Outline- Ch.22.4 Part I

1. Review: Nucleotide structure and nomenclature
2. *De novo* nucleotide biosynthesis and regulation

References

1. Nelson and Cox (2013) Lehninger Principles of Biochemistry, 6th ed, Freeman
2. Berg et al. (2015) Biochemistry, 8th ed, Freeman
3. Appling et al. (2016) Biochemistry Concepts and Connections, Pearson

Review: Nucleotide structure and nomenclature

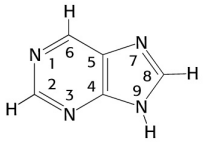
Know names

TABLE 25.1 Nomenclature of bases, nucleosides, and nucleotides

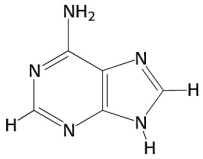
Base	Ribonucleoside	Ribonucleotide (5′-monophosphate)
RNA		
Adenine (A)	Adenosine	Adenylate (AMP)
Guanine (G)	Guanosine	Guanylate (GMP)
Uracil (U)	Uridine	Uridylate (UMP)
Cytosine (C)	Cytidine	Cytidylate (CMP)
Hypoxanthine	Inosine	Inosinate (IMP)
Xanthine	Xanthosine	Xanthylate (XMP)
Orotate	Orotidine	Orotidylate (OMP)
DNA		
Base	Deoxyribonucleoside	Deoxyribonucleotide (5′-monophosphate)
Adenine (A)	Deoxyadenosine	Deoxyadenylate (dAMP)
Guanine (G)	Deoxyguanosine	Deoxyguanylate (dGMP)
Thymine (T)	Thymidine	Thymidylate (TMP)
Cytosine (C)	Deoxycytidine	Deoxycytidylate (dCMP)

Review: Nucleotide structure and nomenclature

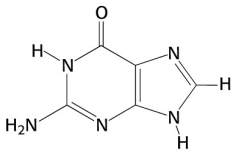
PURINES



Purine



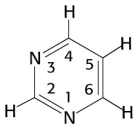
Adenine



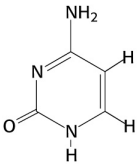
Guanine

Hypoxanthine, Xanthine

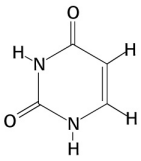
PYRIMIDINES



Pyrimidine

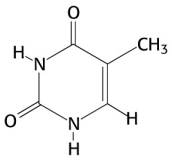


Cytosine



Uracil

Orotate



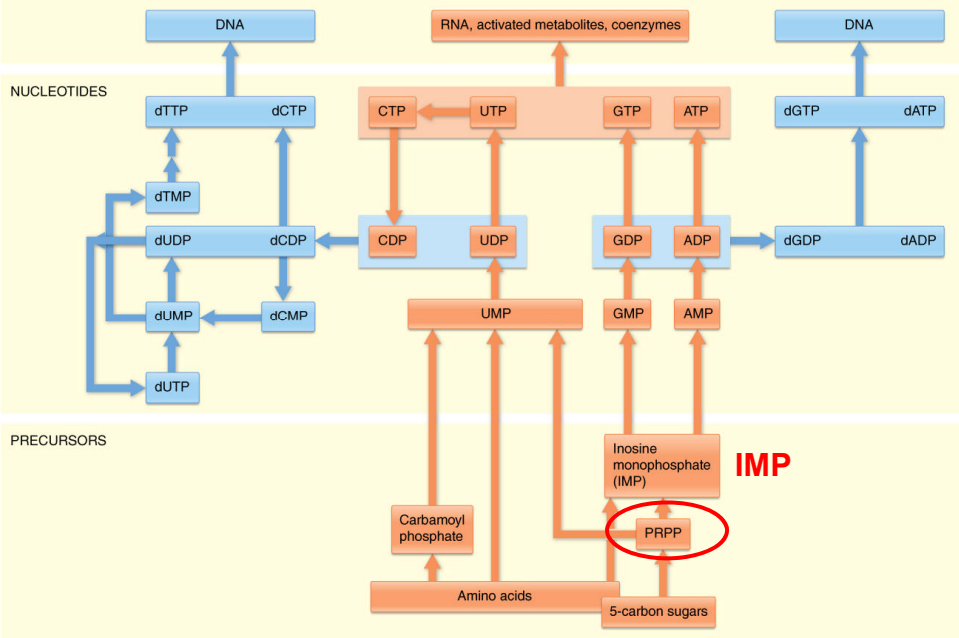
Thymine

Know names and structures

Nucleotide biosynthesis

- **Two pathways**:
  - *De novo*: Synthesis from amino acids, ribose-5-phosphate, CO_2, and NH_3
  - Salvage: Recycle free bases and nucleosides (mostly from diet)
- ***De novo* pathways are nearly universally conserved in all organisms**

