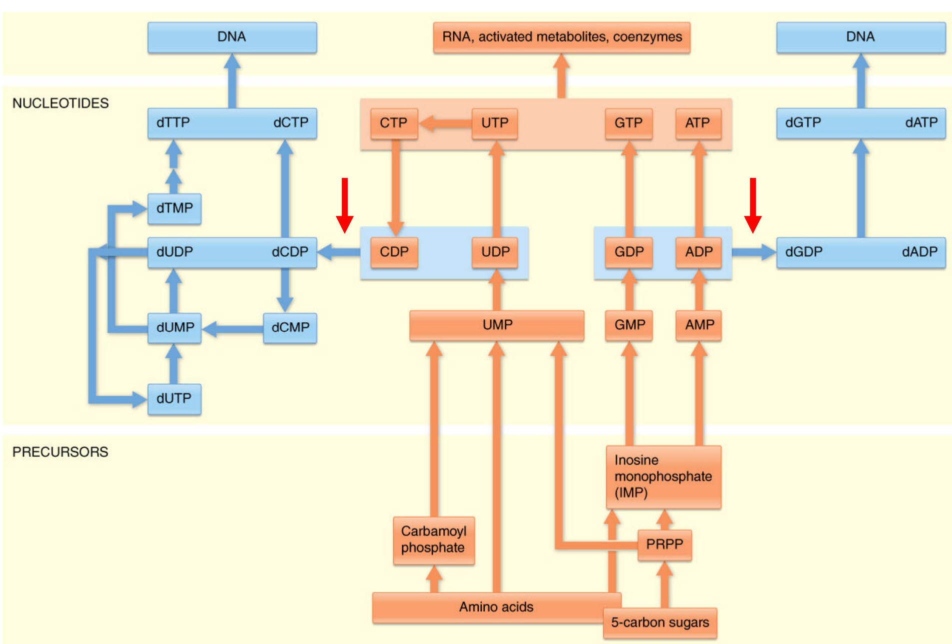
Outline- Ch.22.4 Part II

1. Deoxynucleotide biosynthesis and regulation

2. Salvage pathways and nucleotide catabolism

Nucleotide biosynthesis: birds-eye view



Making deoxyribonucleotides: ribonucleotide reductase (RNR)

- Synthesized from NDPs via free radical mechanism
- Several different classes of enzymes that utilize different cofactors and free radical generating mechanisms
  - *E. coli* Class I (aerobic) binuclear iron center, Tyrosine radical, 5 Cysteines
- Reactive cysteine pair provides H to replace 2'-OH

