OUTLINE:

Introduction and review
Transport
Glycogenolysis
Glycolysis
Other sugars
Pasteur: Anaerobic vs Aerobic
Fermentations

Exam-1 material

Pyruvate
Krebs' Cycle
Oxidative Phosphorylation
- Electron transport
- Chemiosmotic theory: Phosphorylation

Exam-2 material

Fat Catabolism
Fatty acid Catabolism
- Mobilization from tissues (mostly adipose)
- Activation of fatty acids
- Transport; carnitine
- Oxidation: β-oxidation, 4 steps:

Protein Catabolism
Amino-Acid Degradation
- Dealing with the nitrogen; Urea Cycle
- Dealing with the carbon; Seven Families

Nucleic Acid & Nucleotide Degradation

Exam-3 material

PHOTOSYNTHESIS:
Overview and Key experiments:
Light Reactions
- energy in a photon/pigments
- Reaction center &Photosystems (PSII & PSI)

Proton Motive Force – ATP
Carbon Assimilation – Calvin Cycle
- **Rubisco/**Oxygenase (Glycolate cycle)
- remaking Ru 1,5P2

Overview and regulation
C4 versus C3 plants
Kornberg cycle – glyoxylate
Carbohydrate Biosynthesis in Animals
- precursors/Cori cycle

Gluconeogenesis
- reversible steps
- irreversible steps – four

Glycogen Synthesis
- UDP-Glc/Glycogen synthase/branching

Pentose-Phosphate Pathway
- oxidative-NADPH
- non-oxidative-Ribose 5-P

Regulation of Carbohydrate Metabolism
Anaplerotic reactions
Biosynthesis of Fatty Acids
- location & transport
- Synthesis; acetyl-CoA carboxylase + FAS
  - (ACP priming & 4 steps)

Exam-4 material

BI/CH 422

ANABOLISM II OUTLINE:

Biosynthesis of Fatty Acids and Lipids
- Fatty Acids
  - contrasts
  - Control of fatty acid metabolism
  - Diversification of fatty acids
    - elongation/desaturation
    - Eicosanoids: Prostaglandins and Thromboxane
- Triacyl glycerides
- Membrane lipids
  - Glycerophospholipids
  - Sphingolipids
  - Isoprene lipids: **Cholesterol**
    - Ketone body synthesis
    - Mevalonate
    - **Cholesterol:** bile acids, steroids
      - control of cholesterol biosynthesis

ANABOLISM III OUTLINE:

Biosynthesis of Amino Acids and Nucleotides
- Nitrogen cycle
- Nitrogen fixation
  - nitrogenase complex
- Nitrogen assimilation
  - Plants
    - Nitrate/nitrite reductases
  - Animals
    - Glutamine synthetase
    - Glutamate synthase (GOGAT)
- Amino-acid Biosynthesis
  - non-essential
  - essential
- Nucleotide Biosynthesis
  - RNA precursors
    - Denovo vs. salvage
      - Purines
      - Pyrimidines
  - DNA precursors
- Control of nitrogen metabolism
- Biosynthesis and degradation of heme; other 2° products of amino acids

Exam-5 material

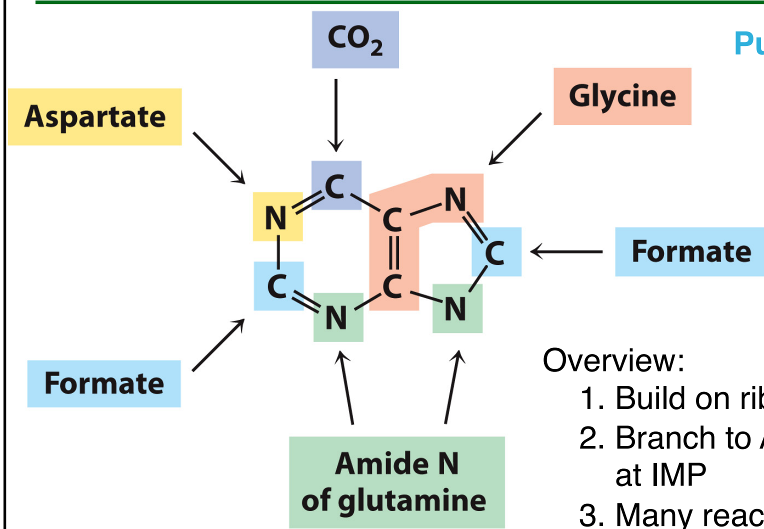
Biosynthesis Amino Acids & Nucleotides

Two major sources of Nucleotides:

1. They can be synthesized *de novo* ("from the beginning")
   - Purine nucleotides: from Gly, Gln(NH_3), Asp(NH_3), THF, and CO_2, and ribose-5-phosphate (PRPP)
   - Pyrimidine nucleotides: from Asp, carbamoyl-phosphate, and ribose-5-phosphate (PRPP)
2. Nucleotides can be salvaged from RNA, DNA, and cofactor degradation and diet.
   - Recall purines are degraded to uric acid (no energy) but pyrimidines can be oxidized to acetyl-CoA and succinyl-CoA
   - Purine salvage is a significant contribution (80-90%)
   - Interesting: Many parasites (e.g., malaria) lack *de novo* biosynthesis and rely exclusively on salvage. Therefore, compounds that inhibit salvage pathways are promising anti-parasite drugs.
3. Because ATP/ADP are involved in so many reactions and regulation mechanisms, the absolute [nucleotide] are kept low; so cells must continually synthesize them.
   - This synthesis may actually limit rates of transcription and replication.
4. Unlike amino-acid biosynthesis, pathways are conserved in ALL organisms.

Biosynthesis Amino Acids & Nucleotides

Purines



Overview:
1. Build on ribose (PRPP)
2. Branch to AMP & GMP at IMP
3. Many reactions like those seen before

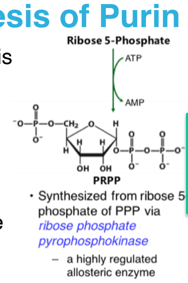
Biosynthesis Amino Acids & Nucleotides

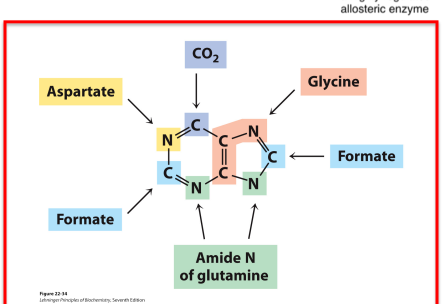
De Novo Biosynthesis of Purin

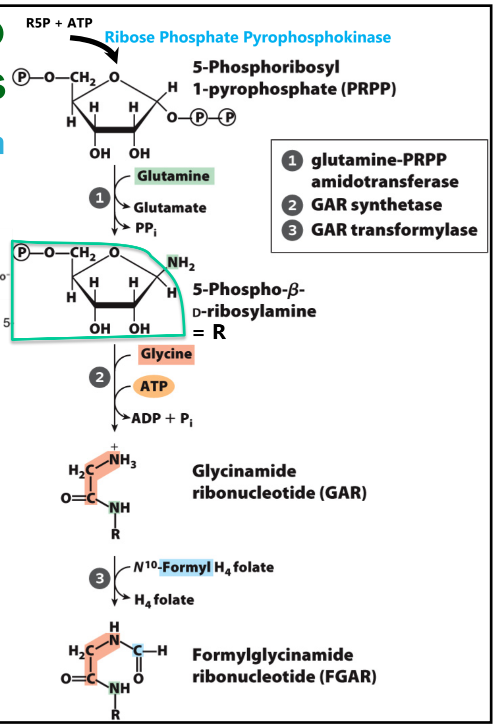
0. Begins with PRPP synthesis
1. PRPP reacts with Gln (like Glu synthase; ammonia channel).