Nucleotide biosynthesis: birds-eye view



De novo purine synthesis

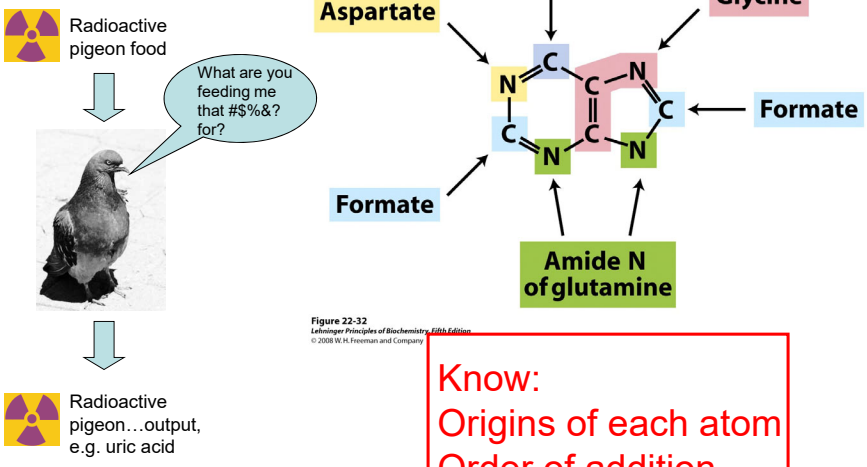


Figure 22-32
Lehninger Principles of Biochemistry, Fifth Edition
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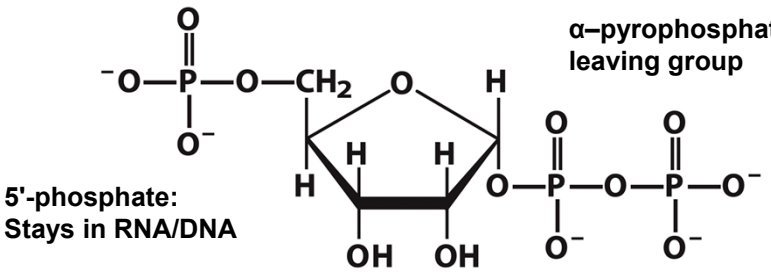
Know:
Origins of each atom
Order of addition

PRPP: Origin of the nucleotide ribose



Unnumbered 22 p892a
Lehninger Principles of Biochemistry, Sixth Edition

- From pentose phosphate pathway
- PRPP needed in other pathways (histidine, tryptophan)
- Rings are built while attached to ribose-5-phosphate

IMP synthesis: Steps 1-3

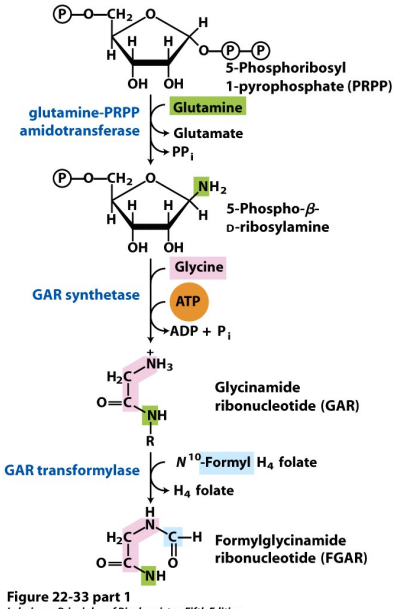


Figure 22-33 part 1
Lehninger Principles of Biochemistry, Fifth Edition

Step 1: form N9

- PRPP is activated precursor (AP)
- inversion of configuration
- committed step, tightly regulated

Step 2: form C4, C5, N7

- phosphorylglycine AP
- Glycine amino: next nucleophile
- all atoms of glycine remain

Step 3: form C8

- N_{10}formyl-THF donates formyl group

IMP synthesis: Steps 4+5

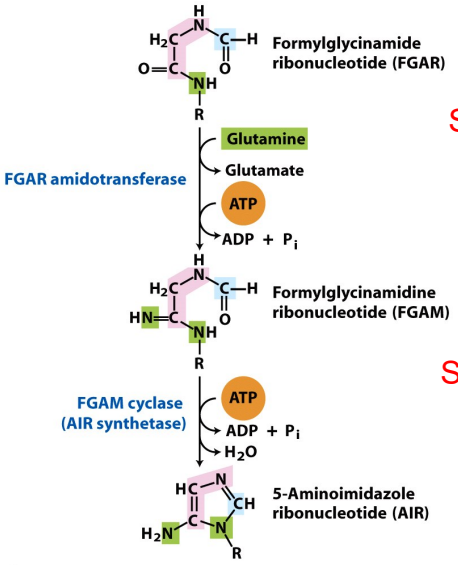


Figure 22-33 part 2
Lehninger Principles of Biochemistry, Fifth Edition

Step 4: form N3

- C4 amide phosphorylation AP
- amidine formed by NH_3 from Gln

Step 5: cyclization

- C8 amide phosphorylation AP
- 5-member (imidazole) ring complete
- activation by phosphorylation ensures irreversibility

