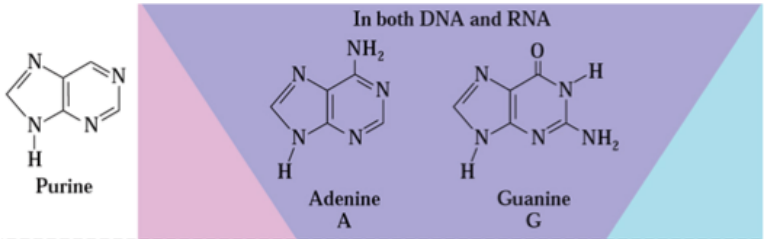
tRNA L-structure

Nucleotides do not provide a significant source of metabolic energy



Quick Summary

- Nucleotides: compositions and types (sugar and base vary)
- Nucleic acids: polymer of nucleotides
  - DNA (w/ deoxyribose): A, G, C, T
  - RNA (w/ ribose): A, G, C, U
  - DNA structures: sequence, double helix, base pairing
  - RNA structures: single-stranded, tertiary structures



Chapter 23.1

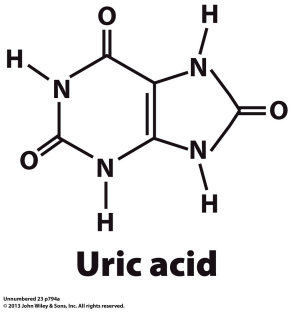
SYNTHESIS OF PURINE RIBONUCLEOTIDES

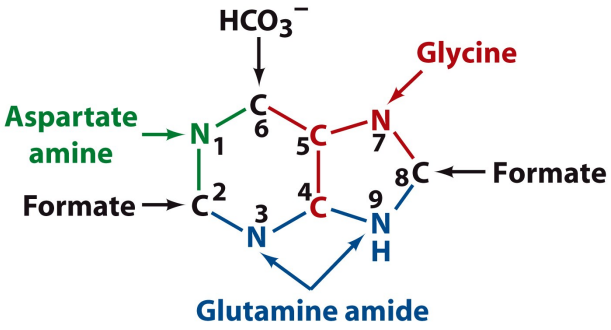
Key Concepts 23.1

- IMP is synthesized through the assembly of a purine base on ribose-5-phosphate.
- Kinases convert IMP-derived AMP and GMP to ATP and GTP.
- Purine nucleotide synthesis is regulated by feedback inhibition & feedforward activation.
- Salvage reactions convert purines to their nucleotide forms.

Atomic Origins of Purine

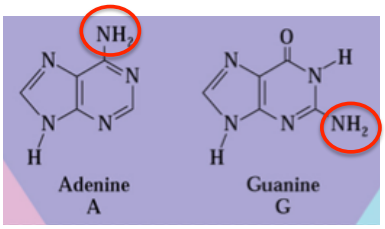
- Isotope labeling analysis of excreted uric acid
- N: from aspartate/glycine/glutamine
- C: from formate/glycine/HCO_3^-
- Glycine likely incorporated as a whole

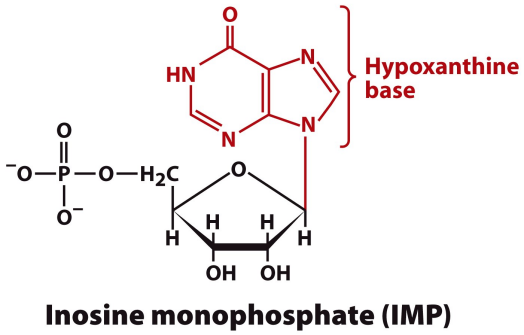




Inosine Monophosphate (IMP)

- Initially synthesized purine derivative
- Precursor to both AMP and GMP
- Key "surprise": purines not synthesized as free base, but as ribonucleotides!
- Pathways highly conserved in different organisms spanning from E. Coli to human!





de novo Biosynthesis of IMP

- 11 reactions
- Starting from activation of ribose-5-phosphate
- Imidozole ring forms first (steps 1-6), followed by pyrimidine ring (steps 7-11)
- Consumes several ATPs along the way
- Several enzymes are multi-functional and rely on channeling for efficiency

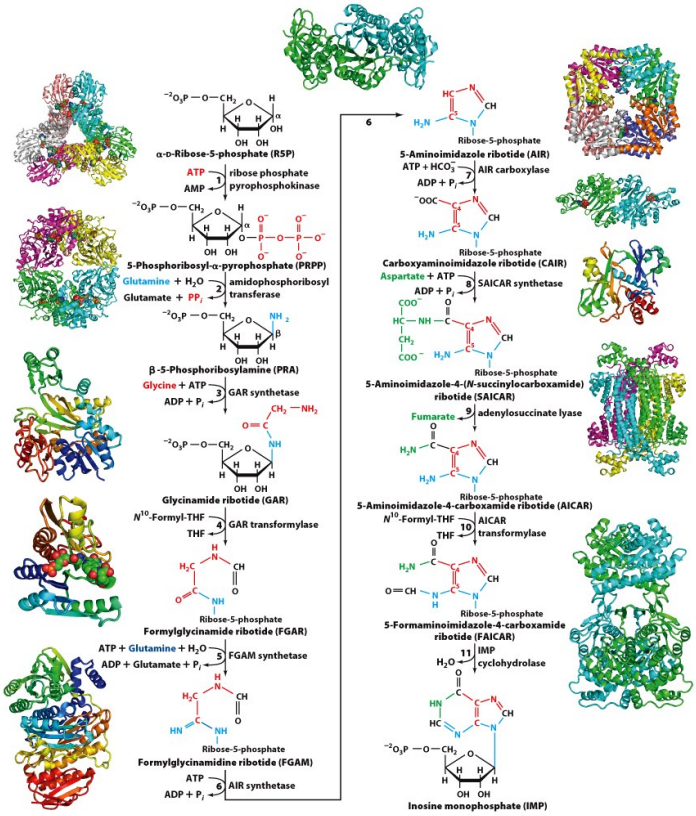
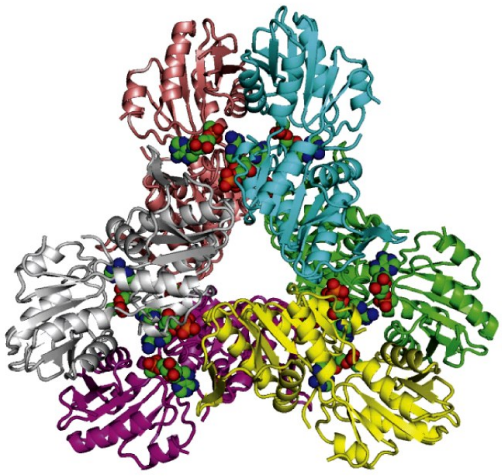


Figure 23-1

de novo Biosynthesis of IMP



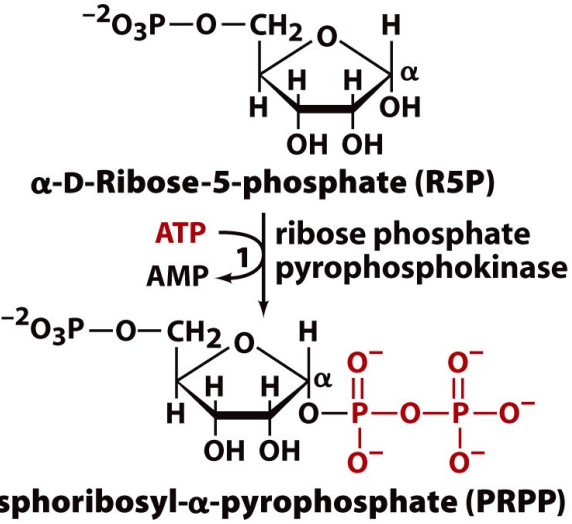
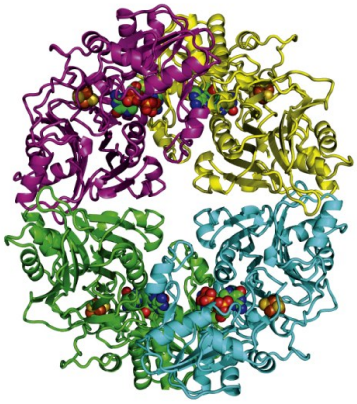


Figure 23-1 part 1

de novo Biosynthesis of IMP



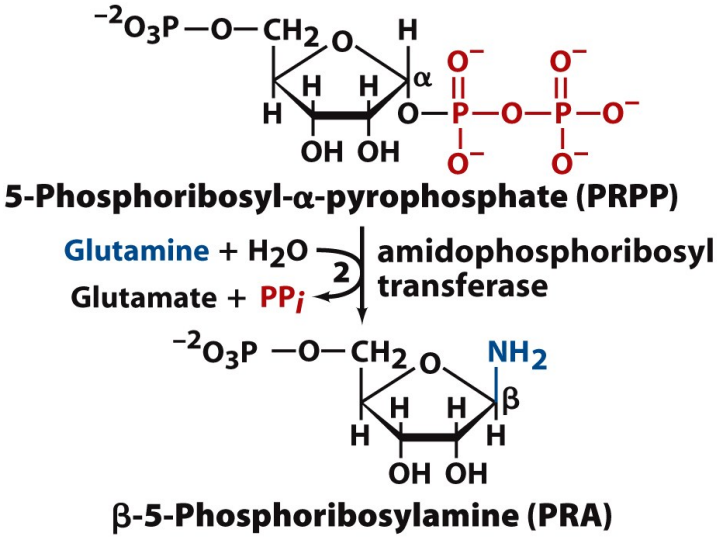
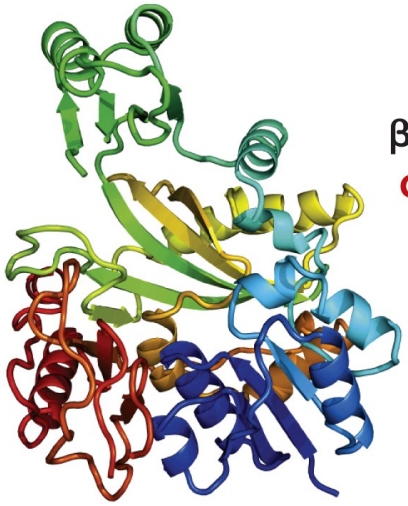