Committal Step

2. Addition of three carbons from glycine by making amide
3. Add C1 from THF



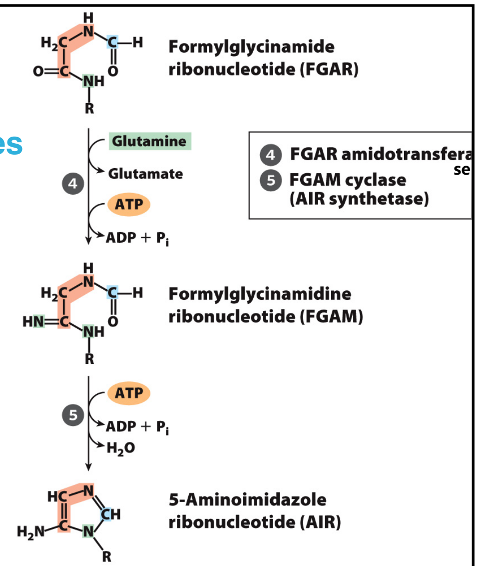


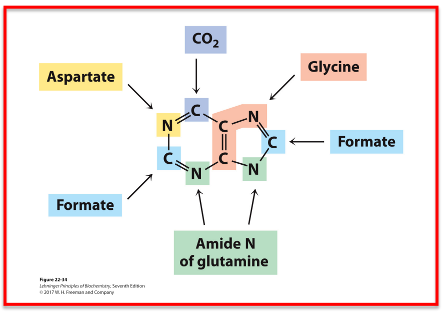


Biosynthesis Amino Acids & Nucleotides

De Novo Biosynthesis of Purines

4. FGAR reacts with Gln (like Glu synthase; ammonia channel).
5. Looks like Schiff base, but its an elimination after phosphorylation.

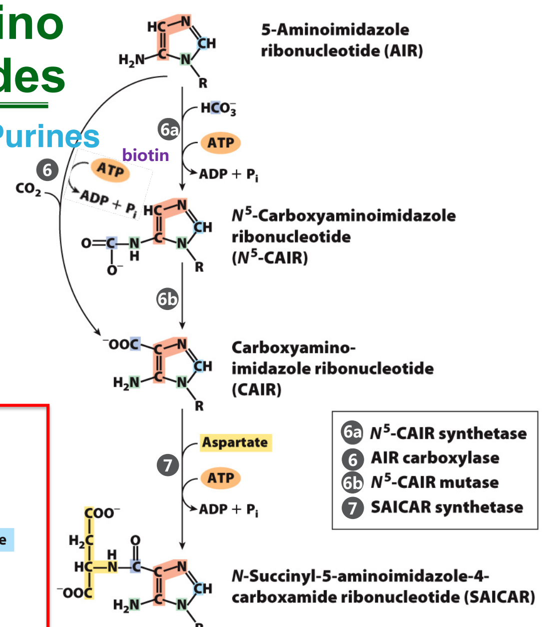


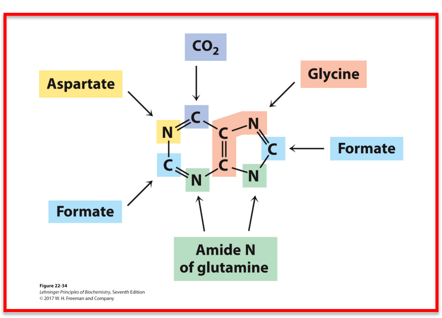


Biosynthesis Amino Acids & Nucleotides

De Novo Biosynthesis of Purines

6. Typical carboxylase (6a/b in microorganisms)
7. Add Nitrogen of Asp (recall Urea Cycle).



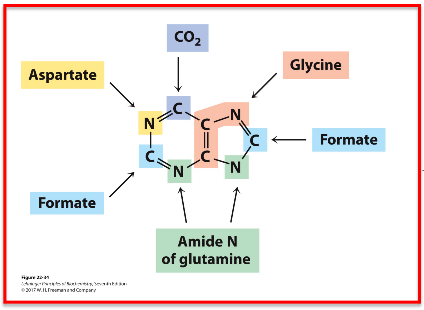


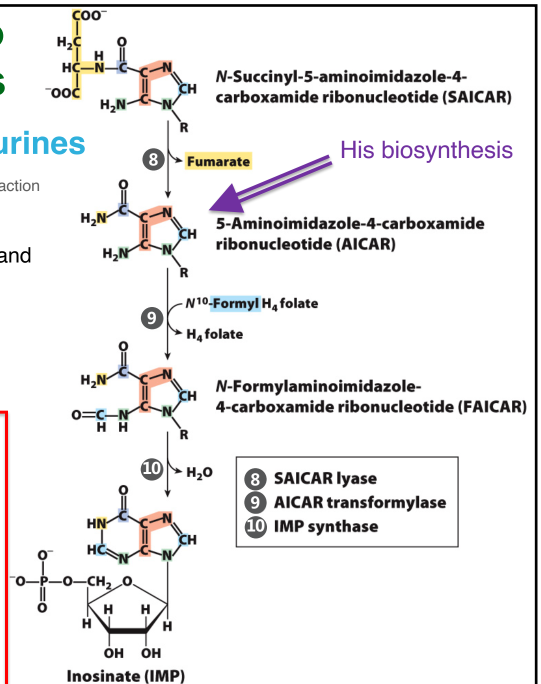
Biosynthesis Amino Acids & Nucleotides

De Novo Biosynthesis of Purines

8. Removal of fumarate (can act as anaplerotic reaction to keep ATP synthesis)
9. Add C1 from THF
10. Schiff base formation gets ring closure and **IMP**