IMP synthesis: Steps 6 + 7

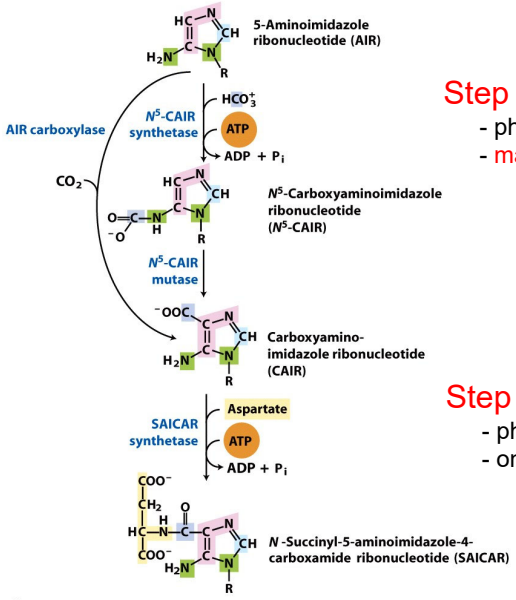


Figure 22-33 part 3
Lehninger Principles of Biochemistry, Fifth Edition

Step 6: form C6

- phosphorylcarbonate AP (bacteria/fungi)
- mammals- no ATP requirement

Step 7: form N1

- phosphorylcarboxylate AP
- only Asp amino will remain

IMP synthesis: Steps 8-10

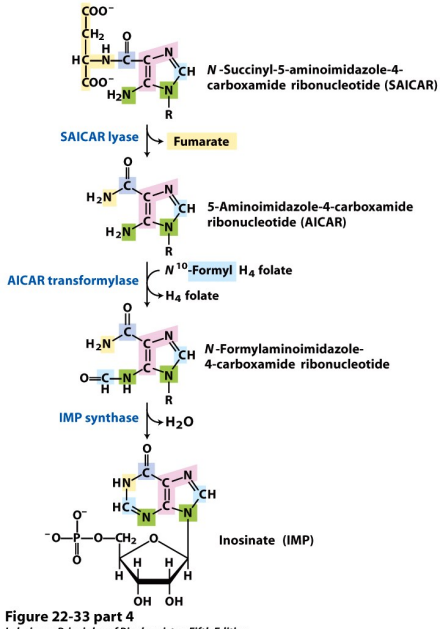


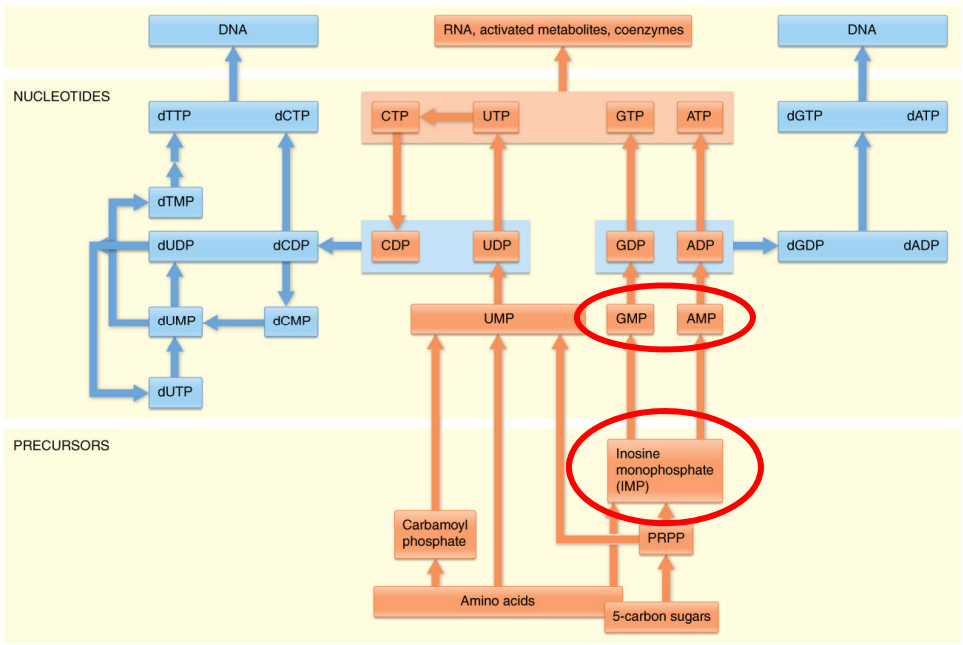
Figure 22-33 part 4
Lehninger Principles of Biochemistry, Fifth Edition

Step 8: eliminate fumarate
- citrulline → arginine (urea cycle)

Step 9: form C2
- N-formyl-THF donor
- second N-formyl THF reaction

Step 10: cyclization #2
- thermodynamically favorable cyclization reaction (no activation)
- 6-member ring complete
- end product: IMP

Nucleotide biosynthesis: birds-eye view



AMP and GMP synthesis: from IMP

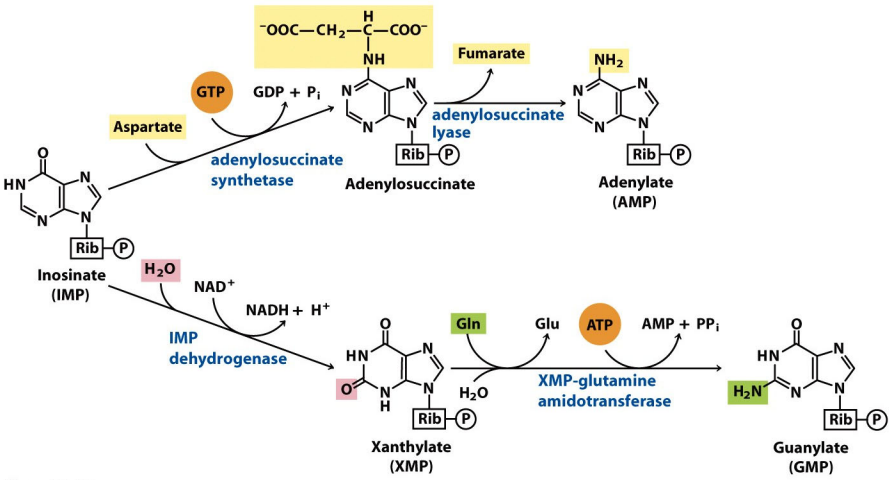


Figure 22-34
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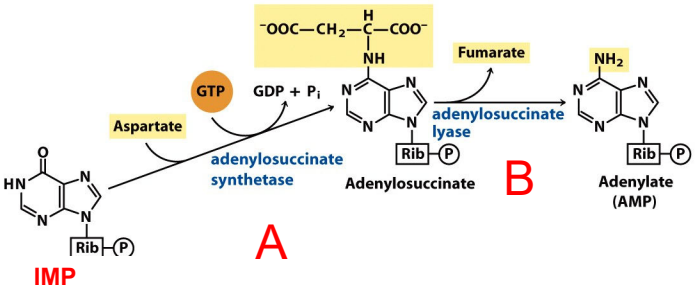
AMP and GMP synthesis: from IMP



