BI/CH 422/622

OUTLINE:

Introduction and review
Transport
Glycogenolysis
Glycolysis
Other sugars
Pasteur: Anaerobic vs Aerobic — Exam-1 material

Fermentations — Exam-2 material
Pyruvate
Krebs' Cycle
Oxidative Phosphorylation
- Electron transport
- Chemiosmotic theory: Phosphorylation

Fat Catabolism — Exam-3 material
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

ANABOLISM I: Carbohydrates

PHOTOSYNTHESIS: — Exam-4 material
- Overview;; Key experiments:
- Light Reactions
- Reaction center
- Photosystems (PSII & PSI – NADPH)
- Proton Motive Force – ATP
- Carbon Assimilation – Calvin Cycle
- Overview and regulation
- C4 versus C3 plants
- Kornberg cycle – glyoxylate

Carbohydrate Biosynthesis in Animals
Gluconeogenesis
Glycogen Synthesis
Pentose-Phosphate Pathway
- oxidative-NADPH
- non-oxidative-Ribose 5-P

Regulation of Carbohydrate Metabolism
Anaplerotic reactions

ANABOLISM II: Lipids

Fatty Acids
- contrasts
- location & transport
- Synthesis: ACC & fatty acid synthase
- Control of fatty acid metabolism
- Diversification of fatty acids
  - elongation
  - desaturation
  - Eicosanoids
    - Prostaglandins and Thromboxane

Triacylglycerides
Membrane lipids
- Glycerophospholipids
- Isoprene lipids:
  - Ketone body synthesis
  - Cholesterol

ANABOLISM III: Nitrogen (Amino Acids & Nucleotides)

Nitrogen cycle – Nitrogen fixation
- nitrogenase

Nitrogen assimilation
- Plants
  - Nitrate/nitrite reductases
- Animals
  - Glutamine synthetase
  - Glutamate synthase

Amino-acid Biosynthesis
- non-essential
- essential

Nucleotide Biosynthesis
Secondary products of amino acids — Exam-5 material

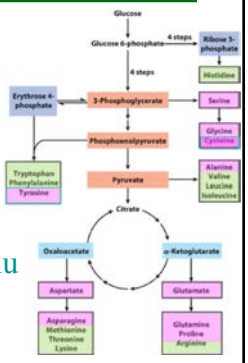
Biosynthesis Amino Acids & Nucleotides

Asp	1	*	OAA
Glu	1	*	α-KG
Ala	1	*	Pyr
Asn	1	–	Asp
Gln	1	–	Glu
Pro	3(1)	(*)	Glu/Arg
Ser	3	–	3PGA
Gly	1	*	Ser
Cys	2	*	Ser/Met
Tyr	1	*	Phe

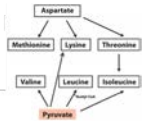
Red=biosynthesis specific *reverse of degradation

Essential Amino acids:

These require many steps and unique to those used for degradation.



Asp/Pyruvate Family



Aromatic Family

Histidine

Met	7	–Asp/Cys/THF/Glu
Thr	5	–Asp/Glu
Lys	9	–Asp/Pyr/Glu
Ile	10	–Asp(Thr)/Pyr/Glu
Val	4	–Pyr/Glu
Leu	7	–Pyr/AcCoA/Glu
Phe	10	–E4P/PEP/Glu
Trp	12	–E4P/PEP/Gln/R5P/Ser
His	10	–R5P/ATP/Gln/Glu