Figure 23-1 part 2

de novo Biosynthesis of IMP



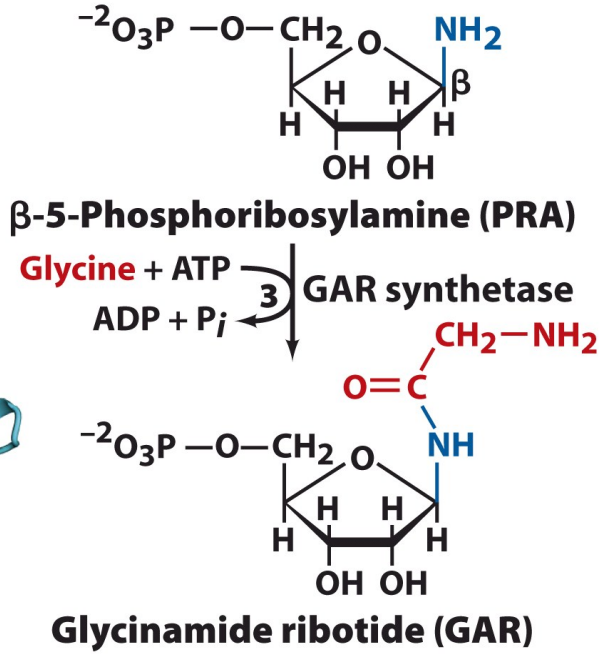
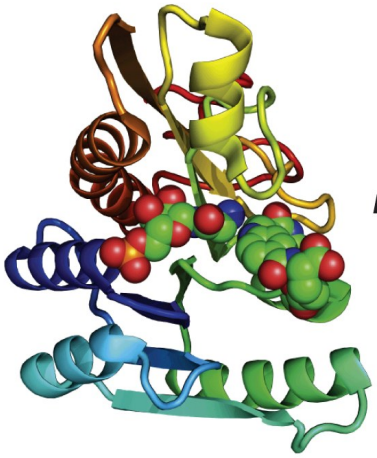


Figure 23-1 part 3

de novo Biosynthesis of IMP



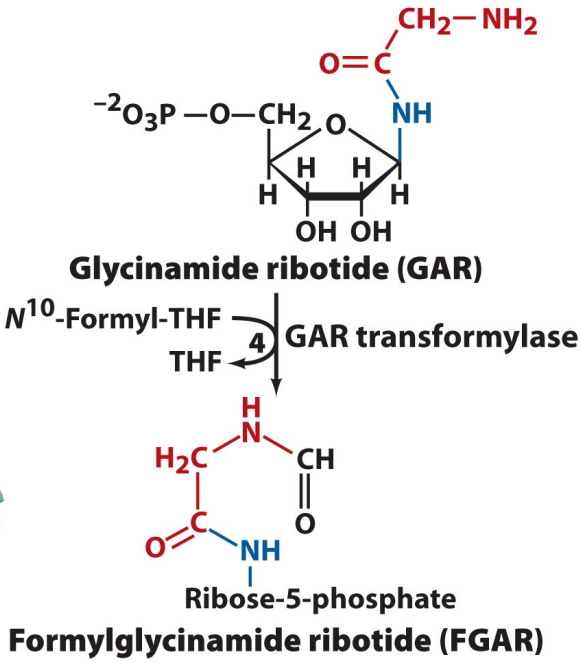
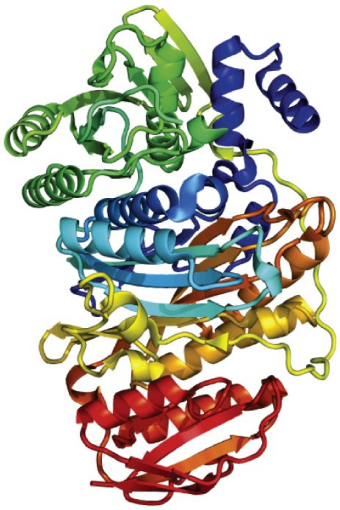


Figure 23-1 part 4

de novo Biosynthesis of IMP



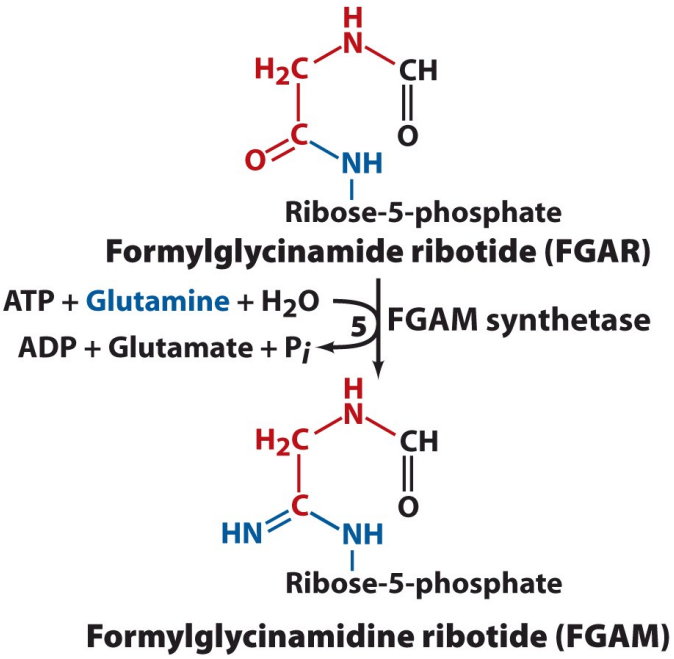
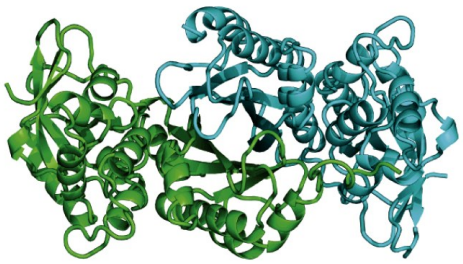


Figure 23-1 part 5

de novo Biosynthesis of IMP



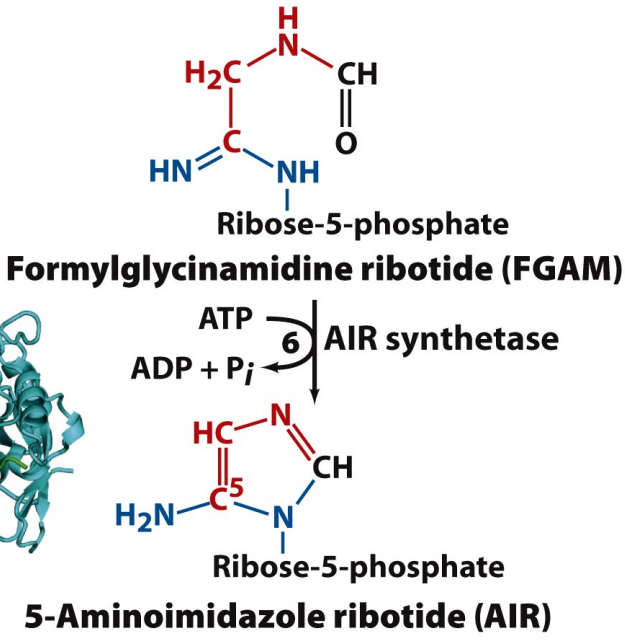
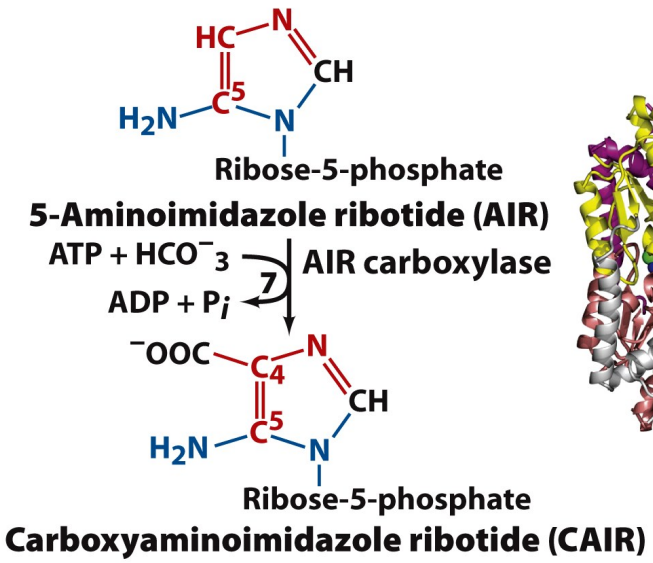