Same as steps 8 and 9 of IMP biosynthesis-
EXCEPT: Step A uses GTP for phosphate activation step

Step B is same enzyme used for IMP pathway

AMP and GMP synthesis: from IMP

Step A: addition of water and dehydrogenation by NAD^+

Step B: nucleophilic displacement of activated carbonyl
EXCEPT: activated with AMP, not phosphate

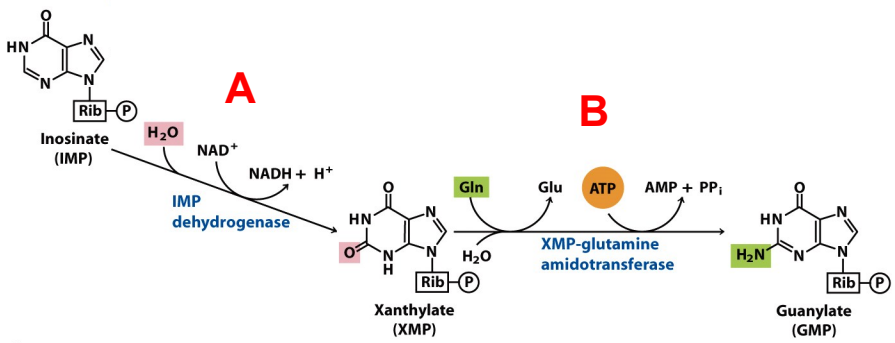


Figure 22-34
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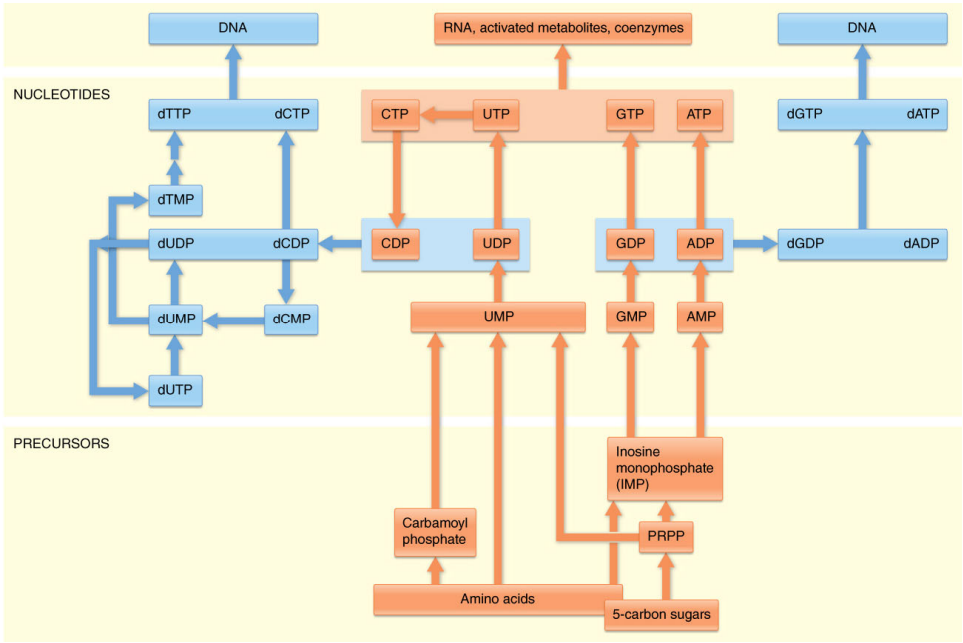
Nucleotide biosynthesis: birds-eye view



Need for precise regulation in vivo

Intracellular pools of NTP/dNTPs (mM):

	E.coli	Mammals
ATP	3.0	2.8
GTP	0.92	0.48
CTP	0.52	0.21
UTP	0.89	0.48
dATP	0.18	0.013
dGTP	0.12	0.005
dCTP	0.07	0.022
dTTP	0.08	0.023

Not much room for error

Regulation of purine synthesis

Important to balance:

- deficiency= lethal
- overabundance= mutagenic

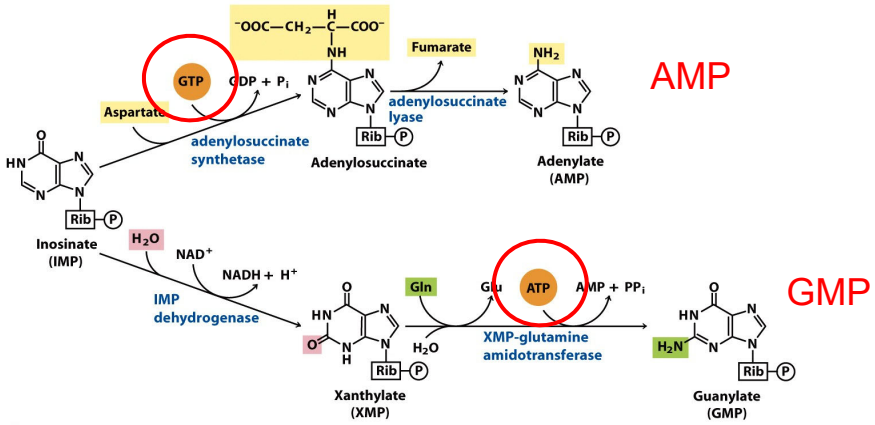
Four points of control in *E. coli*

three mechanisms:

- Reciprocal control
- Feedback inhibition (3 steps)
- Feedforward activation

Regulation of purine synthesis

Purine regulation #1: Reciprocal control

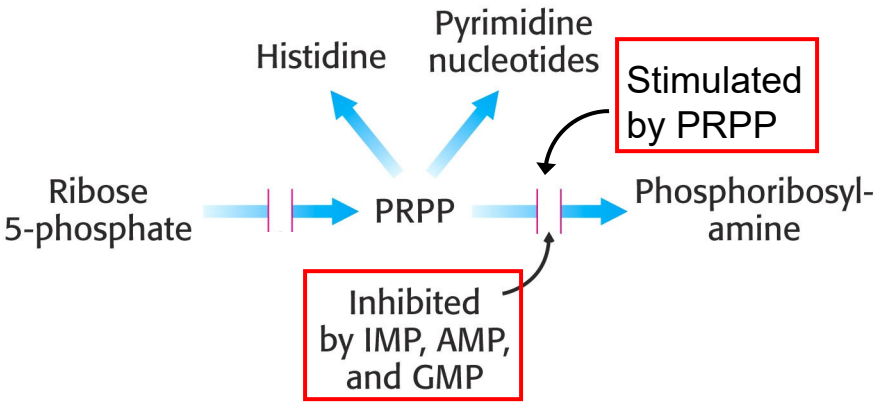


Regulation of purine synthesis

Purine regulation #2: Committed step

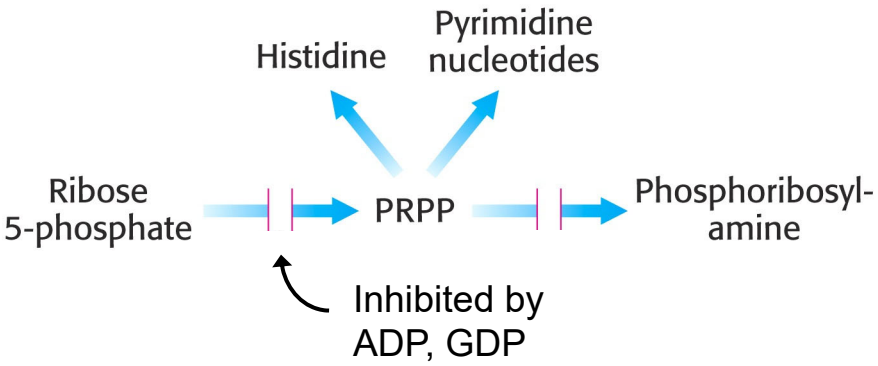
Feedback inhibition: AMP and GMP bind to different sites

Feedforward activation: PRPP



Regulation of purine synthesis

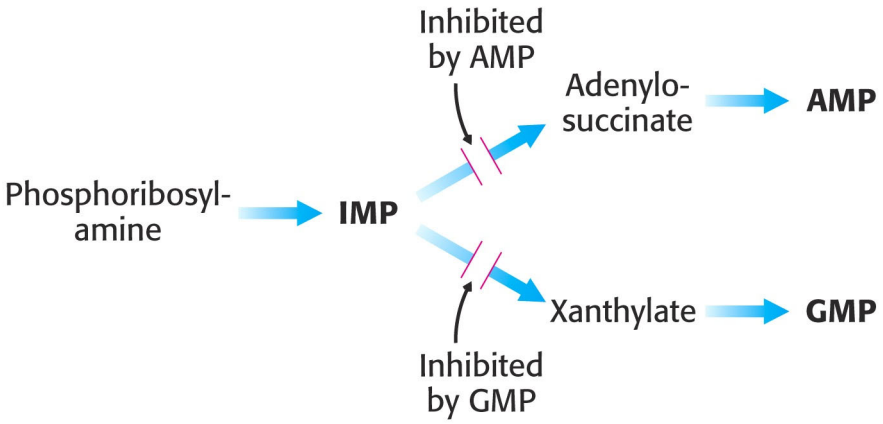
Purine regulation #3: Feedback inhibition of PRPP formation