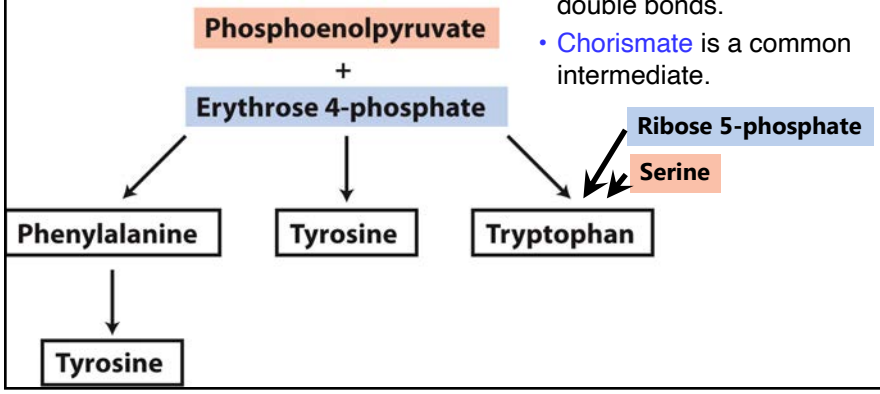
Biosynthesis Amino Acids & Nucleotides

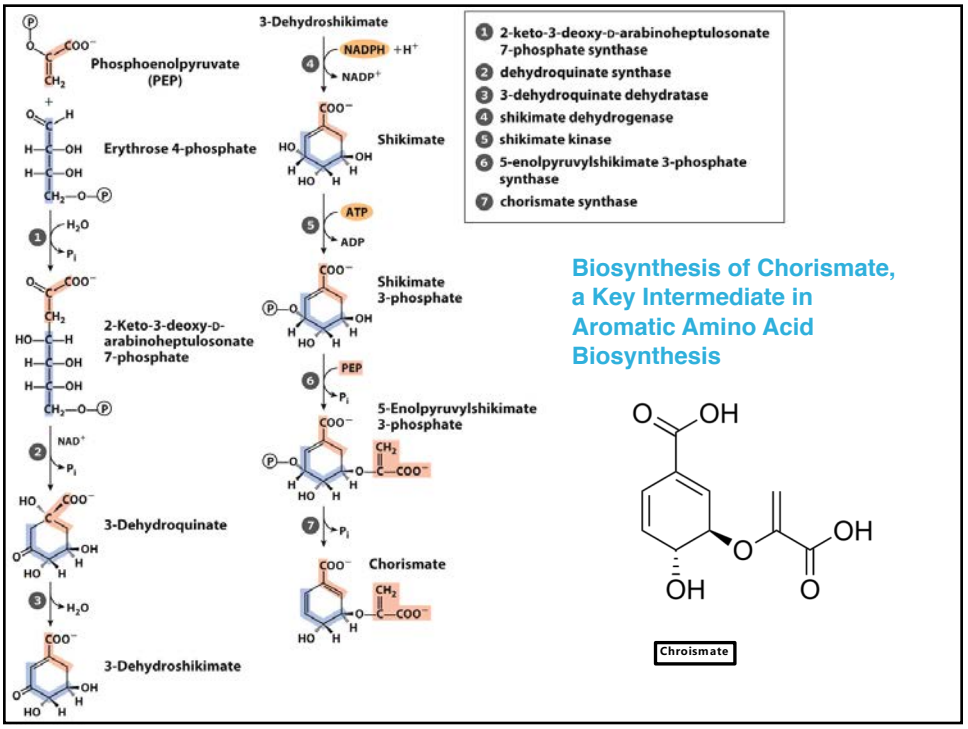
Aromatic Family: Phe, Trp

Aromatic Amino Acids Derive from PEP and Erythrose 4-Phosphate

(and Rib5P and Ser)

- Very complicated and amazing chemistry!
- Rings must be synthesized and closed and then oxidized to create double bonds.
- Chorismate is a common intermediate.



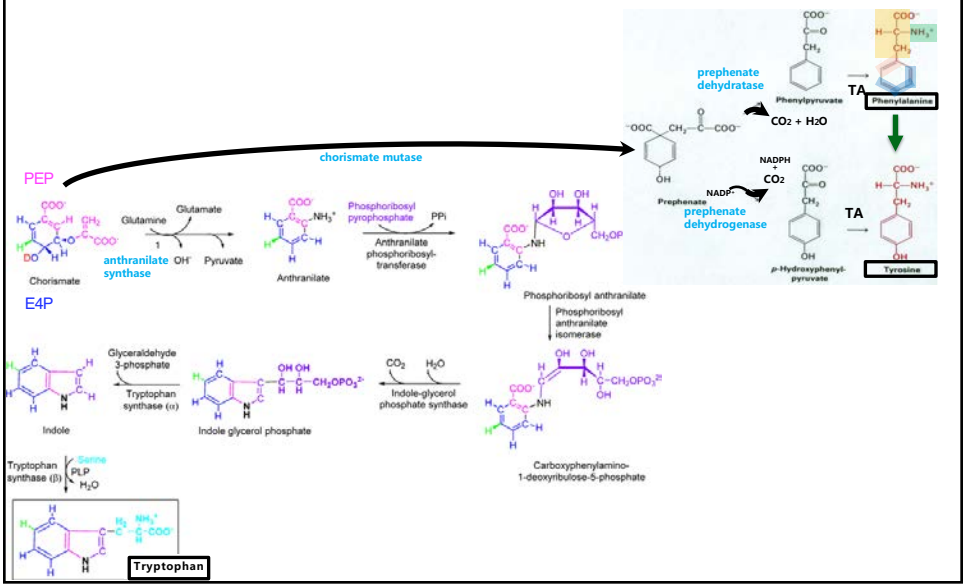


Biosynthesis of Chorismate, a Key Intermediate in Aromatic Amino Acid Biosynthesis