Total ATP=10 (2 for N^{10}-formylTHF)

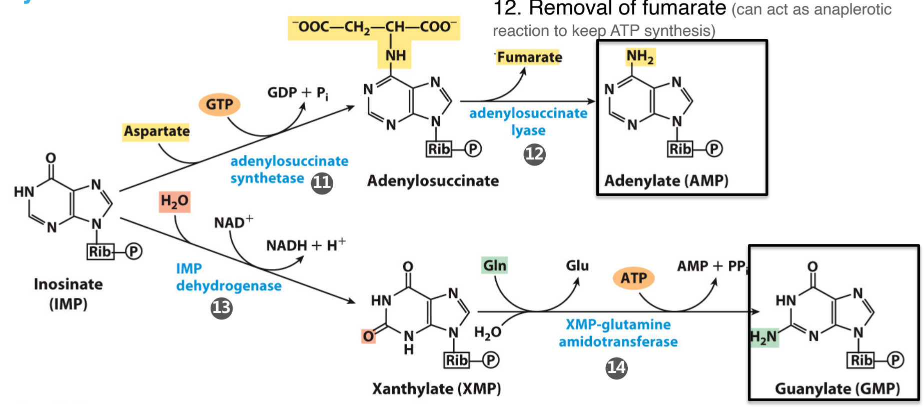




Biosynthesis Amino Acids & Nucleotides

Synthesis of AMP and GMP from IMP

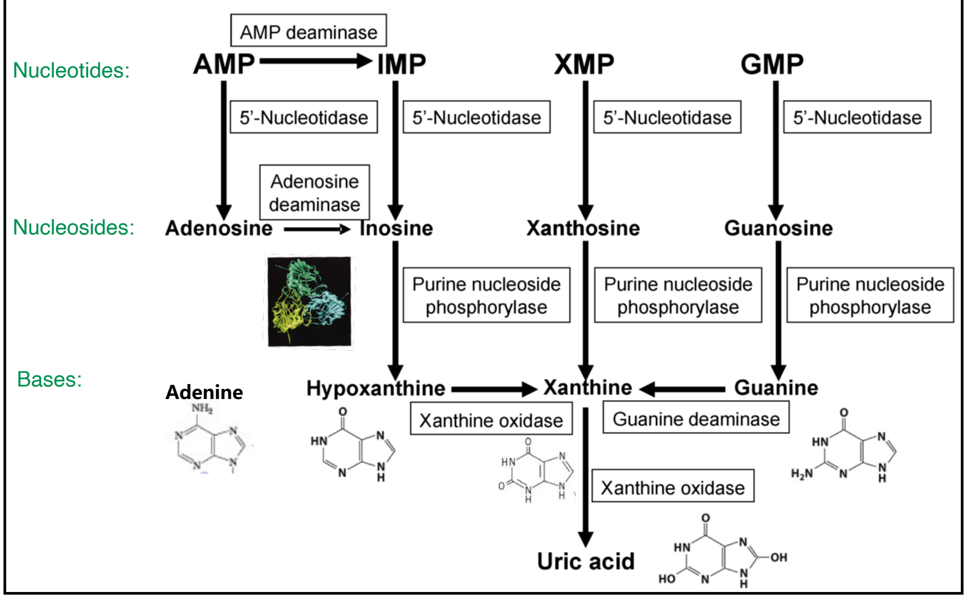
11. Add Nitrogen of Asp (recall Urea Cycle)
12. Removal of fumarate (can act as anaplerotic reaction to keep ATP synthesis)



13. Add water cross imine and oxidize to keto (recall fatty acid oxidation, except at imine not alkene)
14. Add nitrogen from Gln (recall Glu synthase (ammonia channel), and 3rd time we saw use of Gln for this)