E. coli RNR is a tetramer (α and β subunits)

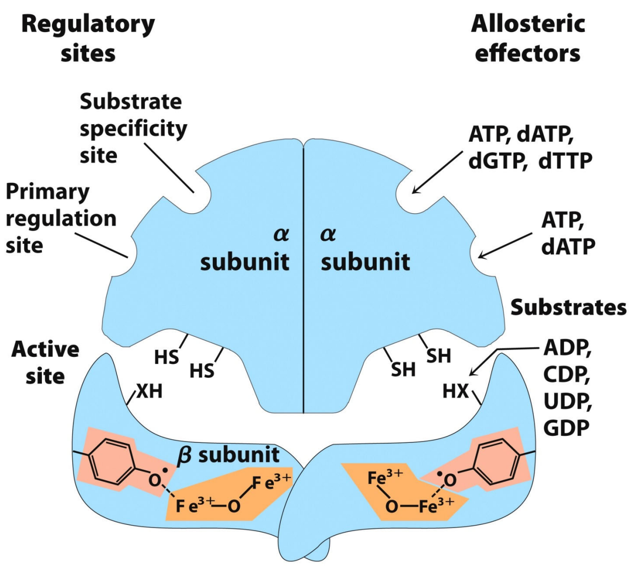


Figure 22-42a
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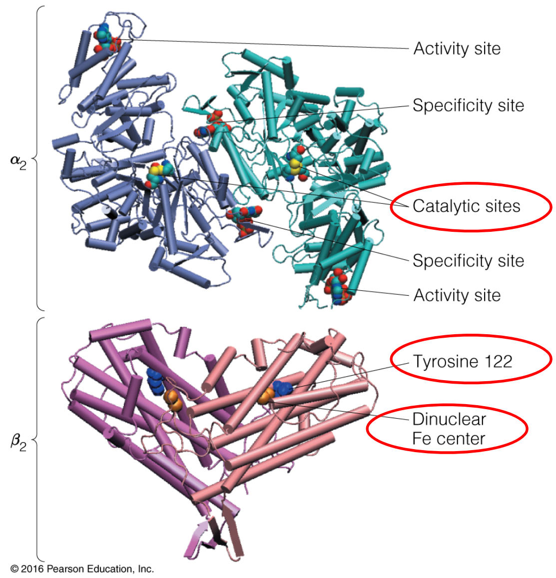
E. coli RNR is a tetramer (α and β subunits)

A better picture…

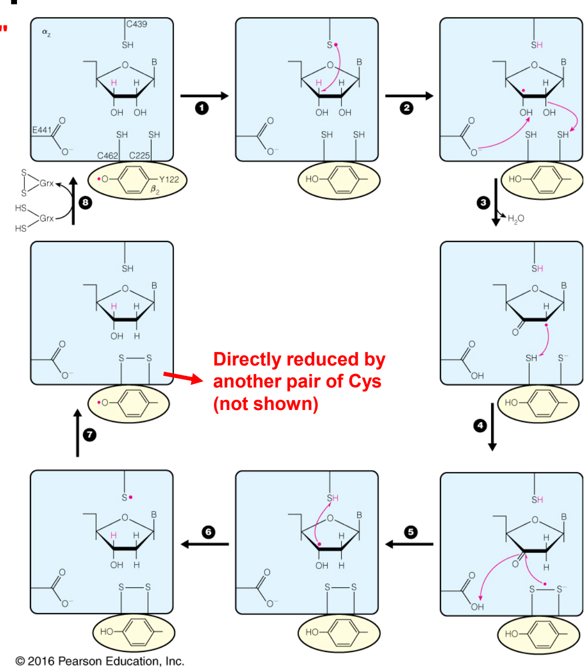
Catalytic groups:

1) **Binuclear Fe^{3+}**
2) **Stable Tyr•**
3) **5 Cys**



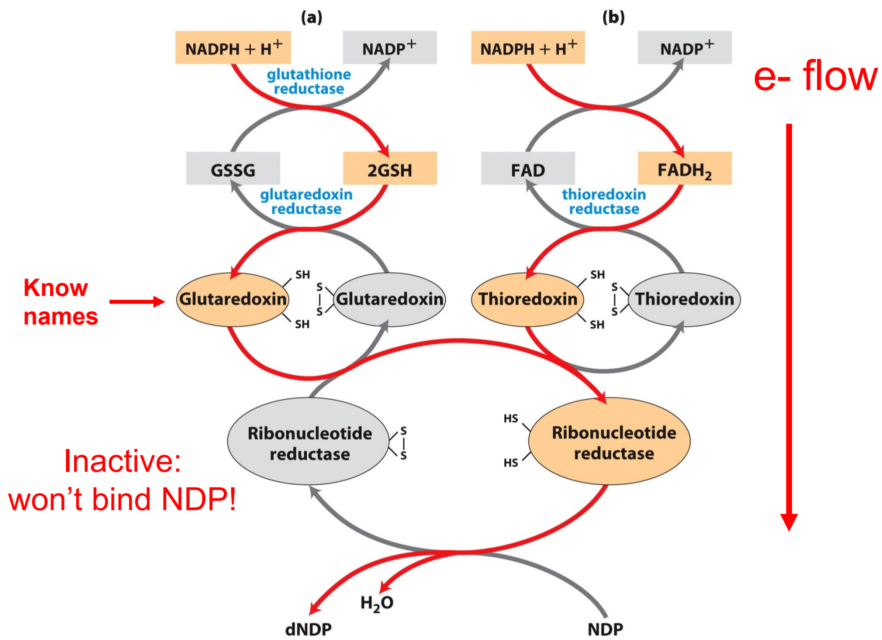
Proposed RNR reaction mechanism

"Catalytic site"