Chroismate

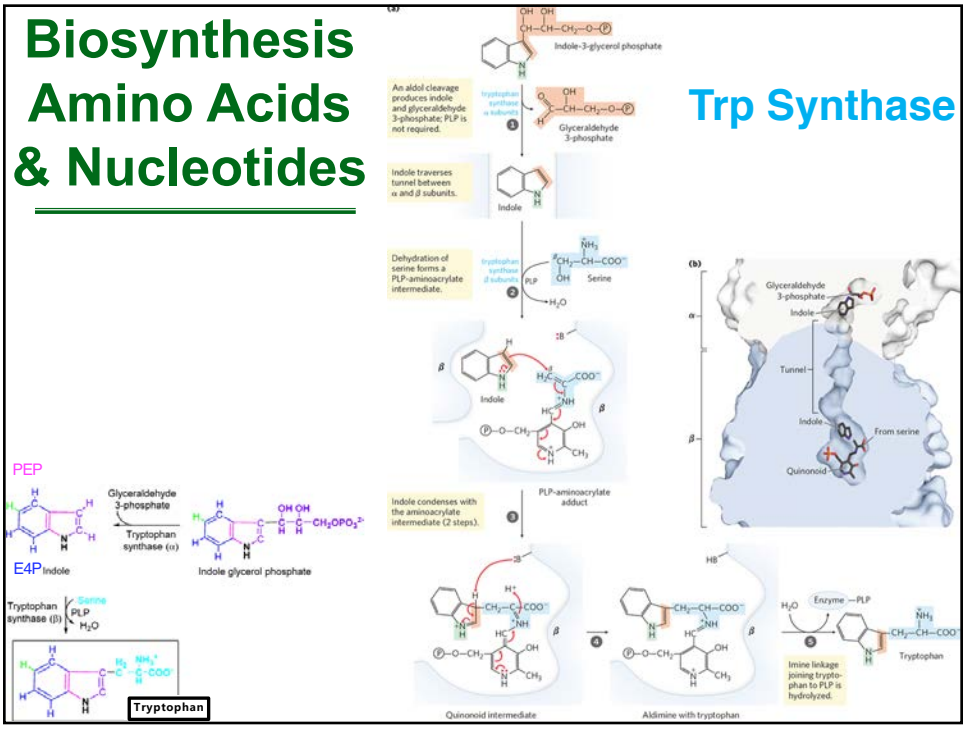
Biosynthesis Amino Acids & Nucleotides

Aromatic Family: Phe, Trp

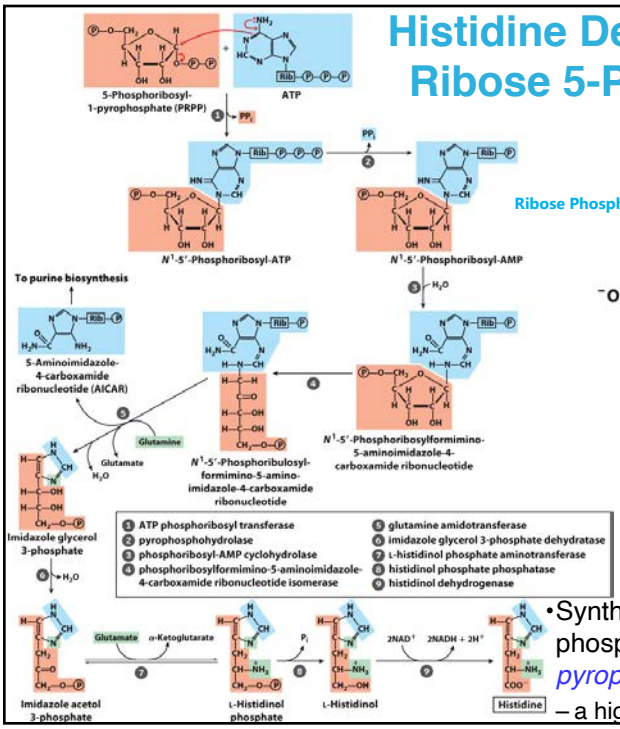


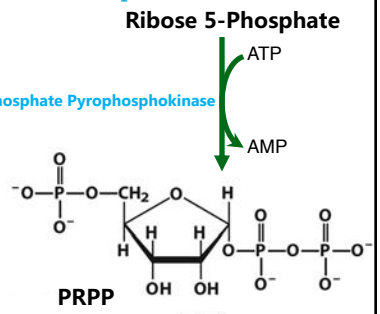
Biosynthesis Amino Acids & Nucleotides

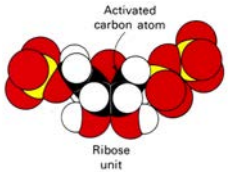
Trp Synthase



Histidine Derives from Ribose 5-Phosphate







- Synthesized from ribose 5-phosphate via *ribose phosphate pyrophosphokinase*
  – a highly regulated allosteric enzyme

Biosynthesis Amino Acids & Nucleotides

Asp	1	*	OAA
Glu	1	*	α-KG
Ala	1	*	Pyr
Asn	1	–	Asp
Gln	1	–	Glu
Pro	3(1)	(*)	Glu/Arg
Ser	3	–	3PGA
Gly	1	*	Ser
Cys	2	*	Ser/Met
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Red=biosynthesis specific *reverse of degradation

Essential vs. Nonessential and Conditionally Essential Amino Acids

- Essential amino acids must be obtained as dietary protein.
- Nonessential amino acids are easily made from central metabolites.
- Consumption of a variety of foods supplies all the essential amino acids.