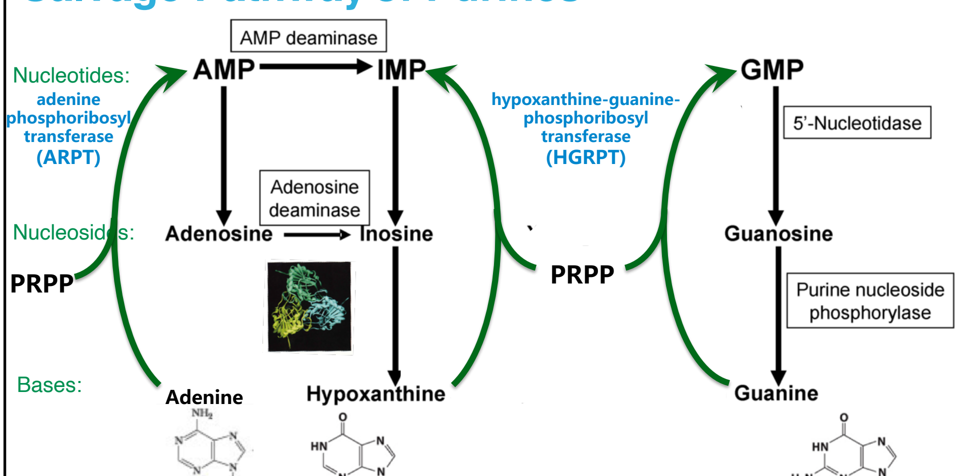
Note that ATP is used to synthesize GMP precursor, while GTP is used to synthesize AMP precursor.

Recall: Nucleotide Degradation



Biosynthesis Amino Acids & Nucleotides

Salvage Pathway of Purines

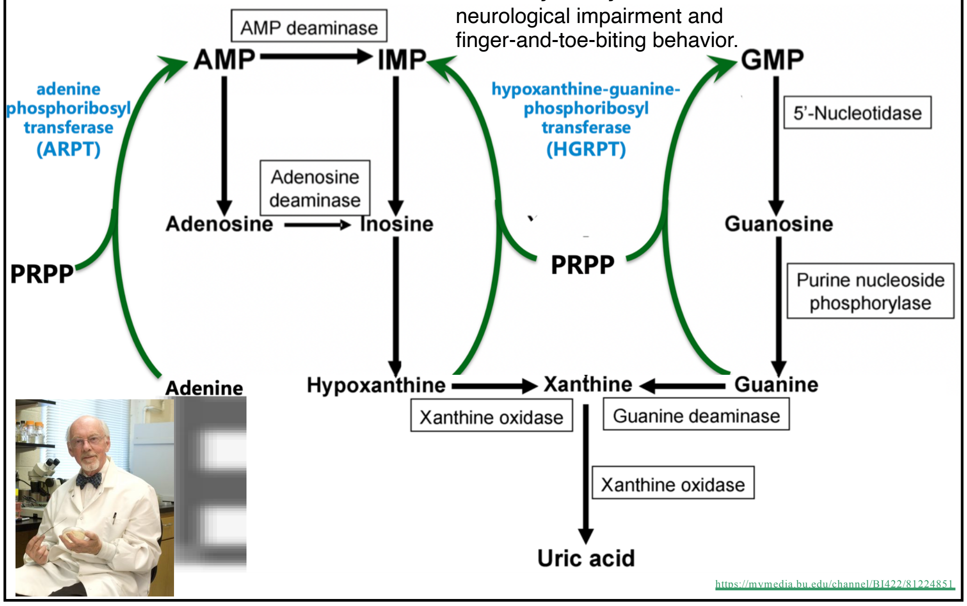


- Over 90% of purine bases are from salvage pathway.
- The brain is especially dependent on salvage pathways.
- The lack of **HGPRT** leads to **Lesch-Nyhan syndrome** with neurological impairment and finger-and-toe-biting behavior.

Biosynthesis Amino Acids & Nucleotides

Salvage Pathway

- The lack of **HGPRT** leads to **Lesch-Nyhan syndrome** with neurological impairment and finger-and-toe-biting behavior.