Directly reduced by another pair of Cys (not shown)

Active RNR must be regenerated after reaction



Regulation of *E. coli* RNR

Close up of active site GDP bound to *E. coli* RNR.

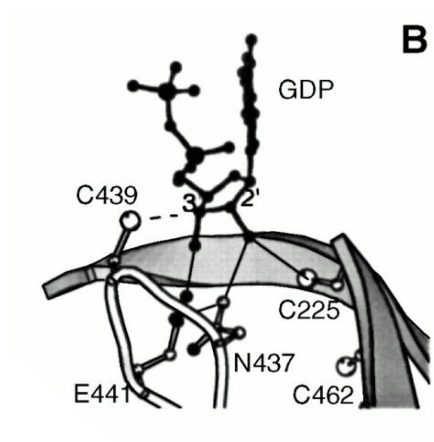
Cys 439 is thiyl radical

Cys 225 and 462 undergo oxidation and reduction during the RNR reaction.

If Cys225-Cys462 disulfide bond:

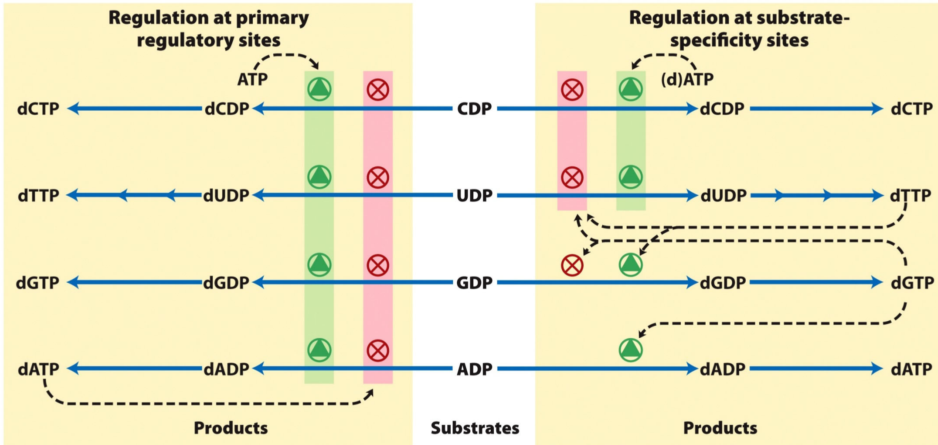
1) movement of C225 S

2) won't allow 2'OH of NDP to enter active site



Regulation #1: oxidized enzyme can't bind S

Regulation of *E. coli* RNR



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Regulation #2: activity site   Regulation #3: specificity site