TABLE 18-1 Nonessential and Essential Amino Acids for Humans and the Albino Rat

Nonessential	Conditionally essential[a]	Essential
Alanine	Arginine	Histidine
Asparagine	Cysteine	Isoleucine
Aspartate	Glutamine	Leucine
Glutamate	Glycine	Lysine
Serine	Proline	Methionine
	Tyrosine	Phenylalanine
		Threonine
		Tryptophan
		Valine

[a]Required to some degree in young, growing animals and/or sometimes during illness.

- Conditionally Essential amino acids are made from essential, or become essential in certain physiological conditions.

Biosynthesis Amino Acids & Nucleotides

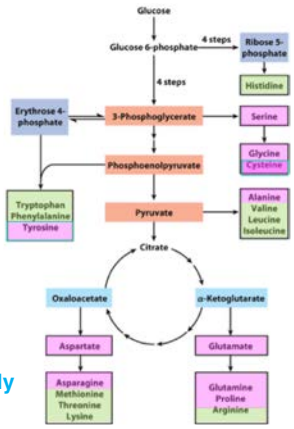
Non-essential Amino acids:

These are very few steps and often the same enzyme(s) used for degradation.

Arg-Val-His-Ile-Leu-Lys-Met-Phe Thr-Trp
Professor A.V.HILL M.P. was a Tea Totaller

AA	#steps	same as degradation	From?	
Asp	1	✓	OAA	Transaminase route
Glu	1	✓	α-KG	
Ala	1	✓	Pyr	
Asn	1	–	Asp	Amidation route
Gln	1	–	Glu	
Pro	3(1)	(✓)	Glu/Arg	Glu Family
Ser	3	–	3PGA	3-PGA Family
Gly	1	✓	Ser	
Cys	2	✓	Ser/Met	From Essential Family
Tyr	1	✓	Phe	

Red=biosynthesis specific Green=essential

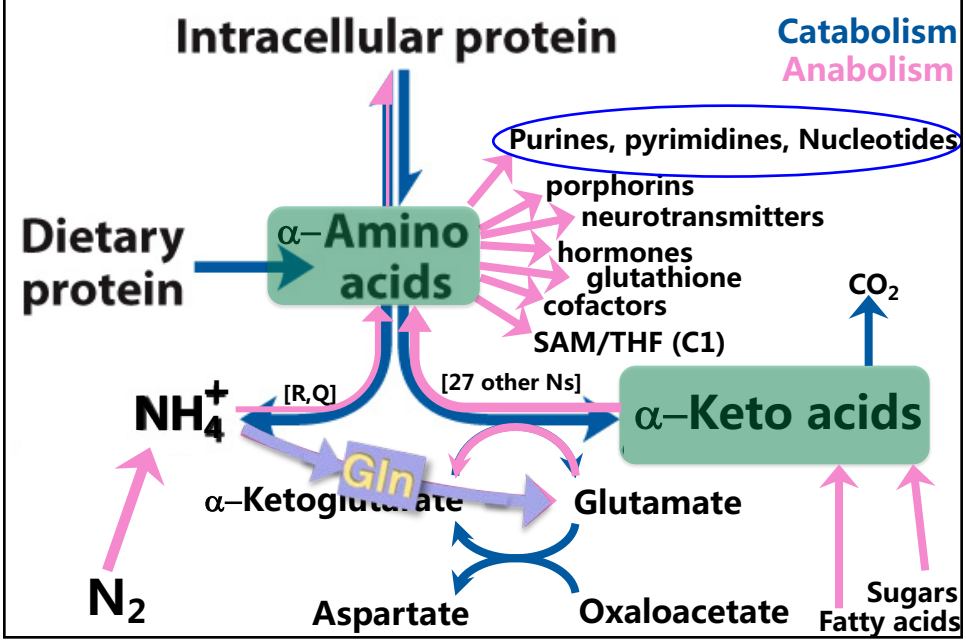


ANABOLISM III: Biosynthesis Amino Acids & Nucleotides

1) Nitrogen fixation: $N_2 \rightarrow NH_4$
2) Nitrogen assimilation: incorporation of ammonia into biomolecules
3) Biosynthesis of amino acids
    a) non-essential
    b) essential
4) Biosynthesis of nucleotides
    a) sources
    b) *de novo* purines (R)(as nucleotides*); salvage; regulation
    c) *de novo* pyrimidines (Y)(as bases); making nucleotide; regulation
    d) deoxy-ribonucleotides, dTMP, and phosphorylation to NTP & dNTP
    e) regulating levels for DNA synthesis
5) Control of nitrogen metabolism
6) Biosynthesis and degradation of heme; other 2° products of amino acids

*Bases synthesized *while* attached to ribose-5-P; products are RMP (R is one-letter code for purine, Y is one letter code for pyrimidine)