Figure 23-1 part 6

de novo Biosynthesis of IMP



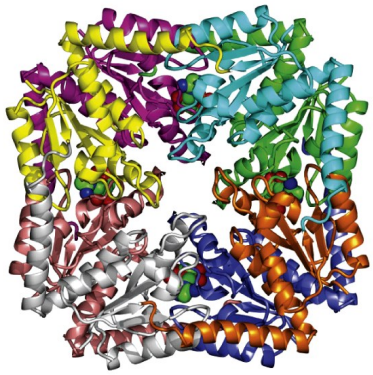
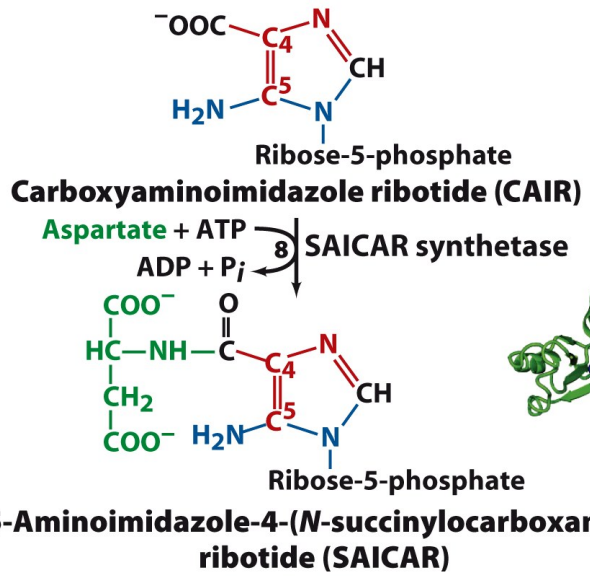


Figure 23-1 part 7

de novo Biosynthesis of IMP



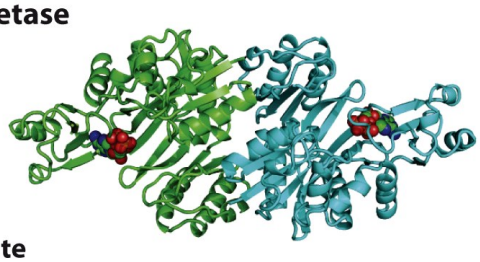
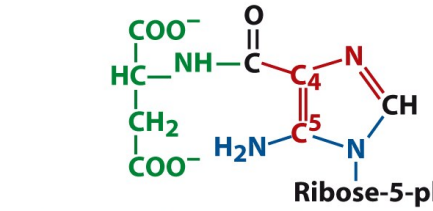


Figure 23-1 part 8

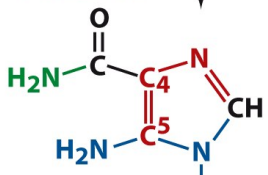
de novo Biosynthesis of IMP



Ribose-5-phosphate

5-Aminoimidazole-4-(*N*-succinylocarboxamide) ribotide (SAICAR)

Fumarate ← 9 | adenylosuccinate lyase



Ribose-5-phosphate

5-Aminoimidazole-4-carboxamide ribotide (AICAR)

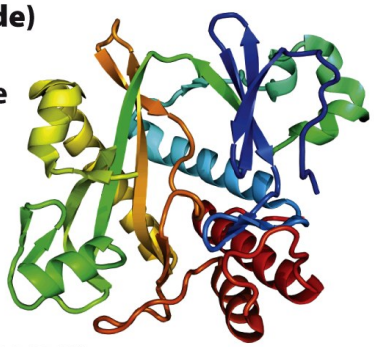
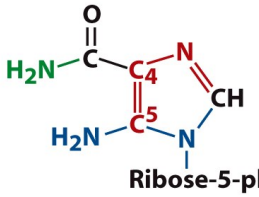


Figure 23-1 part 9

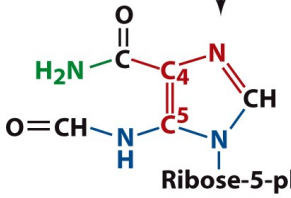
de novo Biosynthesis of IMP



Ribose-5-phosphate

5-Aminoimidazole-4-carboxamide ribotide (AICAR)

N^{10}-Formyl-THF → THF | 10 | AICAR transformylase



Ribose-5-phosphate

5-Formaminoimidazole-4-carboxamide ribotide (FAICAR)

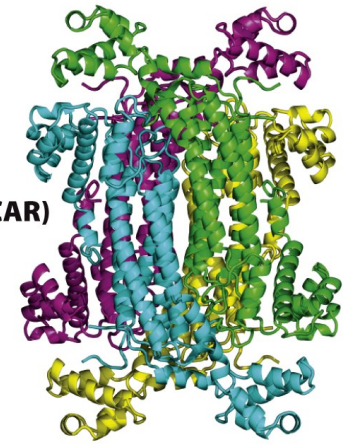


Figure 23-1 part 10

de novo Biosynthesis of IMP

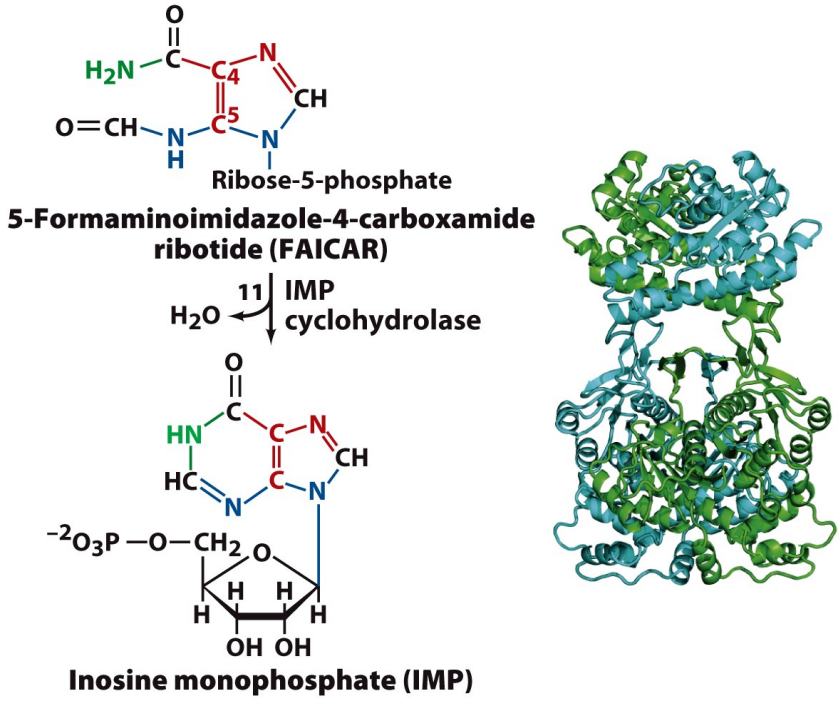


Figure 23-1 part 11

IMP to AMP/GMP

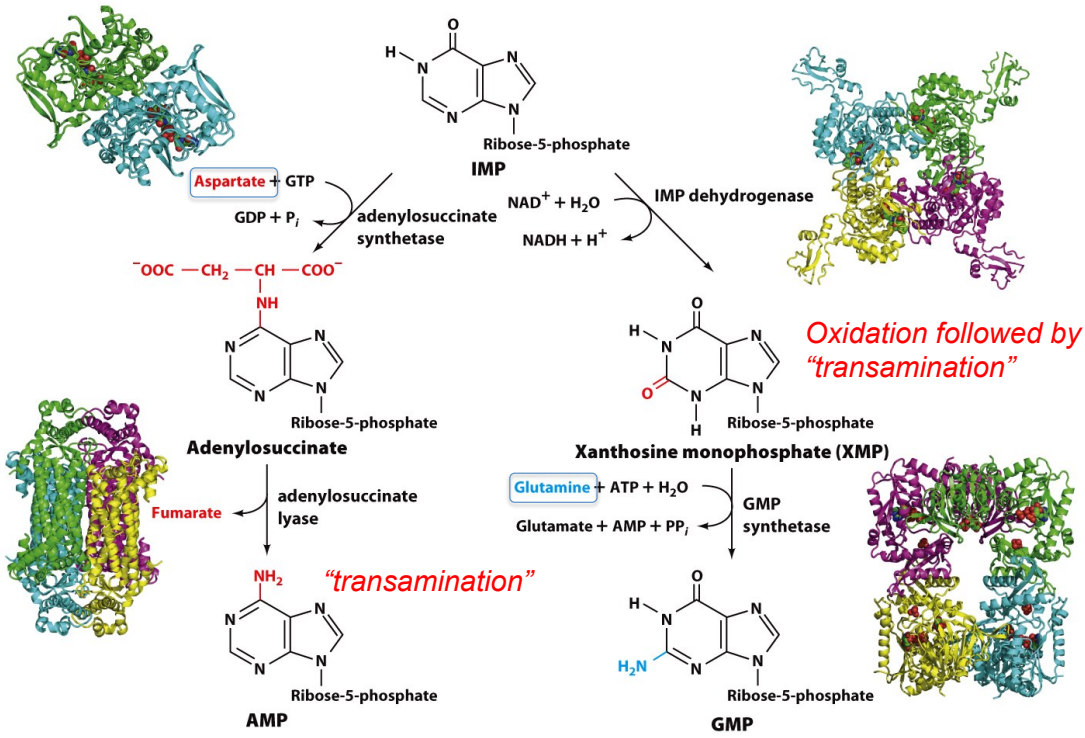


Figure 23-3

AMP/GMP to ATP/GTP

- Nucleoside triphosphates required for NA synthesis
- Kinases convert AMP/GMP to ADP/GDP and ATP/GTP

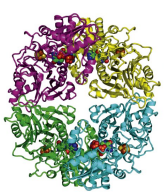
- Adenylate kinase: AMP + ATP <-> 2 ADP
- Guanylate kinase: GMP + ATP <-> GDP + ADP
- Nucleoside diphosphate kinase:

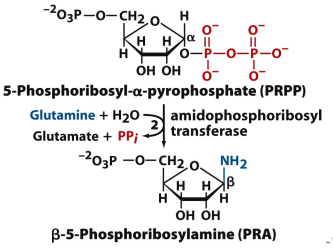
  GDP + ATP <-> GTP + ADP

- ATP synthesized by various energy-generating reactions such as glycolysis and oxidative phosphorylation
  - ATP generation ultimately drive all above kinase reactions!