Regulation of purine synthesis

Purine regulation #4: Feedback inhibition

AMP, GMP competitive inhibitors of branchpoint enzymes



Regulation of purine synthesis in *E. coli*

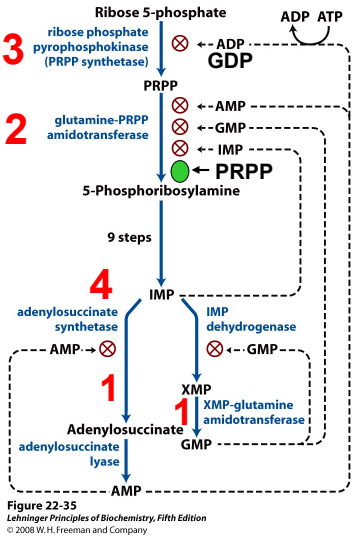


Figure 22-35
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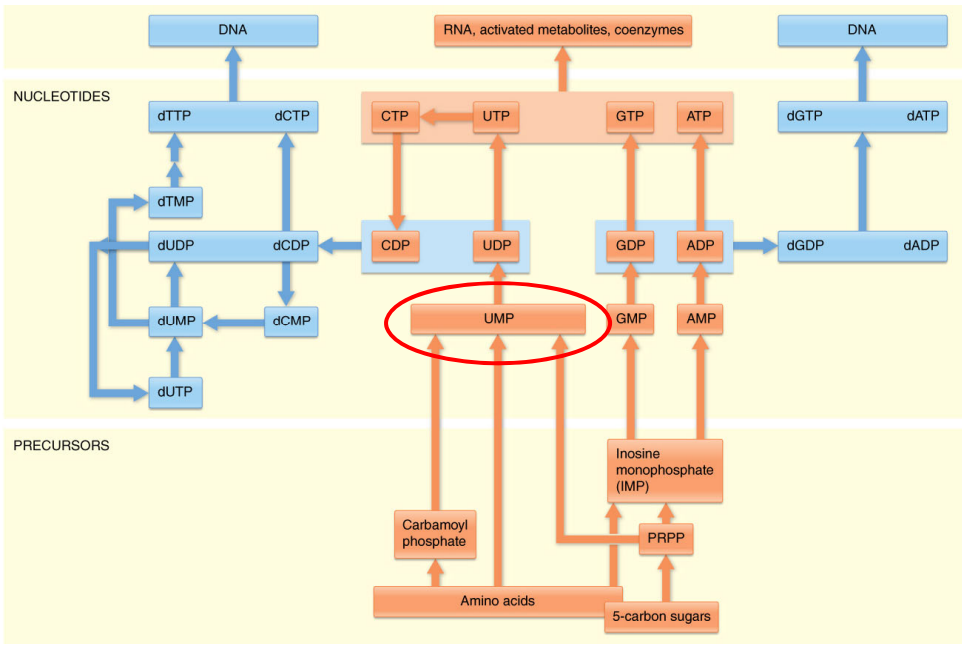
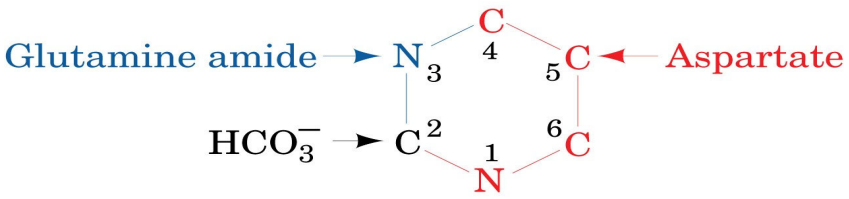
Multiple points of control

Nucleotide biosynthesis: birds-eye view



De novo pyrimidine synthesis

- *De novo* pathway leads to UMP
  UMP phosphorylated to UTP, then → CTP
- Build nucleobase (orotate) first, then attach to PRPP



De novo pyrimidine synthesis

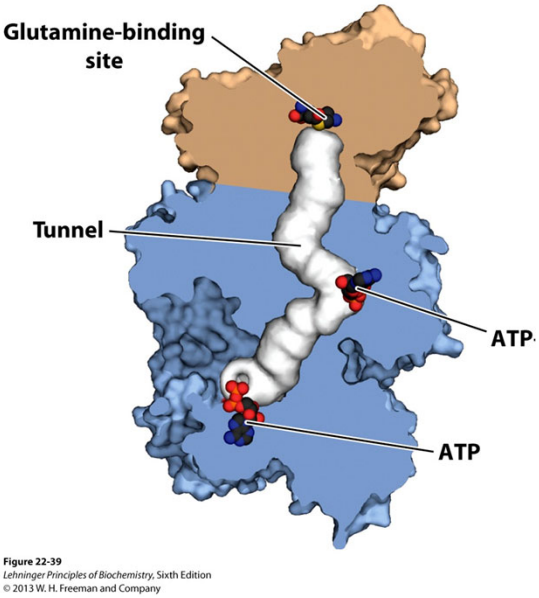


Figure 22-39
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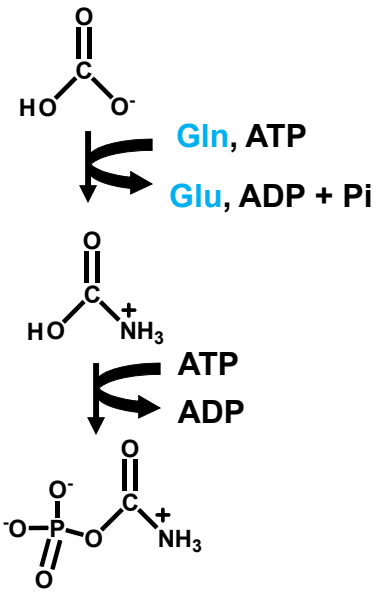
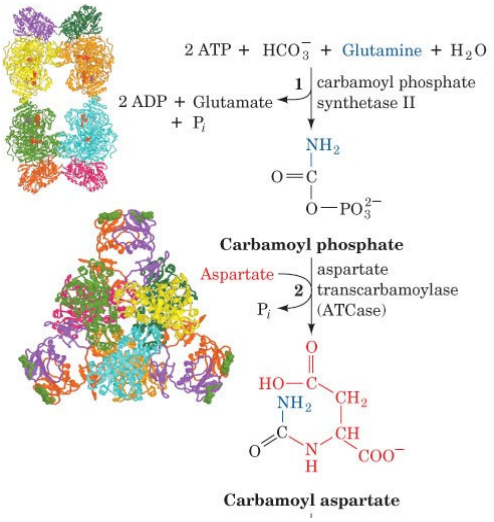