Biosynthesis Amino Acids & Nucleotides

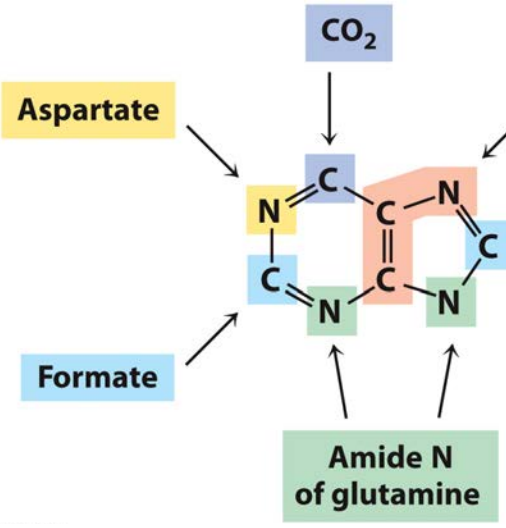


Biosynthesis Amino Acids & Nucleotides

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 antiparasite drugs.
3. Because ATP/ADP are involved in so many reactions and regulation mechanisms, the [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, conserved in all organisms studied.

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

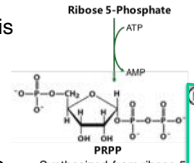
Biosynthesis Amino Acids & Nucleotides

De Novo Biosynthesis of Purines

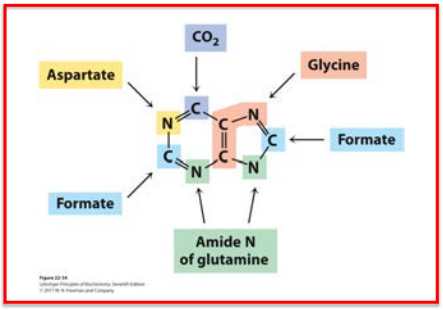
0. Begins with PRPP synthesis
1. PRPP reacts with Gln

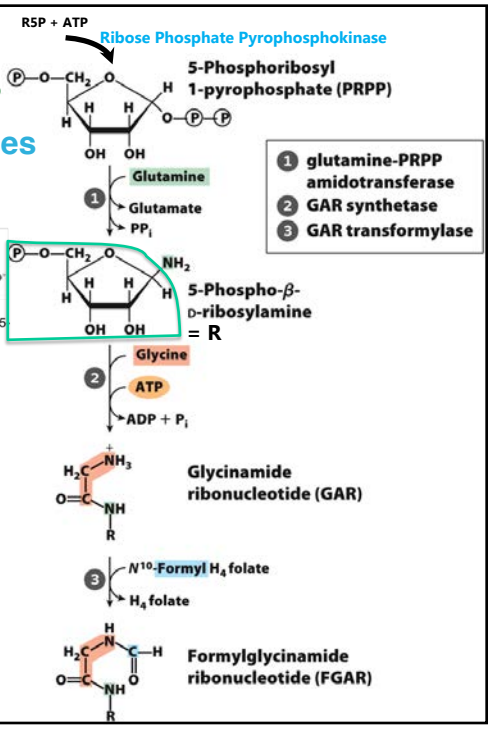
Committal Step

2. Addition of three carbons from glycine by making amide (like Glu synthase; ammonia channel).
3. Add C1 from THF



- Synthesized from ribose 5-phosphate of PPP via *ribose phosphate pyrophosphokinase*
  - a highly regulated allosteric enzyme

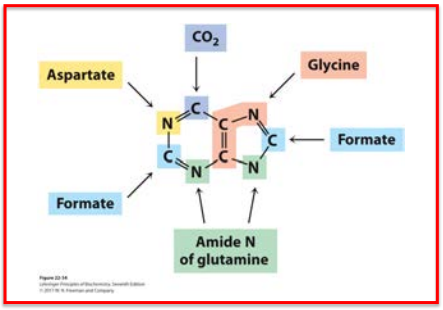


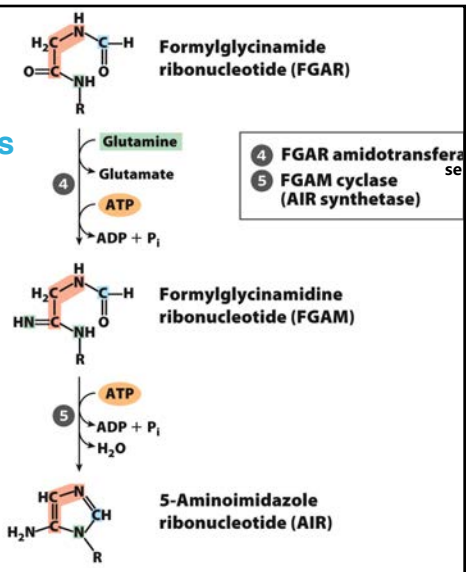


Biosynthesis Amino Acids & Nucleotides

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.

