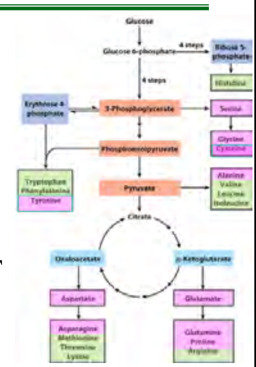
ANABOLISM III: Biosynthesis Amino Acids & Nucleotides

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

<u>Essential Amino acids</u>:
These require many steps and unique to those used for degradation.

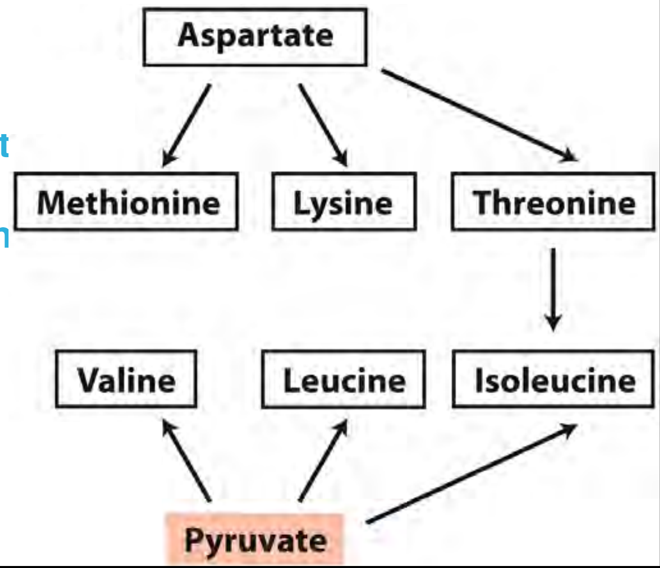


Asp/Pyruvate Family	Met	7	–Asp/Cys/THF
	Thr	5	–Asp/
	Lys	9	–Asp/Pyr/Glu
	Ile	10	–Asp(Thr)/Pyr/Glu
	Val	4	–Pyr/Glu
	Leu	7	–Pyr/AcCoA/Glu
Aromatic Family	Phe	10	–E4P/PEP/Glu
	Trp	12	–E4P/PEP/Gln/R5P/Ser
Histidine	His	10	–R5P/ATP/Gln/Glu