Regulation of Purine Nucleotide Synthesis

- Levels of nucleotides in cell are critical
  - Energy balance
  - Too low: impede transcription and replication and others!
  - Too high: mutation elevated!
- IMP pathway: regulated at first two steps
  - Feedback inhibition
  - Feedforward activation!





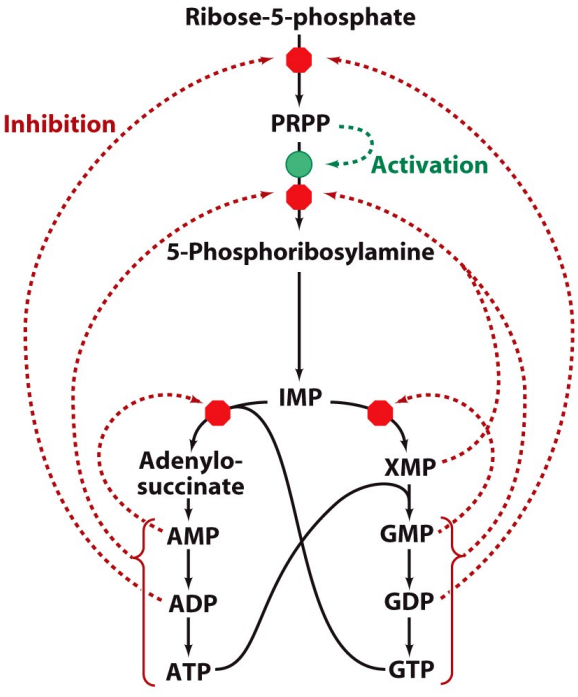


Figure 23-4

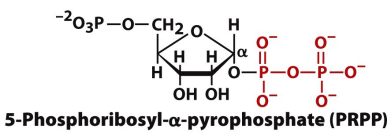
Purine Salvaging

- Free purines released in nucleic acid degradation
- Can be converted into corresponding nucleotides through salvage pathways
  - Diverged in different species
- Two enzymes in human
  - Adenine phosphoribosyltransferase (APRT):

    Adenine + PRPP <-> AMP + Ppi
  - Hypoxanthine-guanine phosphoribosyltransferase (HGPRT)

    Hypoxanthine + PRPP <-> IMP + PPi

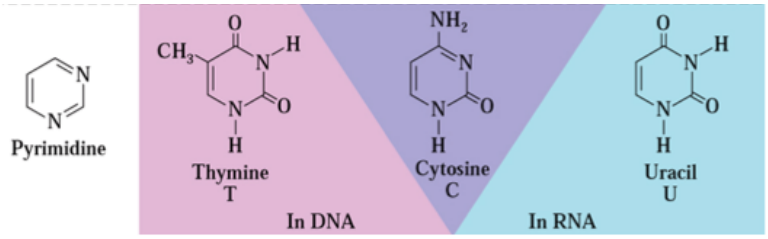
    Guanine + PRPP <-> IMP + PPi
  - HGPRT Deficiency: Lesch-Nyhan synthdrome
    - Affects mostly males
    - Neurological abnormalities: retardation, self-injuring and others
    - Increase uric acid excretion: why?



5-Phosphoribosyl-α-pyrophosphate (PRPP)

Quick Summary

- List the starting materials and cofactors required for IMP biosynthesis.
  - the importance of multifunctional enzymes in nucleotide biosynthesis.
- Describe the reactions that convert IMP to ATP and GTP.
- How do guanine and adenine nucleotides inhibit their own synthesis? How do they promote synthesis of each other?
- Describe how free purines are converted back to nucleotides.



Chapter 23.2

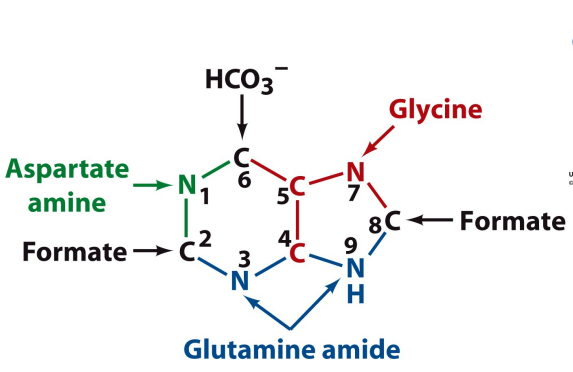
SYNTHESIS OF PYRIMIDINE RIBONUCLEOTIDES

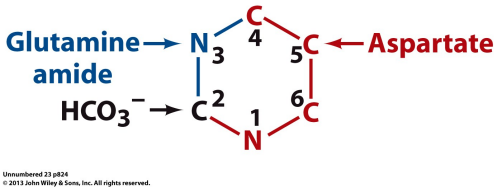
Key Concepts 23.2

- UMP is synthesized as a pyrimidine base to which ribose-5-phosphate is added.
- CTP and UTP are derived from UMP.
- The early steps of pyrimidine nucleotide synthesis are the major control points.

Atomic Origins of Pyrimidines

- Isotope labeling analysis of excreted uric acid
- Aspartate + Glutamine (amide) + HCO_3-
- Initially synthesized as uridine monophosphate (UMP)
- Simpler and quite different from IMP: prymidine ring synthesized first before coupled to ribose-5-phosphate (from PRPP)





De novo Synthesis of UMP

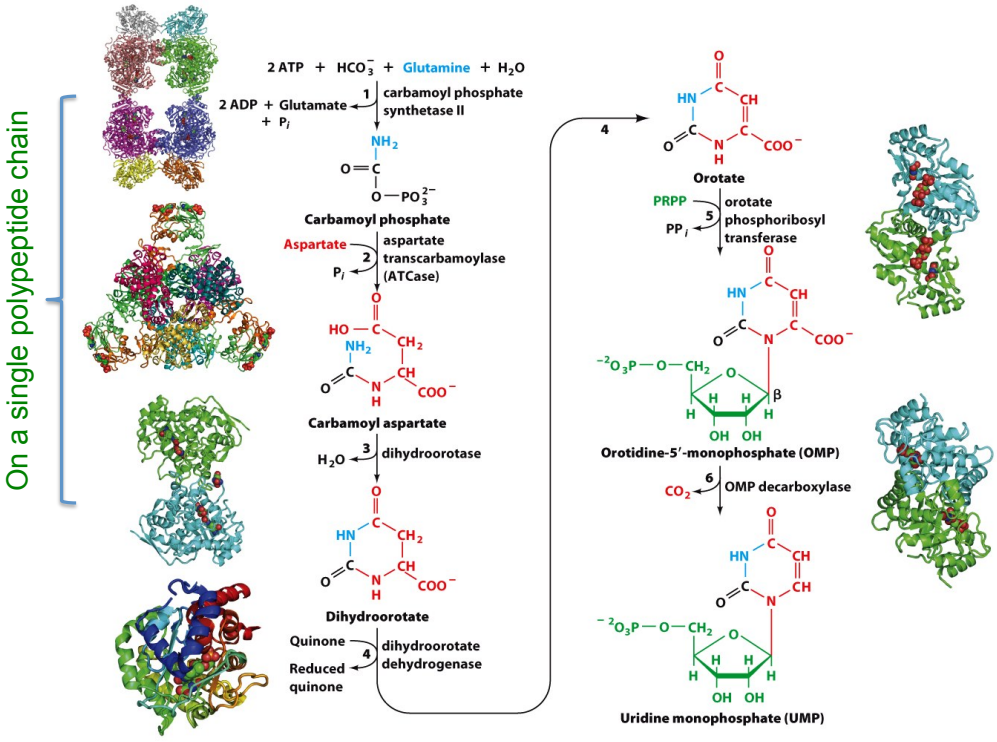
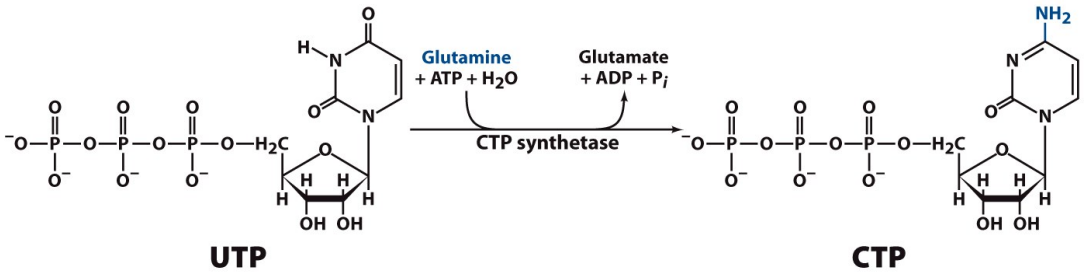
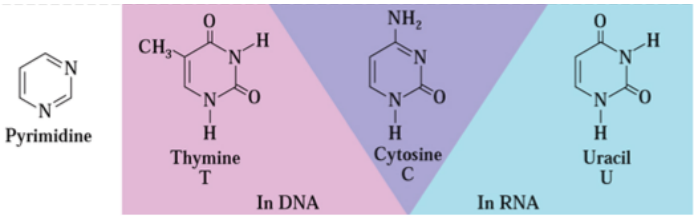


Figure 23-5

UMP to UTP/CTP

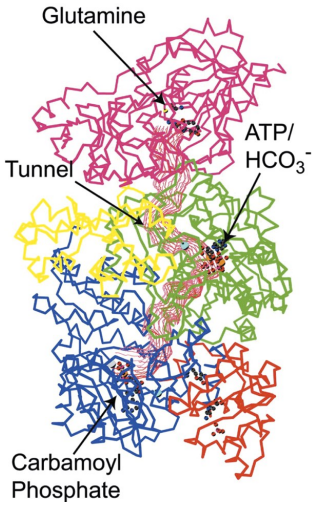
- UMP + ATP -> UDP + ATP -> UTP
- CTP synthetase aminates UTP to produce CTP
  - GLN provides the amino group