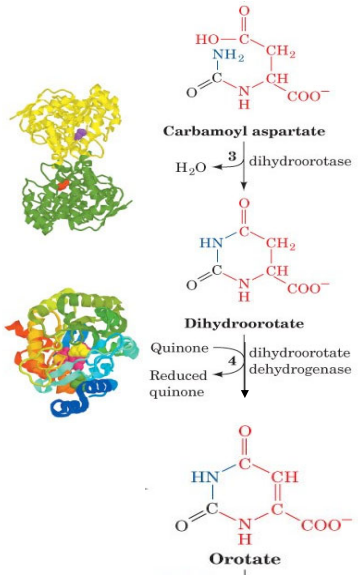
UMP synthesis steps 1-2



Step 1: form N3, C2
- phosphoryl carbonate AP
- 2 enzymes in mammals (one cyto, one mito)
- 2 molecules of ATP

Step 2: form N1, C4,5,6
- phosphoryl carbamate AP
- all atoms of ring complete

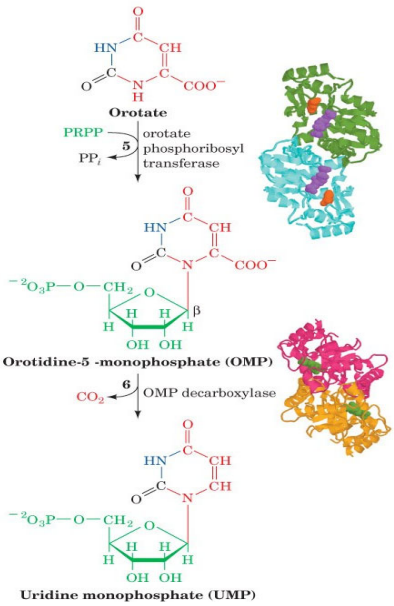
UMP synthesis steps 3-4



Step 3: cyclization
- ring closure with loss of H_2O

Step 4: oxidation
- key intermediate (orotate)
- target for treatment of rheumatoid arthritis

UMP synthesis steps 5-6



Step 5: attach to ribose

- PRPP is AP
- also participates in salvage pathways

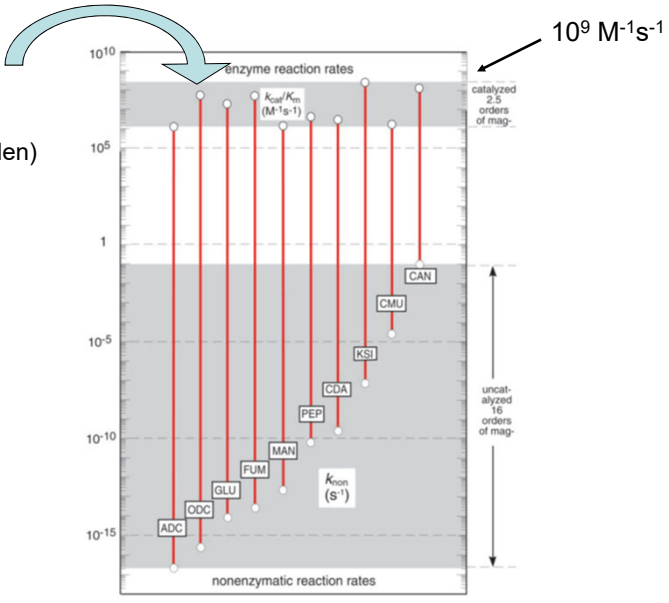
Step 6: decarboxylation

- UMP is final product
- one of most catalytically proficient enzymes known

Enzymes produce large rate enhancements over non-catalyzed rates

ODC:
enhances k_{cat}/K_M 10^{23} over uncatalyzed reaction (Wolfenden)

uncatalyzed $t_{1/2}$= 87 million years
catalyzed $t_{1/2}$ = 18 ms



Richard Wolfenden and co-workers

Regulation of pyrimidine synthesis

Different committed step: eukaryotes vs. prokaryotes
mammals: step 1 (CPSII)
bacteria: step 2 (ATCase)