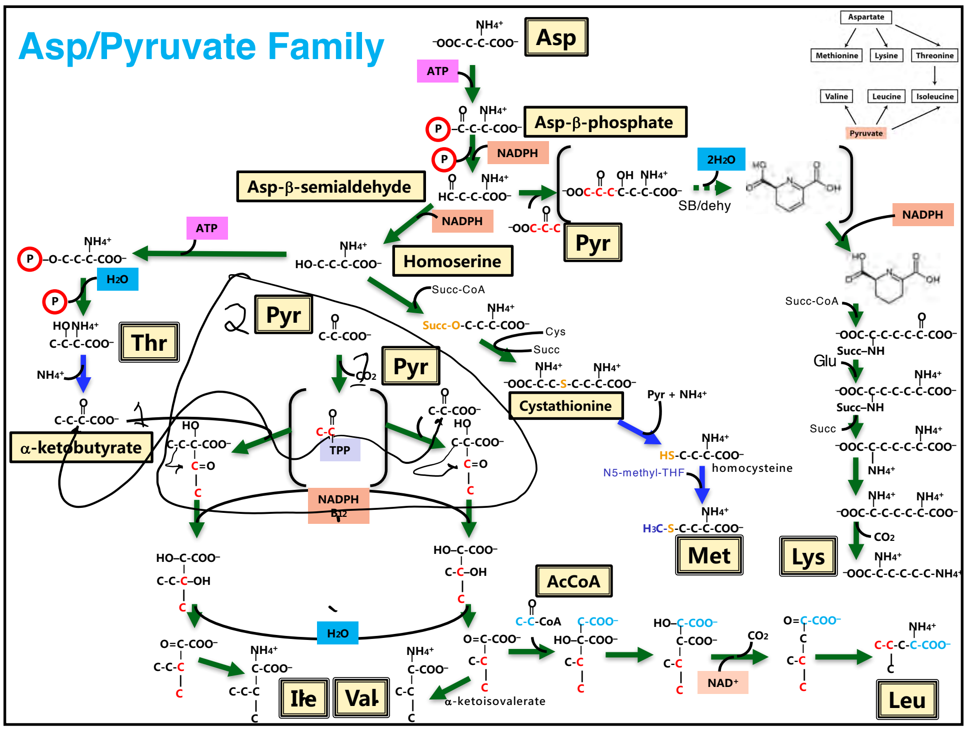
Biosynthesis Amino Acids & Nucleotides

Asp/Pyruvate Family

Oxaloacetate Yields Asp, which Yields Met Lys, and Thr, which along with Pyruvate and Acetyl-CoA Yields Val, Leu, and Ieu



Asp/Pyruvate Family

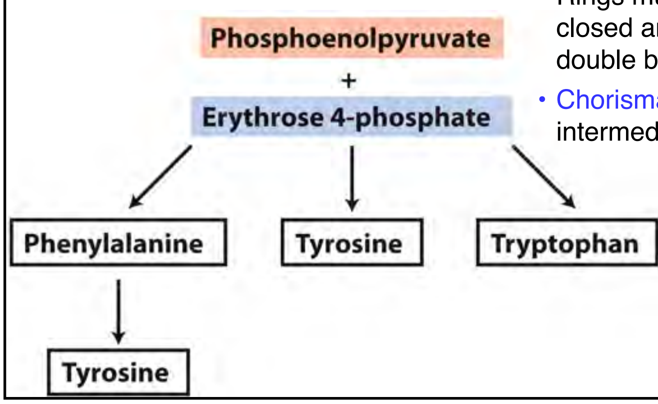


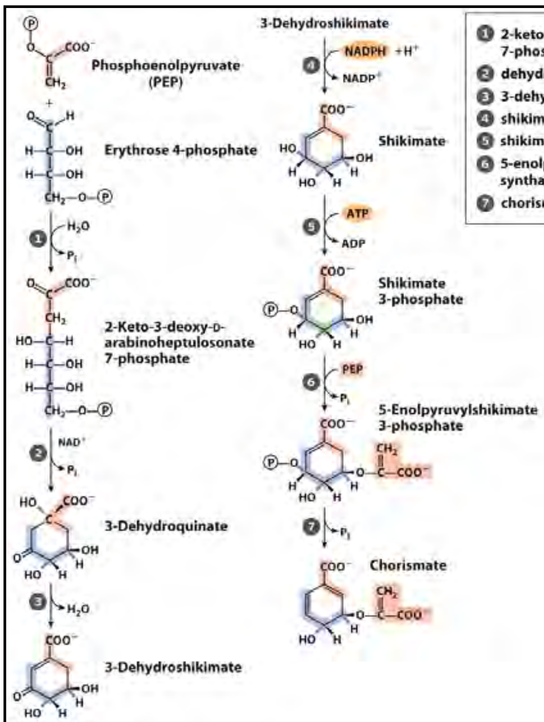
Biosynthesis Amino Acids & Nucleotides

Aromatic Family: Phe, Trp

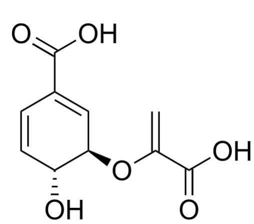
Aromatic Amino Acids Derive from PEP and Erythrose 4-Phosphate