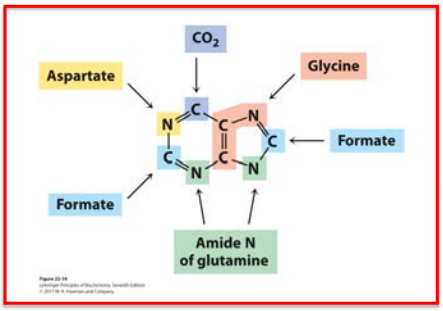


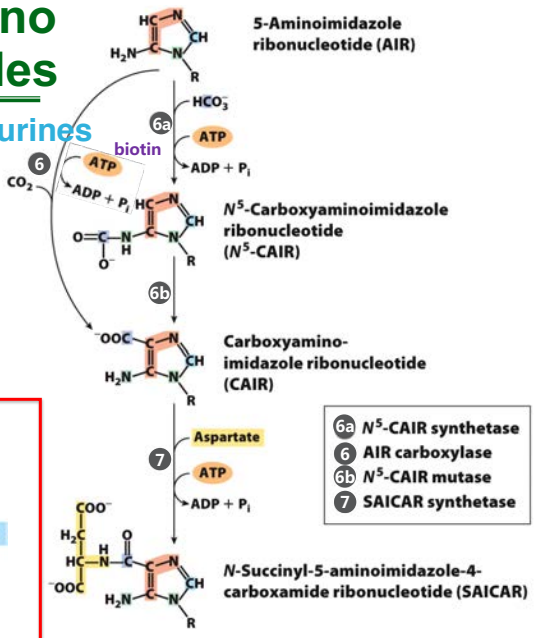


Biosynthesis Amino Acids & Nucleotides

De Novo Biosynthesis of Purines

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



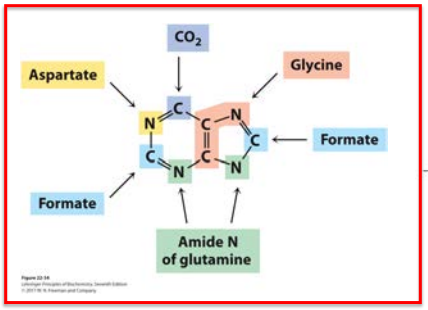


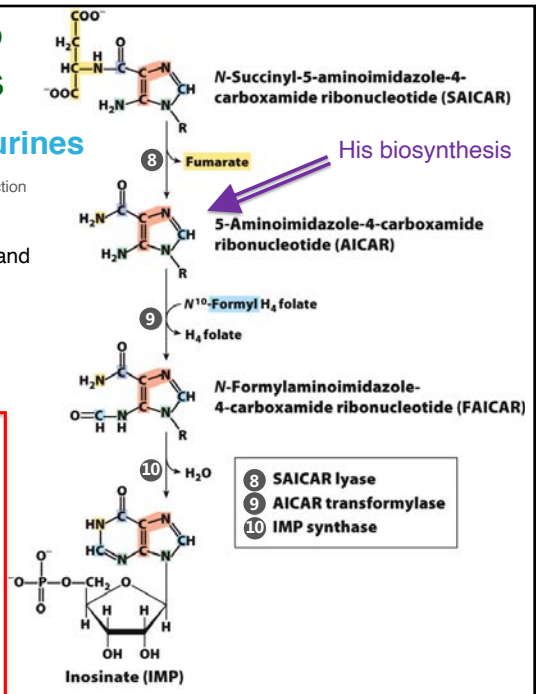
Biosynthesis Amino Acids & Nucleotides

De Novo Biosynthesis of Purines

8. Removal of formate (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 N10-formylTHF)

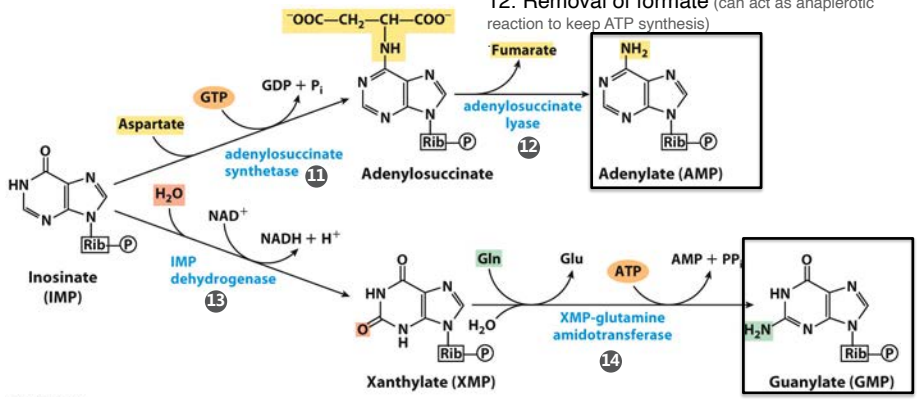




Biosynthesis Amino Acids & Nucleotides

Synthesis of AMP and GMP from IMP

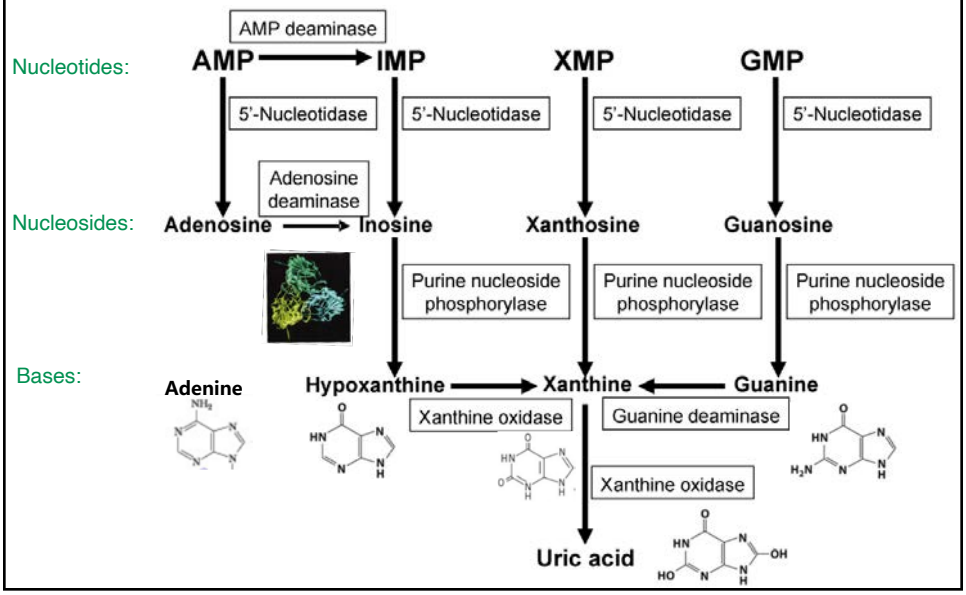
11. Add Nitrogen of Asp (recall Urea Cycle)