Regulation of UMP Synthesis

- Mainly regulated at step 2 (ATCase reaction) in bacteria
- In animals: controlled by regulation of CPS II



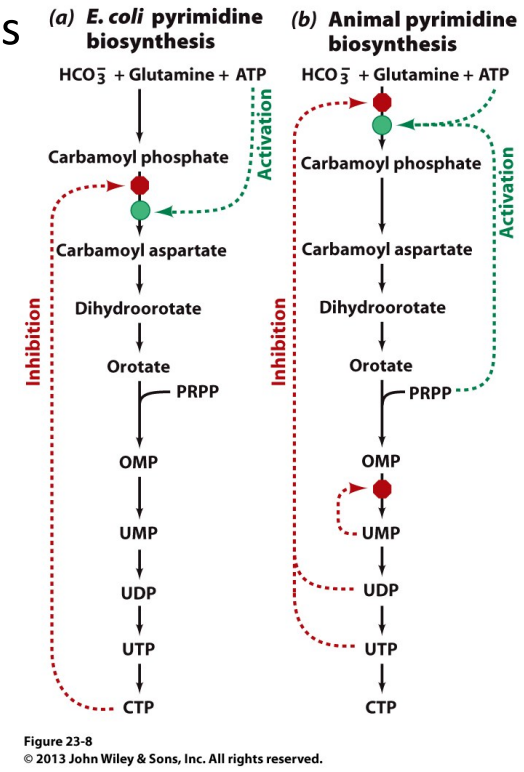


Figure 23-8

Quick Summary

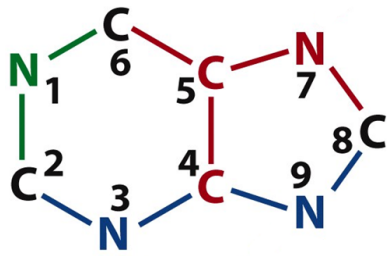
- Compare the pathways of purine and pyrimidine nucleotide synthesis with respect to (a) precursors, (b) energy cost, (c) acquisition of the ribose moiety, and (d) number of enzymatic steps.
- How are CTP and UTP derived from UMP?
- How does regulation of pyrimidine synthesis differ in bacteria and animals?

What general pathway(s) can eukaryotic cells use to produce nucleotides?

A. Anabolic and amphibolic pathways.
B. Salvage and *de novo* pathways.
C. Catabolic and *de novo* pathways.
D. Salvage pathways only.
E. Anabolic pathways.

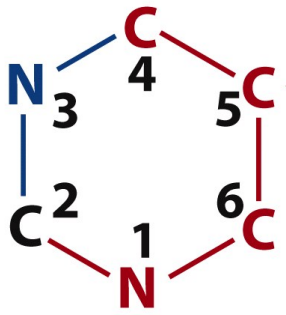
The N7 in the purine ring is contributed by which of the following molecules?

A. Aspartate
B. Glycine
C. Glutamate
D. Glutamine
E. Arginine

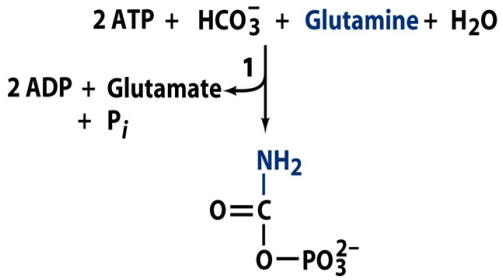


The N3 in the pyrimidine ring is contributed by which of the following molecules?

A. Aspartate
B. Glycine
C. Glutamate
D. Glutamine
E. Arginine



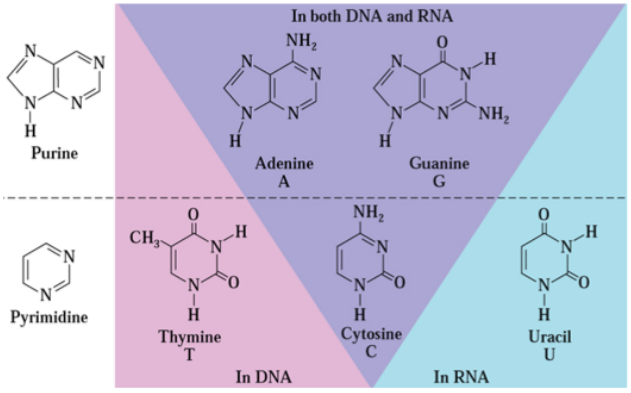
Which enzyme catalyzes the reaction shown below?



A. Carbamoyl phosphate synthetase I
B. Carbamoyl phosphate synthetase II
C. Glutamine transcarbamoylase
D. Glutamate synthase

Which of the following statements is true regarding regulation of purine nucleotide biosynthesis?

A. Glutamine-PRPP amidotransferase is inhibited by purine monophosphates.
B. IMP dehydrogenase is inhibited by AMP.
C. Adenylosuccinate synthetase is inhibited by ATP.
D. A and C are correct.
E. All the above statements are correct.



Chapter 23.3

FORMATION OF DEOXYRIBONUCLEOTIDES

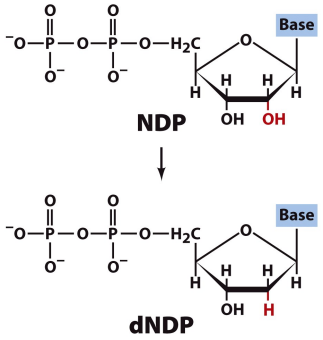
Key Concepts 23.3

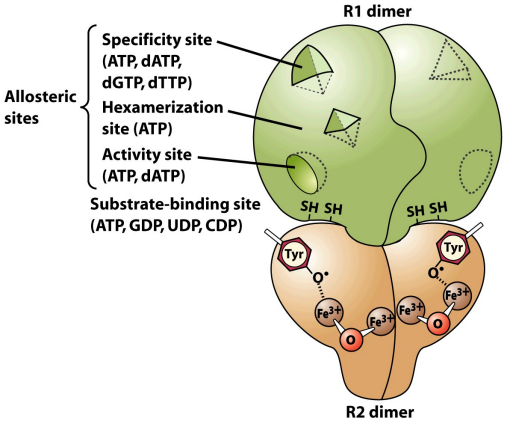
• Ribonucleotide reductase uses a free radical mechanism to convert ribonucleotides to deoxyribonucleotides.

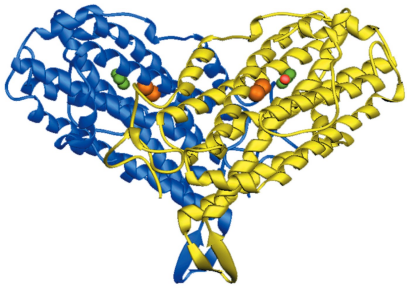
• Thymidylate synthase transfers a methyl group to dUMP to form thymine.

Ribonucleotide Reductase (RNR)

- No de novo synthesis
- Three classes of RNRs: replace 2'–OH of NDP with H via a free radical mechanism
- Class I RNR: heterotetramer with a Fe/Mn prosthetic group



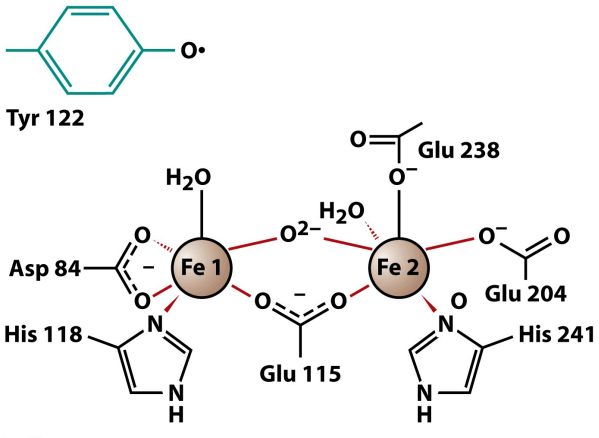


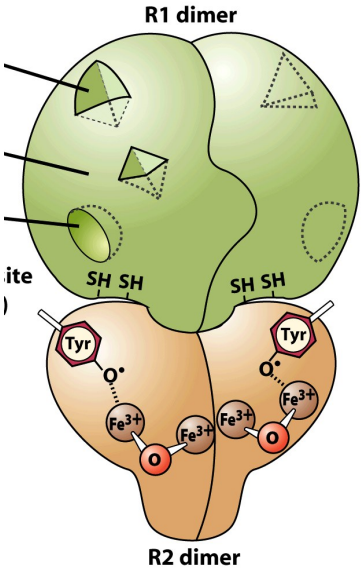


$R2_2$ (PDB: 1R1B)

Class I RNRs

- R1 subunit contains several redox-active thiol groups
- Novel binuclear Fe(III) prosthetic group
- Coordinated by a Tyr radical!