Inactivation of RNR by dATP

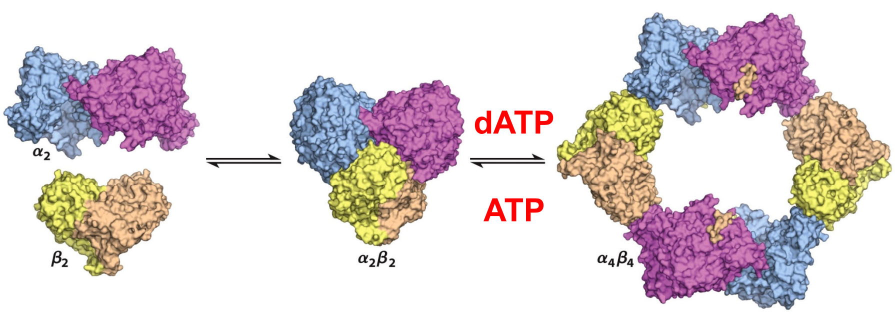

Figure 22-45
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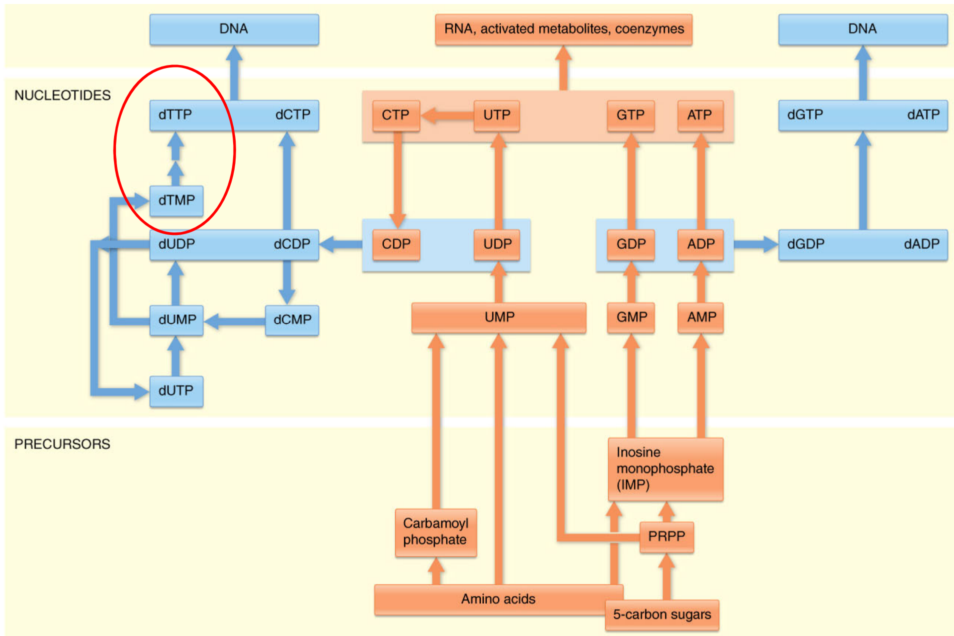
Nucleotide biosynthesis: birds-eye view