12. Removal of formate (can act as anaplerotic reaction to keep ATP synthesis)



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)

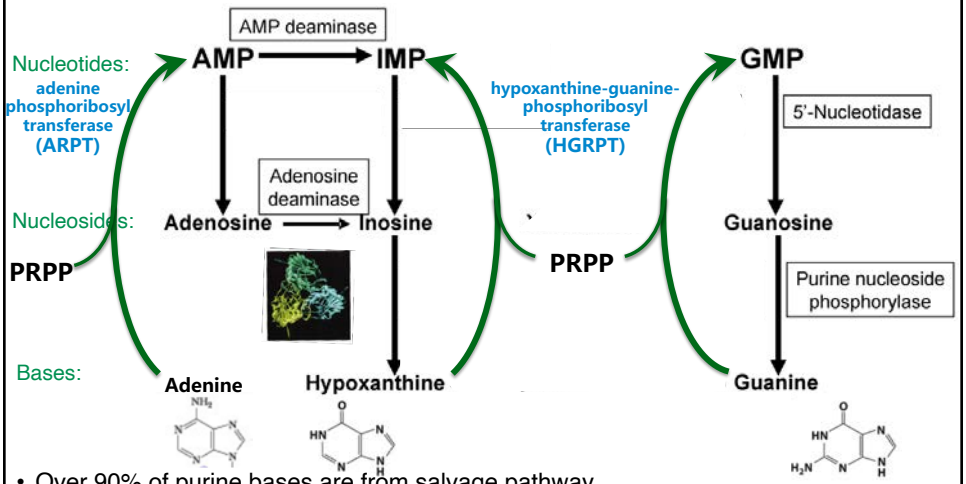
Note that ATP is used to synthesize GMP precursor, while GTP is used to synthesize AMP precursor.

Recall: Nucleotide Degradation



Biosynthesis Amino Acids & Nucleotides

Salvage Pathway of Purines

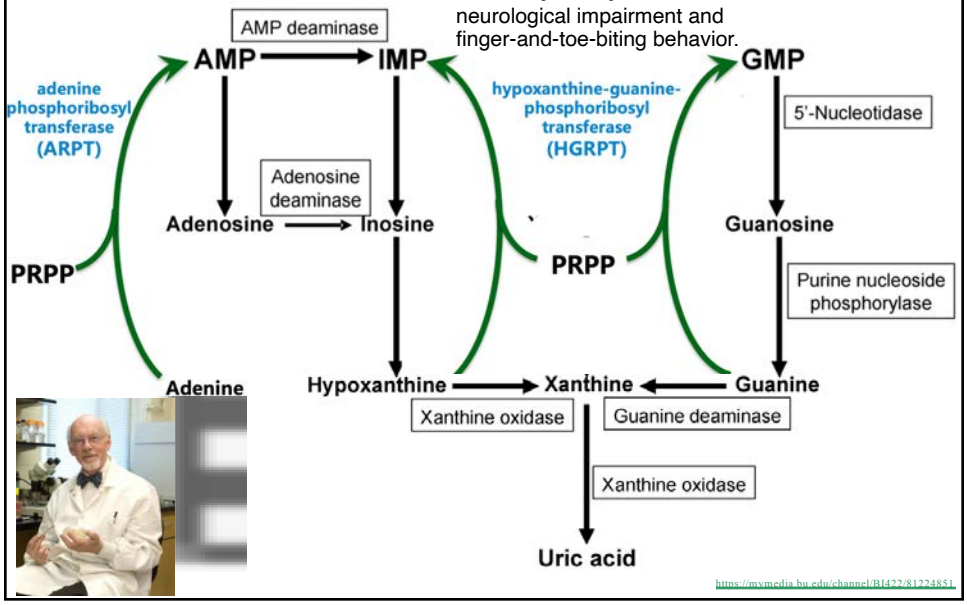


- Over 90% of purine bases are from salvage pathway.
- The brain is especially dependent on salvage pathways.
- The lack of **HGPRT** leads to **Lesch-Nyhan syndrome** with neurological impairment and finger-and-toe-biting behavior.