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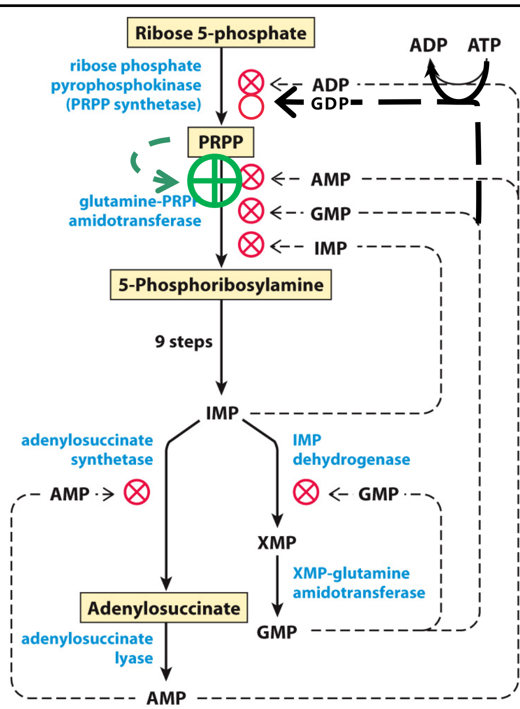
Biosynthesis Amino Acids & Nucleotides

Regulation of Purine Biosynthesis

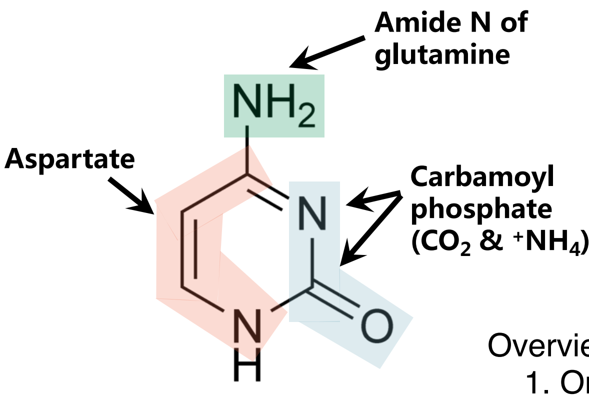
Four Major Sites of Allosteric Regulation

1. *PRPP synthetase* is inhibited by ADP and GDP.
2. *Glutamine-PRPP amidotransferase* is inhibited by end-products IMP, AMP, and GMP.
3. Excess GMP inhibits formation of xanthylate from inosinate by *IMP* dehydrogenase.
4. Excess AMP inhibits formation of adenysuccinate from inosinate by *adenylsuccinate synthetase*.



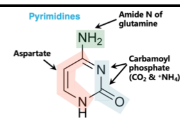
Biosynthesis Amino Acids & Nucleotides

Pyrimidines



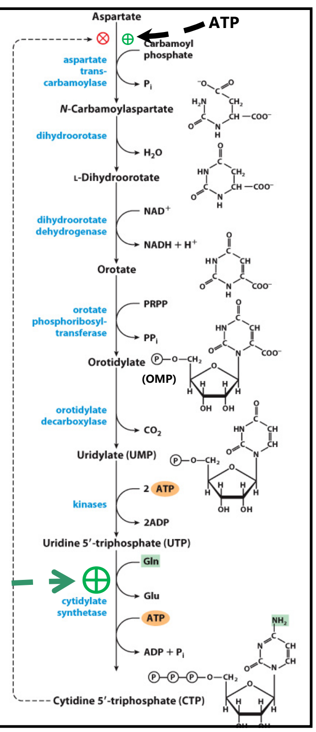
Overview:
1. Orotic acid
2. Add ribose (PRPP), make UMP
3. CTP made from UTP

Biosynthesis Amino Acids & Nucleotides



De Novo Biosynthesis of Pyrimidines

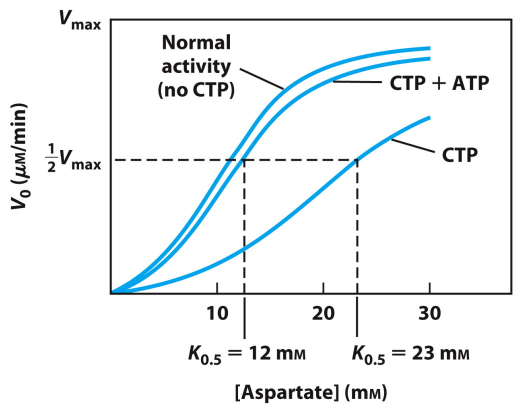
- Unlike purine synthesis, pyrimidine synthesis proceeds by *first making the pyrimidine ring* (in the form of orotic acid) and *then* attaching it to ribose 5-phosphate using PRPP.
- Aspartate and carbamoyl phosphate provide all the atoms for the heterocycle or pyrimidine. The first pyrimidine is Orotate.
- This is converted to a nucleotide using PRPP, resulting nucleotide (orotidylate; OMP).
- OMP is decarboxylated to form uridylate (UMP).
- The other pyrimidine nucleotide used in RNA is made at the triphosphate level; UMP is phosphorylated twice to make UTP.
- UTP is converted to CTP by amination using Gln similar to making AMP from XMP.
- The biosynthesis of CTP is the CLASSIC feedback inhibition by the allosteric negative effector (CTP) on ATCase. Also, activation by GTP