Formation of dTMP by methylation of dUMP

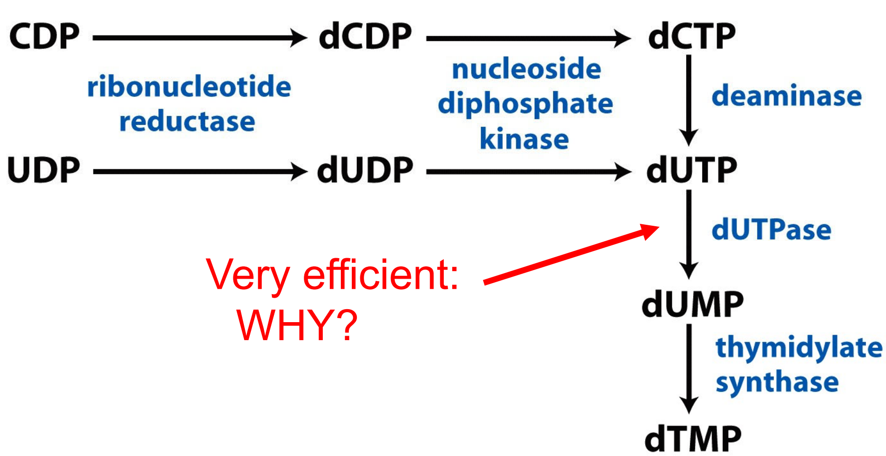


Figure 22-43
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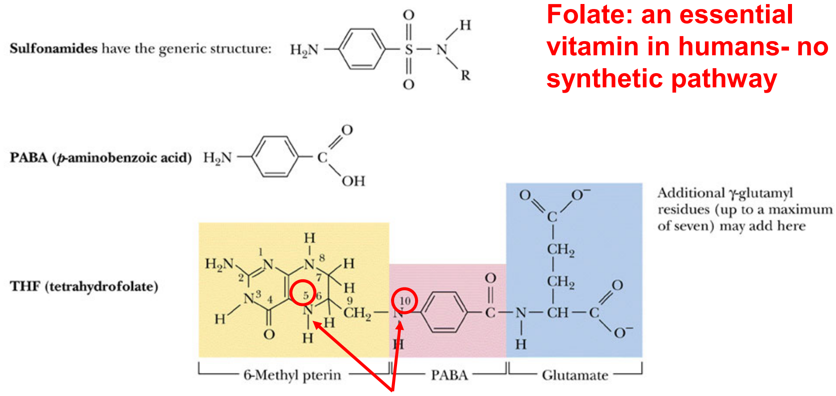
Tetrahydrofolate: A one-carbon carrier

Folate: an essential vitamin in humans- no synthetic pathway

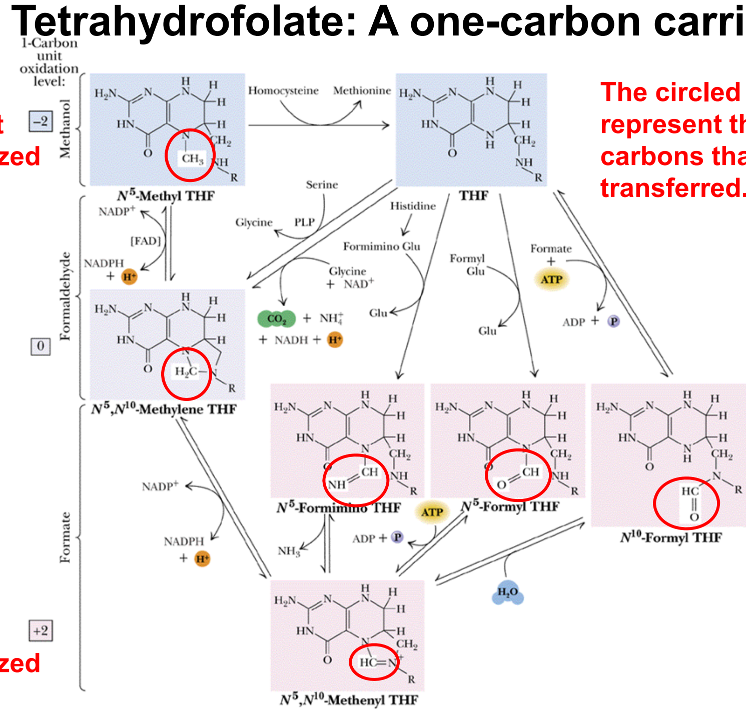


Carbon attachment points

Tetrahydrofolate: A one-carbon carrier

Least oxidized

most oxidized



The circled carbons represent the carbons that get transferred.

Formation of dTMP by methylation of dUMP

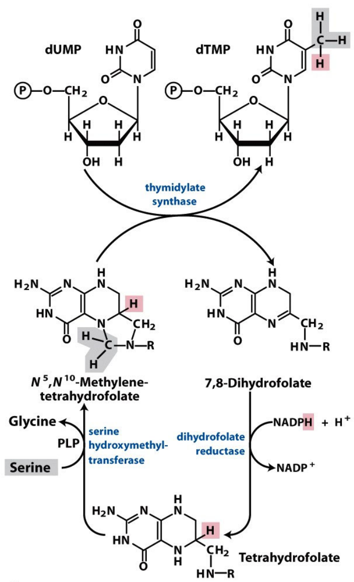


Figure 22-44
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Thymidylate synthase (TS):
Catalytic features:
- covalent intermediate
- methyleneTHF donor
- reduction/oxidation THF
  TS reaction only!
- DHF→THF requires DHFR

Important cancer/antibacterial target!