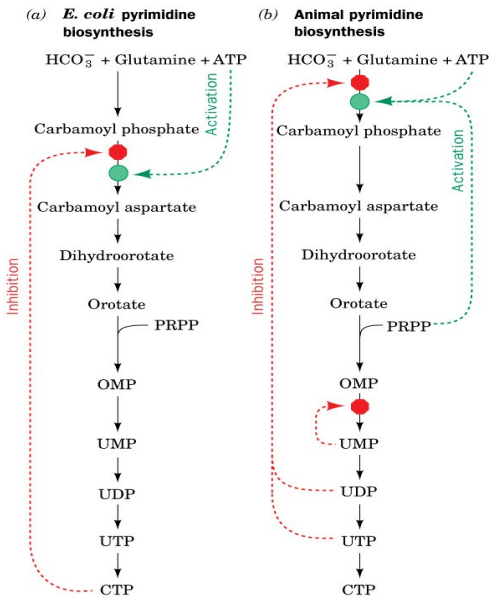
Mammals:
1) CPSII
2) OMP decarboxylase

Bacteria:
ATCase: classic allosteric control

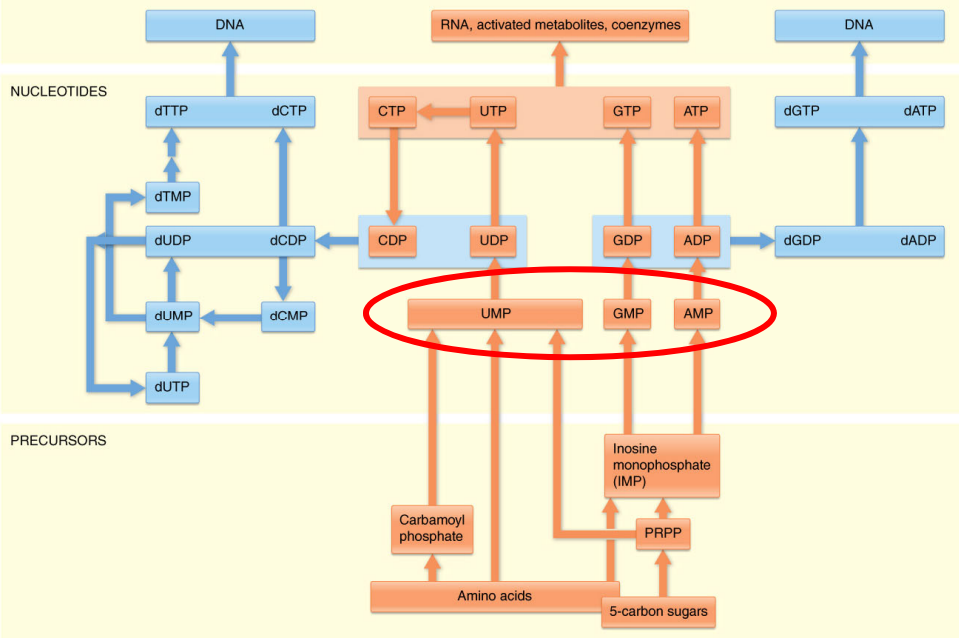
Regulation of pyrimidine synthesis

Bacteria:
1) ATCase
ATP activates
CTP inhibits

Mammals:
1) CPSII
ATP, PRPP activate
UDP, UTP inhibit
2) OMP decarboxylase
UMP (CMP) compet inh.



Nucleotide biosynthesis: birds-eye view



Conversion to nucleoside triphosphate

1) Nucleoside monophosphate kinases (NMP kinase):
   Specific for each base
   Not specific for sugar (ribo or deoxy)

Adenylate kinase: AMP + ATP → 2 ADP

Guanylate kinase: GMP + ATP → GDP + ADP

Uridylate kinase: UMP + ATP → UDP + ADP

Cytidylate kinase: CMP + ATP → CDP + ADP

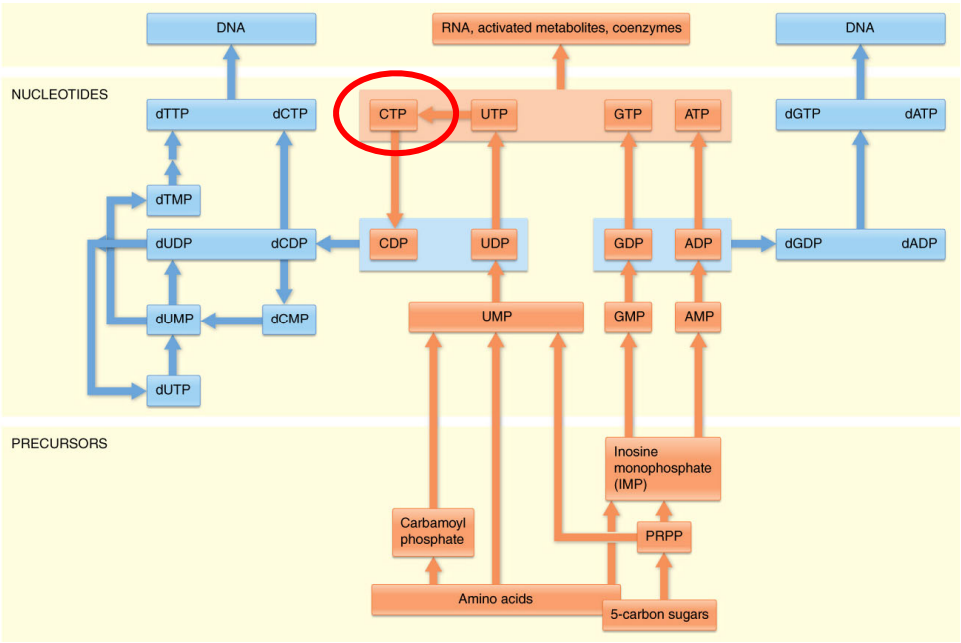
Conversion to nucleoside triphosphate

2) Nucleoside diphosphate kinases (NDP kinase):
   Not specific for each base
   Not specific for sugar (ribo or deoxy)
   ATP is *de facto* phosphate donor
      ATP abundance drives reaction (ΔG ~ 0)

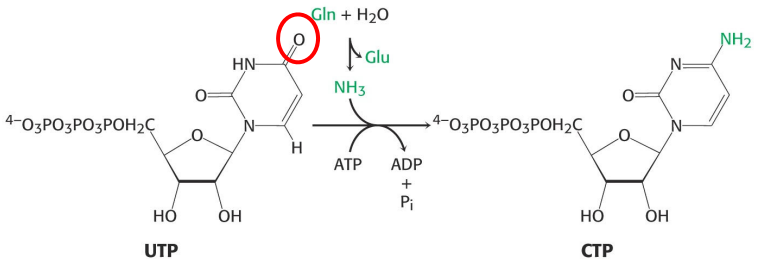
$$XDP + YTP \rightleftharpoons YDP + XTP$$

$$\text{i.e. } GDP + ATP \rightleftharpoons ADP + GTP$$

Nucleotide biosynthesis: birds-eye view



Finally: Synthesis of CTP!



Cytidylate synthetase:

- C4 carbonyl phosphate is AP
- nucleophilic displacement by NH_3

Nucleotide biosynthesis: birds-eye view