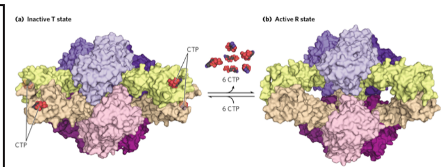


Biosynthesis Amino Acids & Nucleotides

Regulation of Pyrimidine Biosynthesis via Feedback Inhibition

Aspartate Transcarbamoylase (ATCase)





$(\alpha_3)_2(\beta_2)_3$

Recall from 421: ATCase is inhibited by end-product CTP and is accelerated by ATP.

ANABOLISM III: Biosynthesis Amino Acids & Nucleotides

Involvement of ribonucleotide-derivatives in all of biology

Dr. Kornberg:
Lecture 04.26.17 (0:00-5:06) 5 min

https://mymedia.bu.edu/channel/BI422/81224851

Biosynthesis Amino Acids & Nucleotides

So far:

GMP→GDP→GTP
AMP→ ADP→ATP
UMP→UDP→UTP
CDP←CTP

Specific kinases, e.g., *UMP kinase*, *GMP kinase*, *Adenylate kinase* etc.

Non-specific kinase, *nucleoside diphosphate kinase* (works on both oxy- and deoxy-ribose nucleosides)

GDP→dGDP
ADP→dADP
UDP→dUDP
CDP→dCDP

How are Ribonucleic Acid Precursors converted to Deoxyribonucleic Acid Precursors?
……..and how is dTTP made?

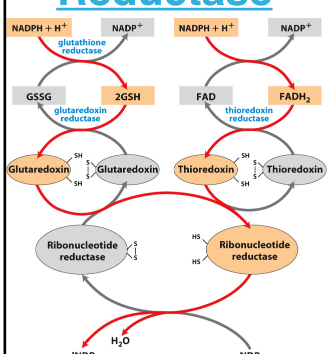
2'C-OH bond is directly reduced to 2'-H bond …without activating the carbon for dehydration, etc.!
catalyzed by *ribonucleotide reductase*

Very unique enzyme in all of biochemistry - use of free radicals (without cofactors)

Mechanism: Two H atoms are donated by NADPH and carried by thioredoxin or glutaredoxin to the active site.
–Substrates are the NDPs and the products are dNDP.

Biosynthesis Amino Acids & Nucleotides

Source of Reducing Electrons for Ribonucleotide Reductase

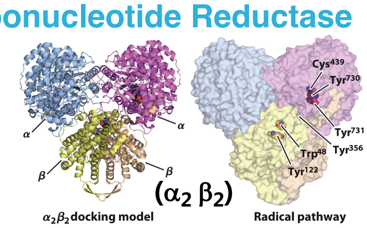


- NADPH serves as the electron donor.
- Funneled through glutathione or thioredoxin pathways

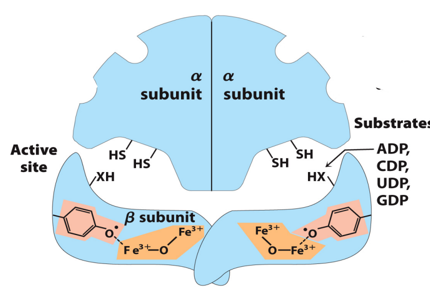
Structure of Ribonucleotide Reductase

α_2 are regulatory and half the catalytic site; need to be reduced.

β_2 are the other half of the active site, and the free-radical generators



JoAnne Stubbe
(1946–)



Proposed Ribonucleotide Reductase Mechanism Involves Free Radicals

- Most forms of enzyme have two catalytic/ regulatory subunits and two radical-generating subunits.
  - contain Fe^{3+} and dithiol groups
  - enzyme creates stable Tyr radical to abstract H• from sugar
- A 3'-ribo-nucleotide radical forms.
- 2'-OH is protonated to help eliminate H_2O and form a radical-stabilized carbocation.
- Electrons are transferred to the 2'-C.