Formation of dTMP is target of many drugs

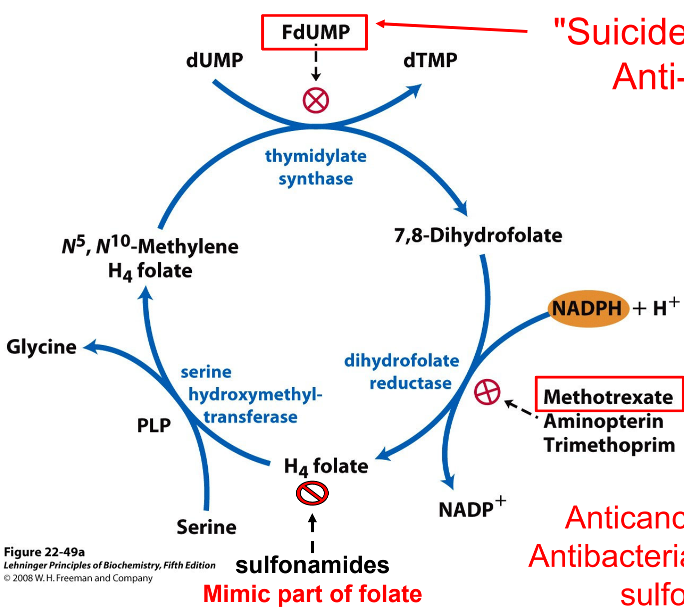

Figure 22-49a
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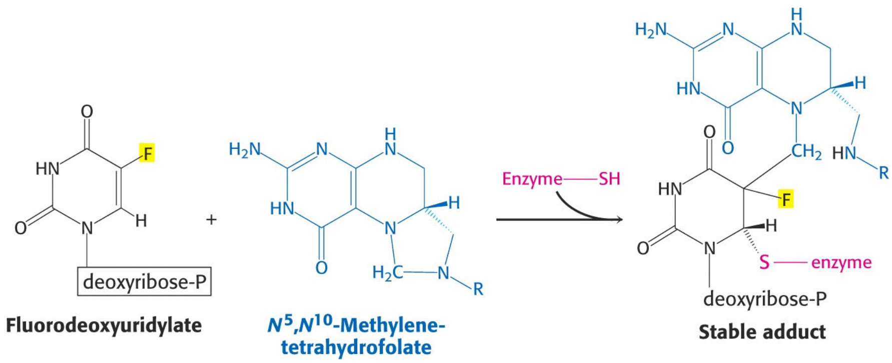
"Suicide inhibitor"
Anti-tumor

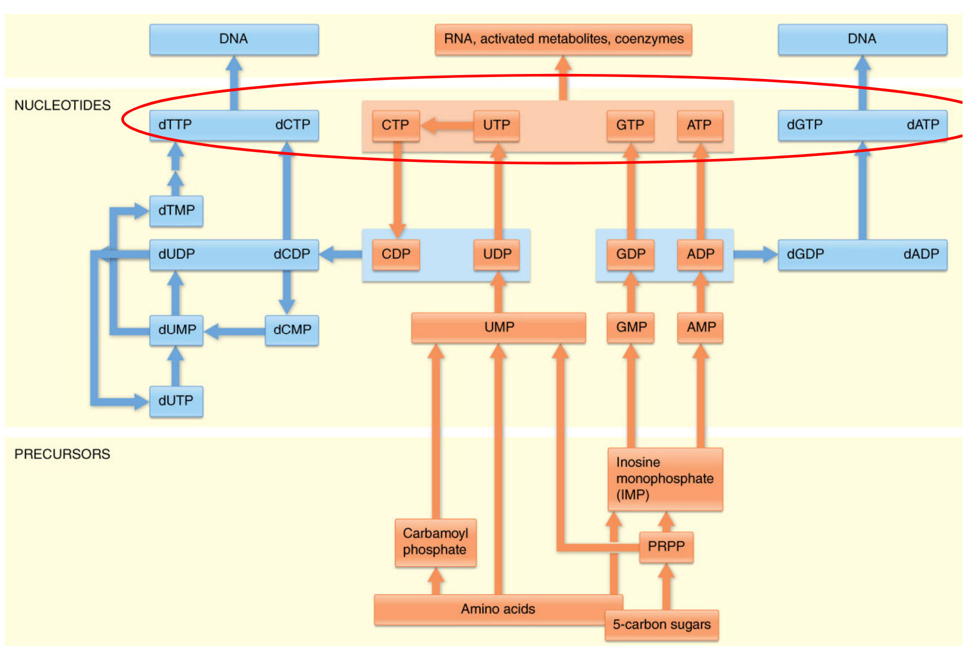
Anticancer (leukemia)
Antibacterial (trimethoprim, sulfonamides)