Proposed RNR Reaction Mechanism

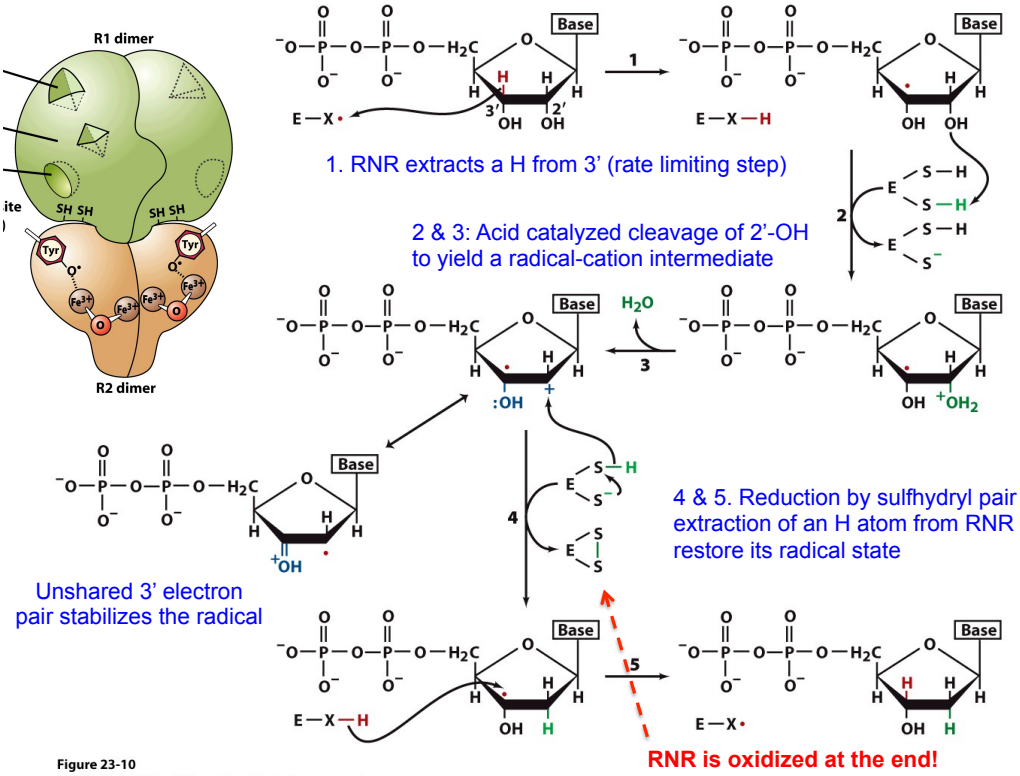
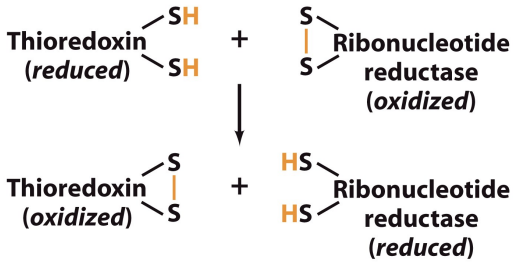
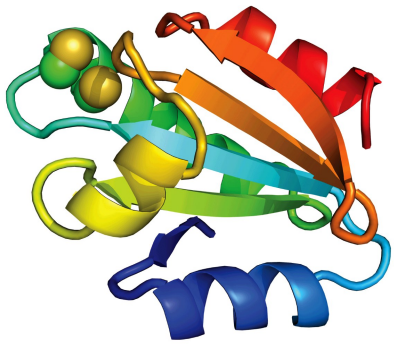


Figure 23-10

Oxidation and Reduction of RNRs

- Oxidized RNRs do not bind NDPs!
  - Conformational switch
  - Conserved the free radicals
  - Would otherwise damage the substrate and enzyme itself
- Thioredoxin reduces RNR to return RNR to the original state
- Thioredoxin reductase recover reduced form of thioredoxin





Electron Transfer Pathway of RNRs

- Thioredoxin reduces RNR to return RNR to the original state
- Thioredoxin reductase recover reduced form of thioredoxin
  - NADPH-mediated reduction with FAD as co-enzyme

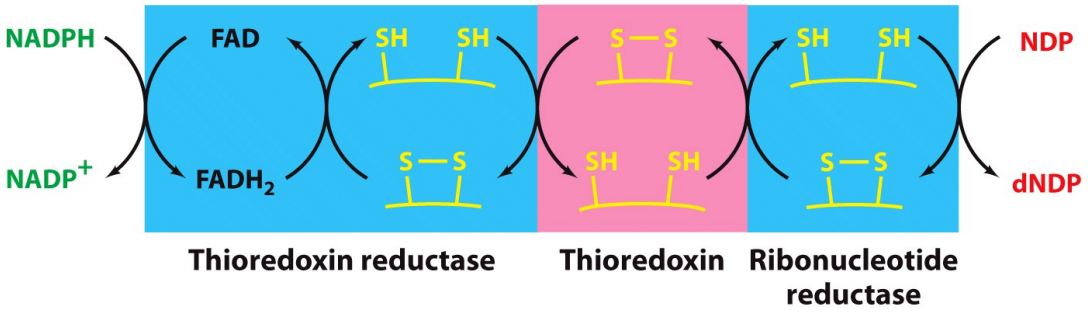


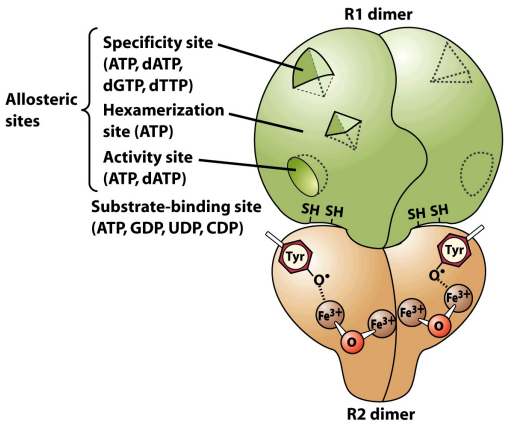
Figure 23-12

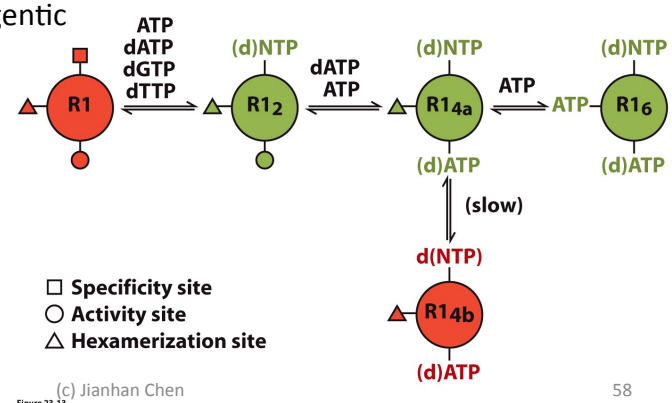
Regulation of RNRs

- Kinases convert dNDPs to dNTPs

  dNDP + ATP <-> dNTP + ADP

- Complex feedback network that is remarkable sensitive to dNTP levels
  - Deficiency of and dNTPs is lethal
  - Excess of dNTPs is mutagentic



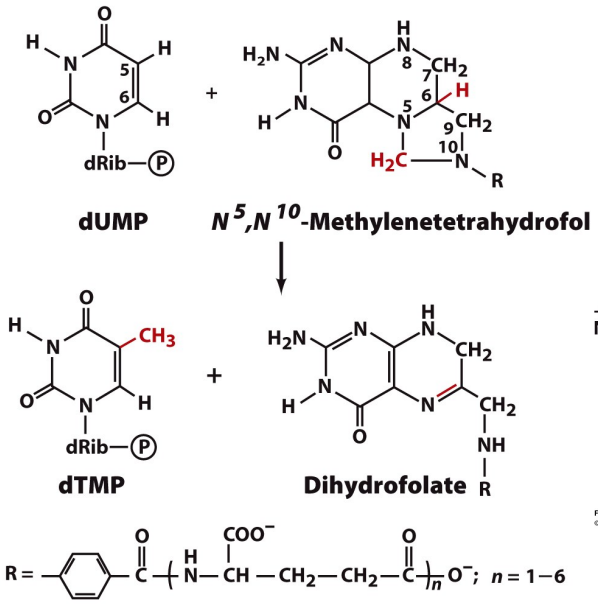


Complex regulation of R1 oligermization and conformational states

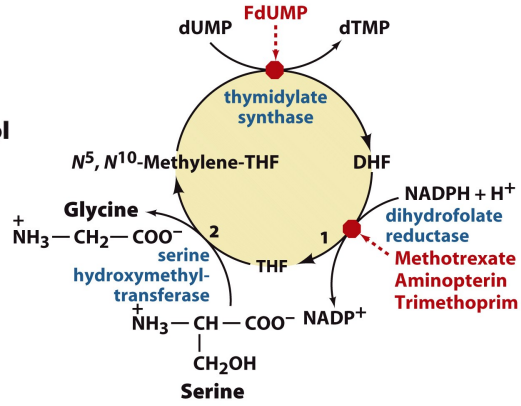
Figure 23-13

dUMP to dTMP via Methylation

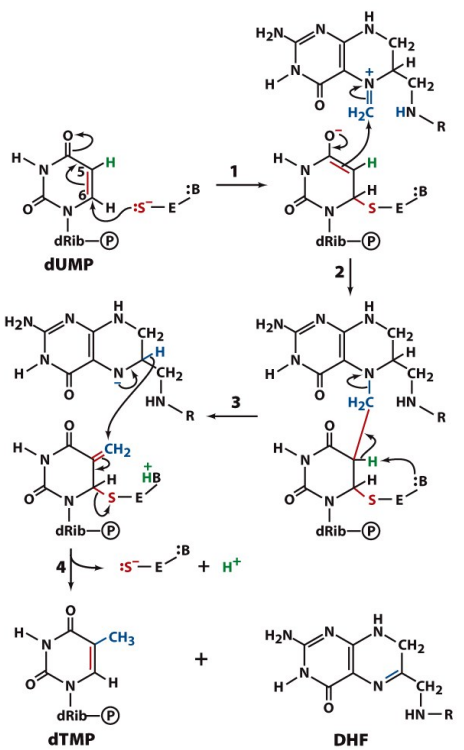
- Catalyzed by thymidylate synthase



Regeneration of THF: two reactions



Thymidylate Synthase: Mechanism



Proposed mechanism

1. Cys 146 attacks C6
2. C5 of enolate ion attacks the CH2 of the iminium cation
3. Enzyme abstracts the proton at C6 position (which is released into solvent)
4. Redox exchange: transfer of methyl group and release of DHF; product released

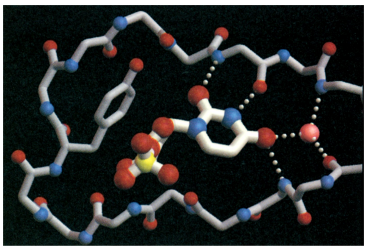
dTTP Generation

- dTMP generated from dUMP, which is generated by hydrolysis of dUTP

  dUTP diphosphohydrolase (dUTPase)

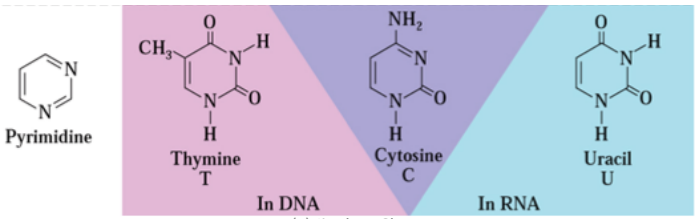
  $dUTP + H_2O$ ------> $dUMP + Ppi$

- dTMP, once generated, is converted back to dTTP.
- Apparently wasteful process. Why?