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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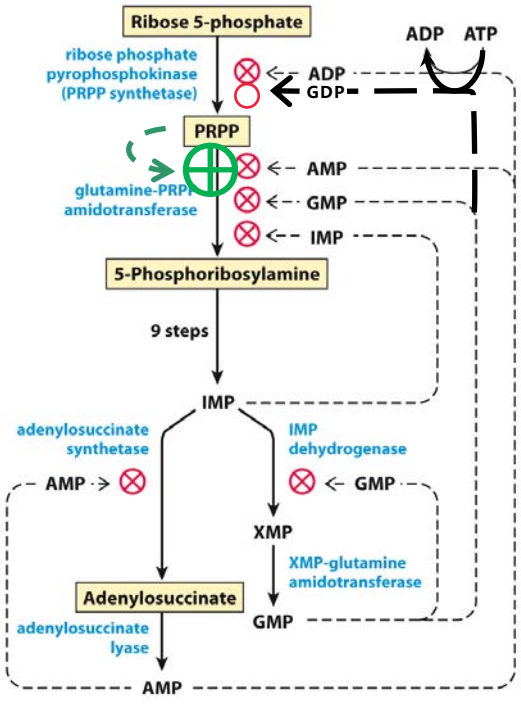
Biosynthesis Amino Acids & Nucleotides

Regulation of Purine Biosynthesis

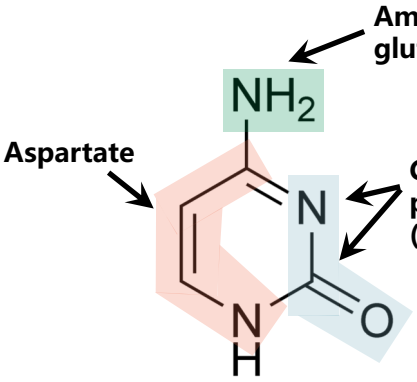
Four Major Sites of Allosteric Regulation

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

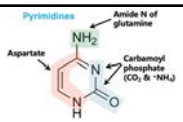
Pyrimidines



Overview:

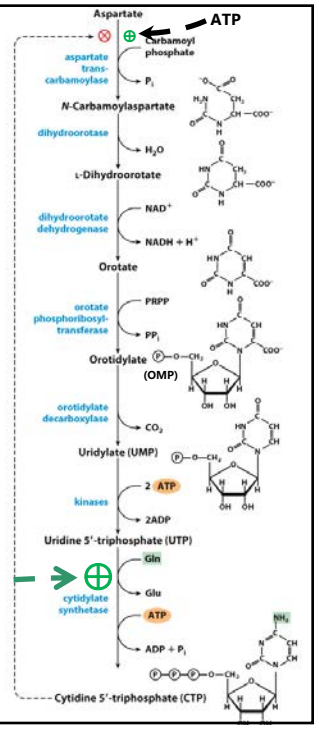
1. Orotic acid
2. Add ribose (PRPP), make UMP
3. CTP made from UTP

Biosynthesis Amino Acids & Nucleotides



De Novo Biosynthesis of Pyrimidines

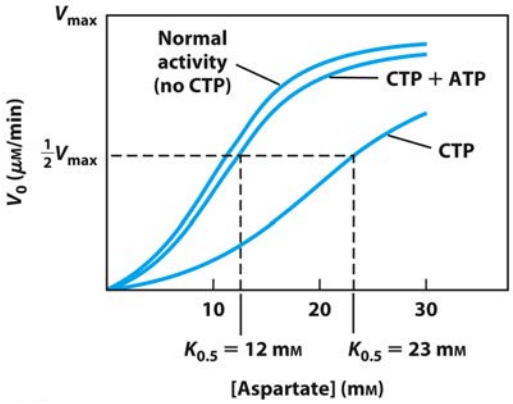
- Unlike purine synthesis, pyrimidine synthesis proceeds by *first making the pyrimidine ring* (in the form of orotic acid) and *then* attaching it to ribose 5-phosphate using PRPP.
- Aspartate and carbamoyl phosphate provide all the atoms for the heterocycle or pyrimidine. The first pyrimidine is Orotate.
- 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

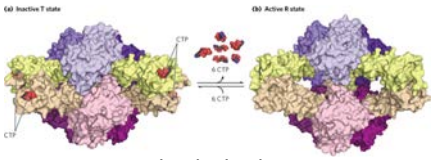


Biosynthesis Amino Acids & Nucleotides

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:

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.