5-FU is suicide inhibitor of TS

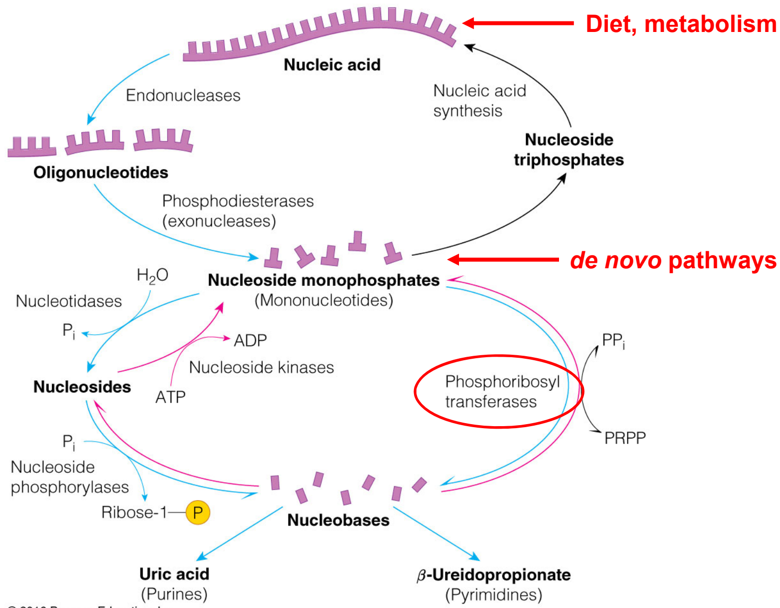


- Forms E-FU covalent intermediate
- Can't abstract Fluorine: STUCK.
- very toxic

Nucleotide biosynthesis: birds-eye view



Salvage pathways of nucleotide synthesis



Salvage pathways of nucleotide synthesis

Purine PRTases:

APRT: Adenine + PRPP → AMP + PPi

HXGPRT: Hypoxanthine + PRPP → IMP + PPi

Guanine + PRPP → GMP + PPi

Deficiency in HXGPRT: Lesch-Nyhan syndrome

- sex-linked, male
- by age 2- signs of mental/physical delay and hostile behavior
- age >2- develop gout, self-destructive, chew off fingers/lips
- underscores importance of purine salvage in mammals

Nucleotide degradation: purine catabolism

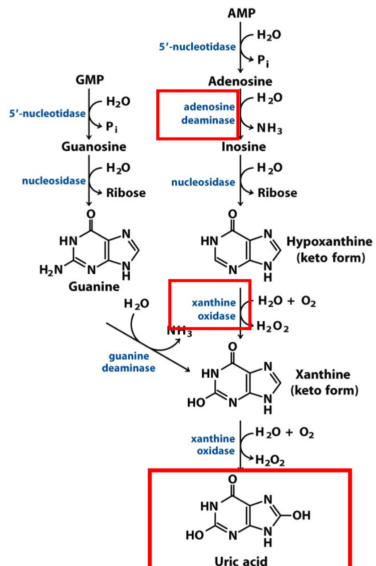


Figure 22-45 part 1
Lehninger Principles of Biochemistry, Fifth Edition

- Nucleotides converted to free base (point of entry into salvage also, but most tissues catabolize)