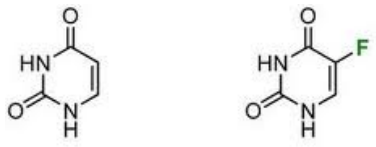
dUMP binding site of dUTPase

DNA polymerases do not discriminate dUTP and dTTP too well; need to suppress dUTP concentration to prevent incorrect incorporation of uracil.



Inhibition of dTMP Synthesis in Cancer Therapy

- dTMP production critical for rapid cell proliferation, such as in cancers: sensitive to dTMP synthesis interruption
- Normal cells slow growing if at all: less sensitive
- Nonreactive dUMP analogs as inhibitors of dTMP synthase
- 5-Fluodeoxyuridylate (5-Fluo dUMP) (suicide substrate)
  - One of the early cancer drugs (approved before 1984)
  - Treat several types of cancer including colon, rectum, and head and neck cancers. It is also used for other types of cancer, and the skin cream is used for other conditions as well.
  - Trade Names: 5-FU, Adrucil, Efudex (topical), 5-fluorouracil
- Inhibition of DHFR blocks THF regeneration: also inhibits dTMP synthesis

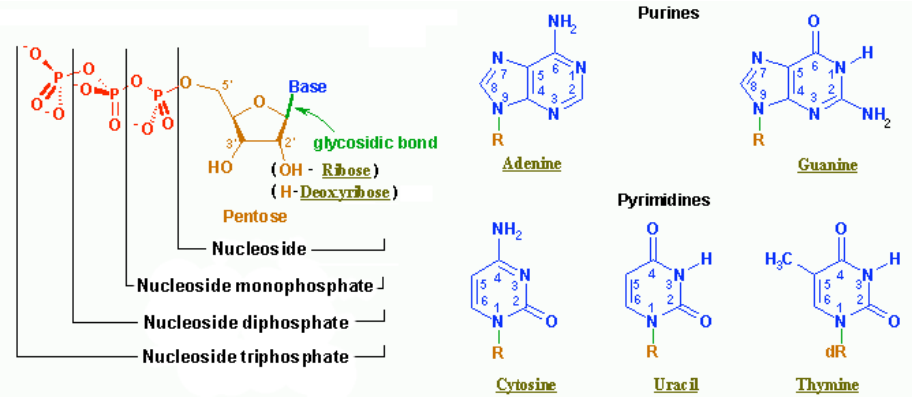


Uracil 5-Fluorouracil

http://www.cancer.org/

Quick Summary

- Describe the production of dNTPs from their corresponding NDPs.
- What is the role of thioredoxin and NADPH in the formation of deoxyribonucleotides?
- Describe the roles of dUTPase, thymidylate synthase, and dihydrofolate reductase in the synthesis of dTMP.
- What is the role of NADPH in the formation of thymidylate?



Chapter 23.4

NUCLEOTIDE DEGRADATION

Key Concepts 23.4

- Purines are broken down to uric acid.
- Uric acid may be further catabolized for excretion.
- Pyrimidines are converted to CoA derivatives for catabolism.

Overview of Nucleotide Metabolism

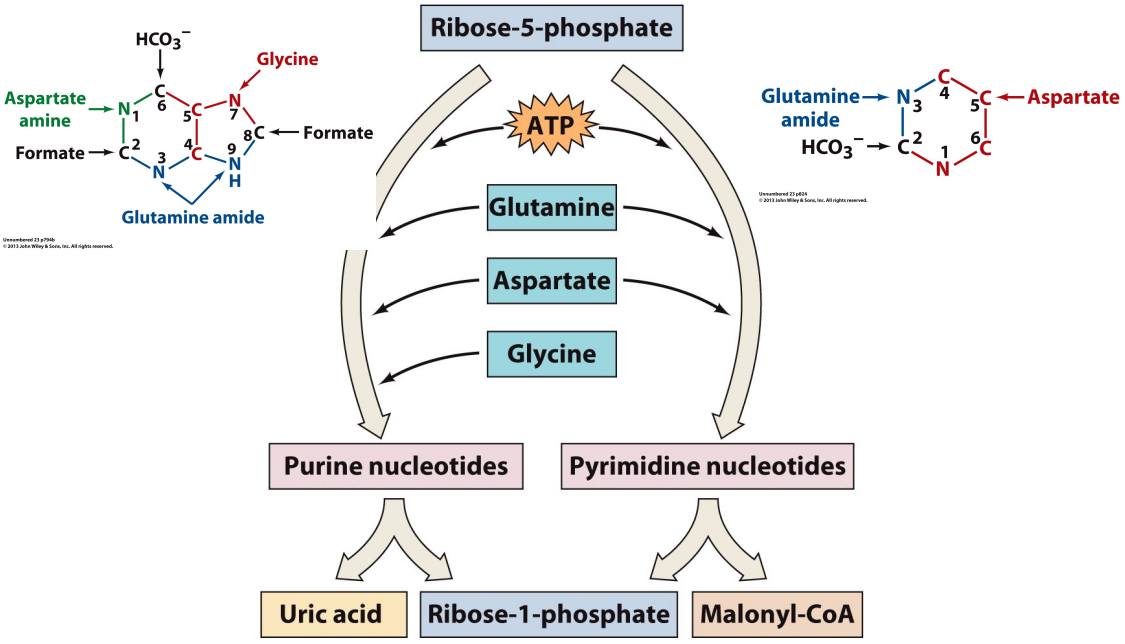


Figure 23-18
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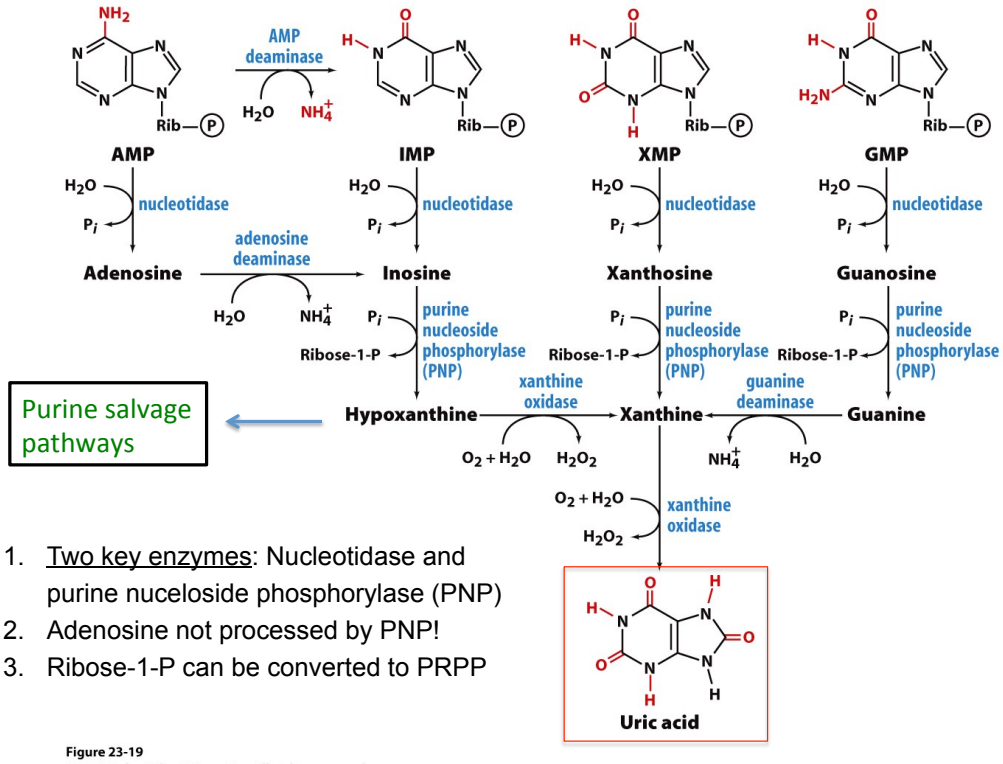
Purine Catabolic Pathways



1. Two key enzymes: Nucleotidase and purine nuceloside phosphorylase (PNP)
2. Adenosine not processed by PNP!
3. Ribose-1-P can be converted to PRPP

Figure 23-19
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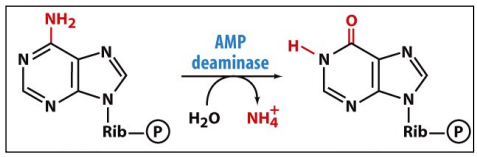
Adenosine Deaminase (ADA)

- 8-stranded beta-barrel with its active side in a pocket at the C-terminal end
- Contains a catalytically active Zn^{2+}
- Defects in ADA active site selectively kill lymphocytes and lead to severe combined immunodeficiency disease (SCID)
  - Invariably fatal;
  - first disease treated successfully by gene therapy (w/ caveats!)
  - Inactive ADA leads to accumulation of dATP (50-fold higher), which in turn inhibits RNRs and prevents synthesis of other dNTPs.
  - Lymphocytes particularly active in dAMP phospholylation