- Deficiency in ADA: SCIDS
  Adenosine → dATP
  "Bubble boy" syndrome

Nucleotide degradation: purine catabolism

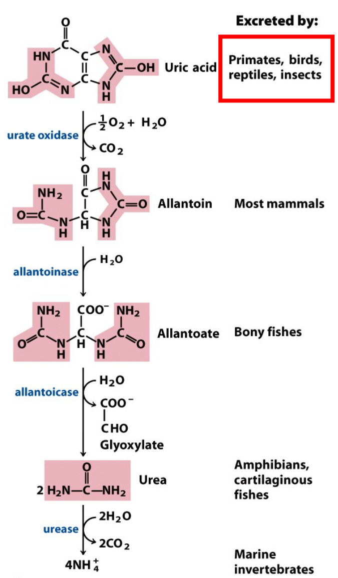


Figure 22-45 part 2
Lehninger Principles of Biochemistry, Fifth Edition

- pKa = 5.4, therefore urate at pH 7
- humans stop here + excrete
- not very soluble, builds up near concentration limit for solubility
- If build up too much: Gout

Interesting Note:
Humans have urate oxidase (uricase) gene, but inactive

Nucleotide degradation: purine catabolism

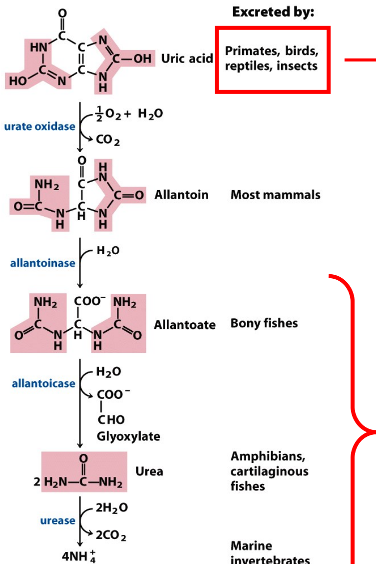


Figure 22-45 part 2
Lehninger Principles of Biochemistry, Fifth Edition

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Fish and marine animals

Nucleotide degradation: purine catabolism

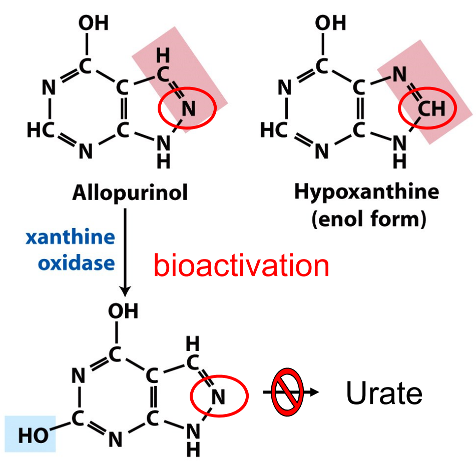

Figure 22-47
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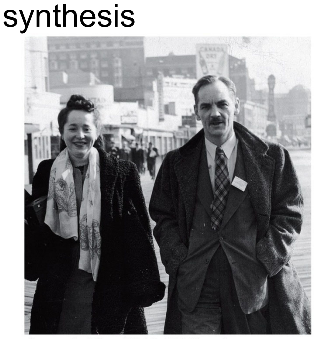
Inhibit xanthine oxidase:
- no xanthine to uric acid
- excrete: X and HX (more soluble)
- inhibits both urate formation and purine synthesis



Gertrude Elion (1918–1999) and
George Hitchings (1905–1998)
Unnumbered 22 p894
Lehninger Principles of Biochemistry, Fifth Edition

Nucleotide degradation: purine catabolism

Other uses for allopurinol:

Antiparasite:
Plasmodium and *Leishmania* no *de novo* pathways; very active purine salvage

Treatment of Lesch-Nyhan:
relieves gout symptoms, but NOT neurological defects!