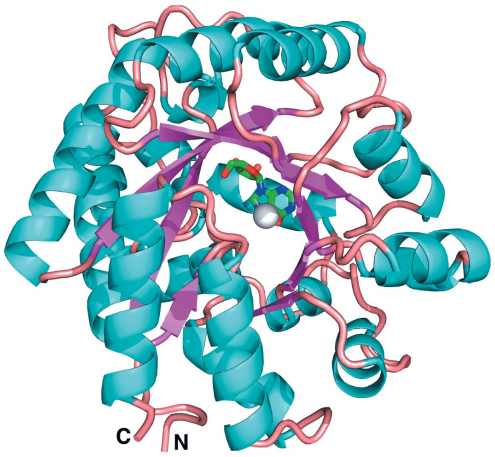
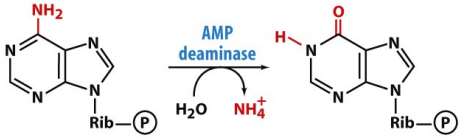


Figure 23-20

Purine Nucleotide Cycle



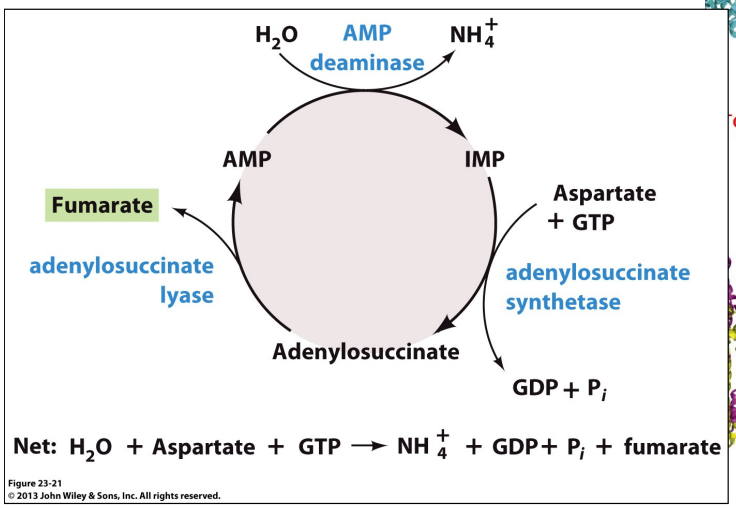
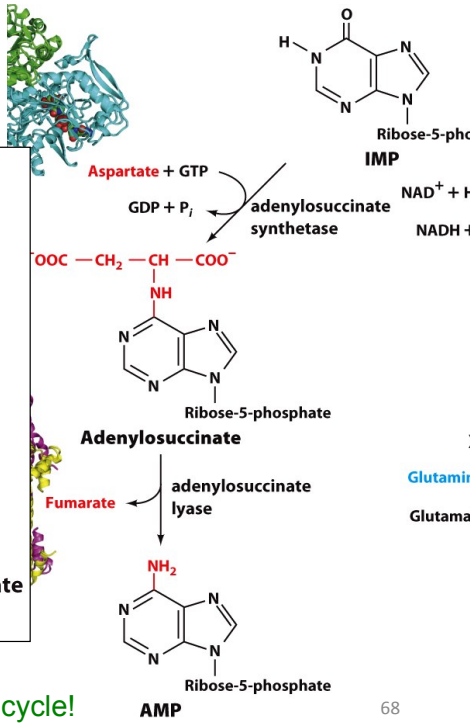
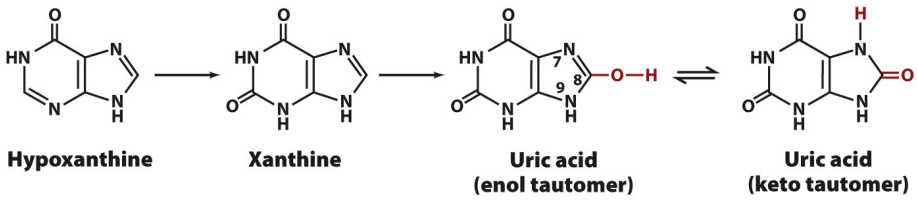


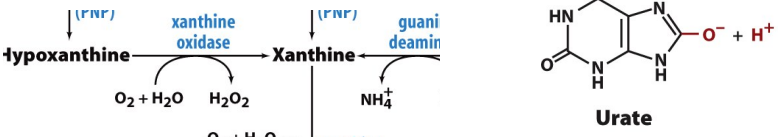
Figure 23-21



Increase skeletal muscle activity increases CTA and replenishes CTA intermediates via purine nucleotide cycle!

Xanthine Oxidase: mini-electron transport system





Occurs exclusively in liver and small intestine

H_2O_2 converted to O2 by catalase

Degradation of Uric Acid

- Excreted or degraded to various levels depending on the species
  - Marine invertebrates can break down uric acid all the way down to ammonia
- Organisms that do not excrete urea can remove excess nitrogen through uric acid
  - Complicated reactions but conserved water (uric acid barely soluble)

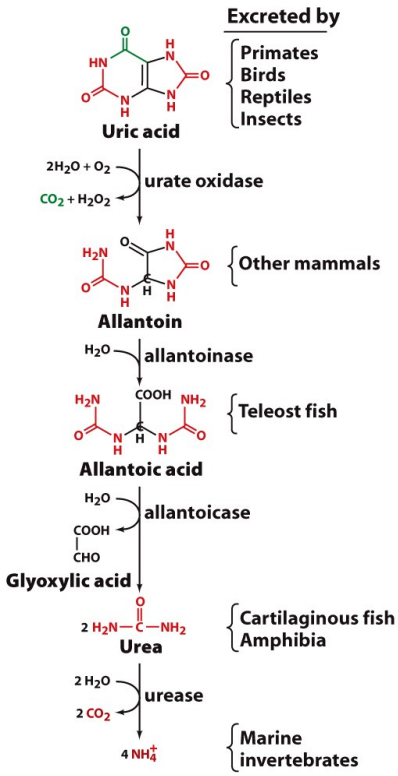


Figure 23-22

Gout: caused by excess of uric acid

- Very painful arthritic joint inflammation
- Caused by deposition of insoluble sodium urate crystal
- Kidney stones, renal damage and urinary tract obstruction
- 3 per 1000 persons

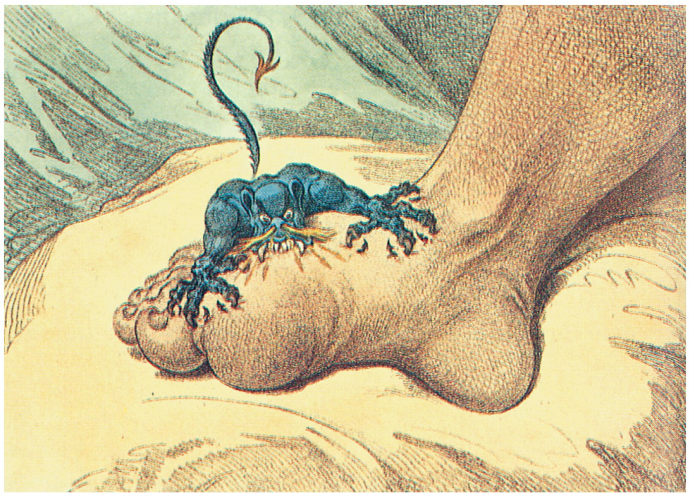


Figure 23-23
Science Source/Photo Researchers, Inc.

Major Pyrimidine Catabolism Pathways

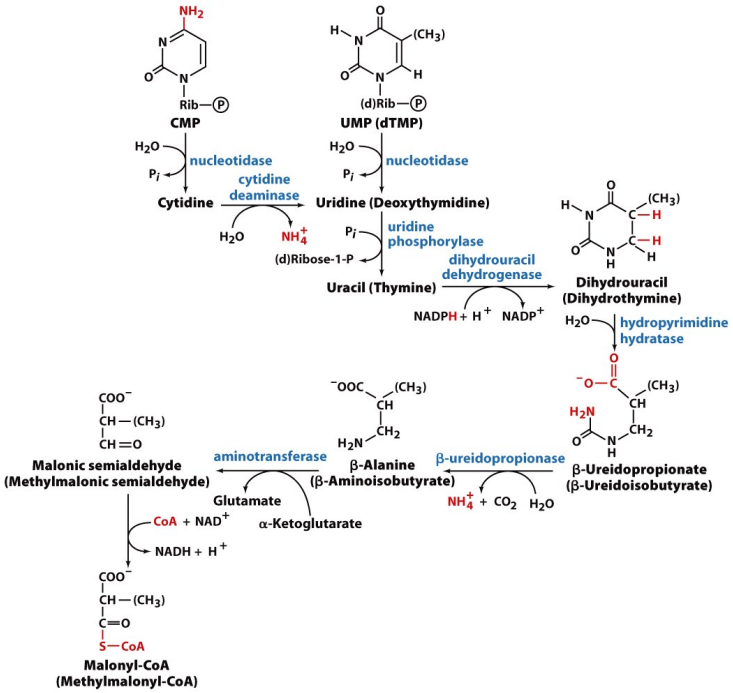


Figure 23-24

Quick Summary

- What compounds are produced by the degradation of purines and pyrimidines?
- Describe the reactions catalyzed by nucleoside phosphorylase, adenosine deaminase, and xanthine oxidase.
- What is the function of the purine nucleotide cycle?
- What are the physiological implications of excreting waste nitrogen in the form or urate, urea, or ammonia?
- Describe how purine catabolism is related to SCID, muscle function, and gout.