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





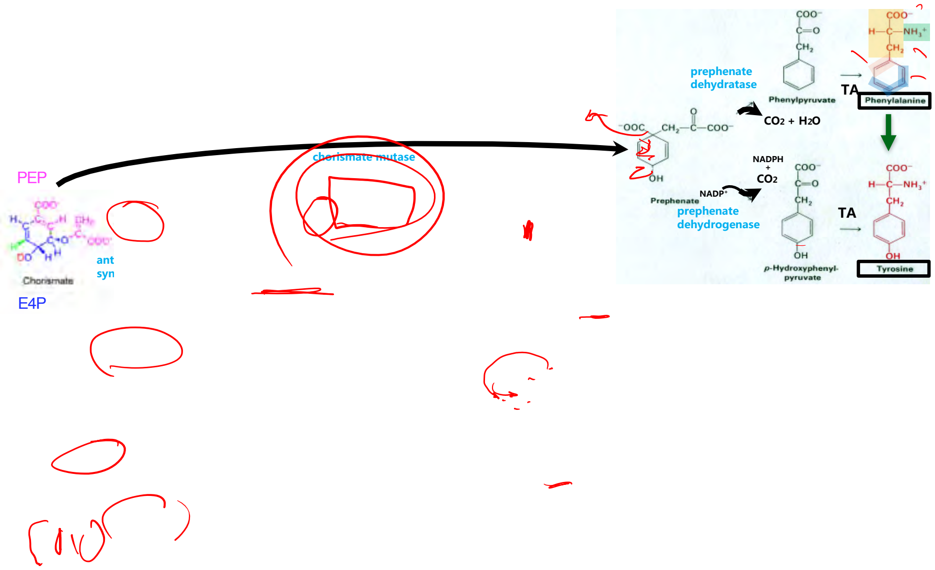
Biosynthesis of Chorismate, a Key Intermediate in Aromatic Amino Acid Biosynthesis



Chroismate

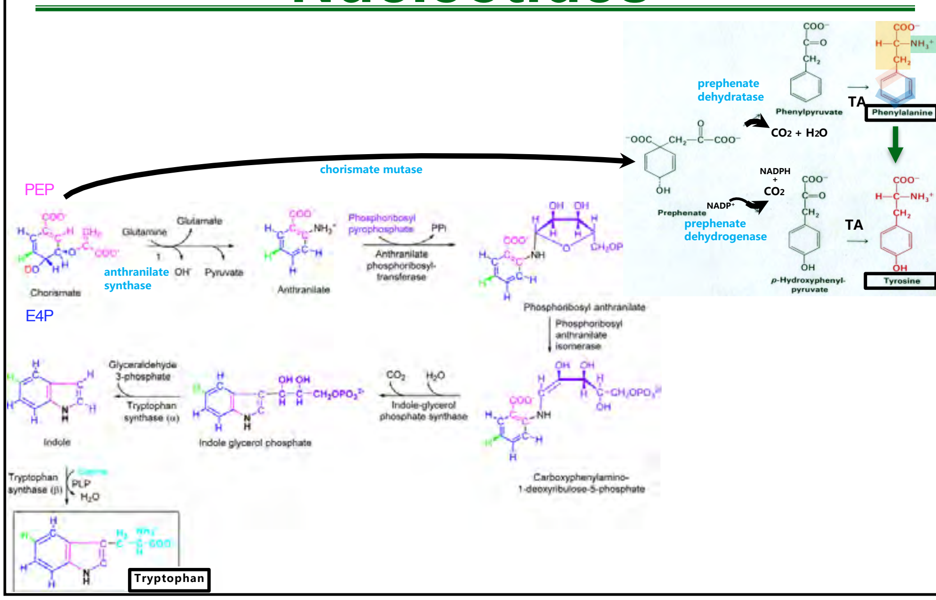
Biosynthesis Amino Acids & Nucleotides

Aromatic Family: Phe, Trp



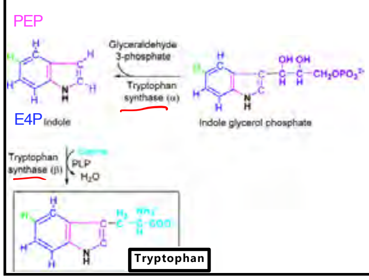
Biosynthesis Amino Acids & Nucleotides

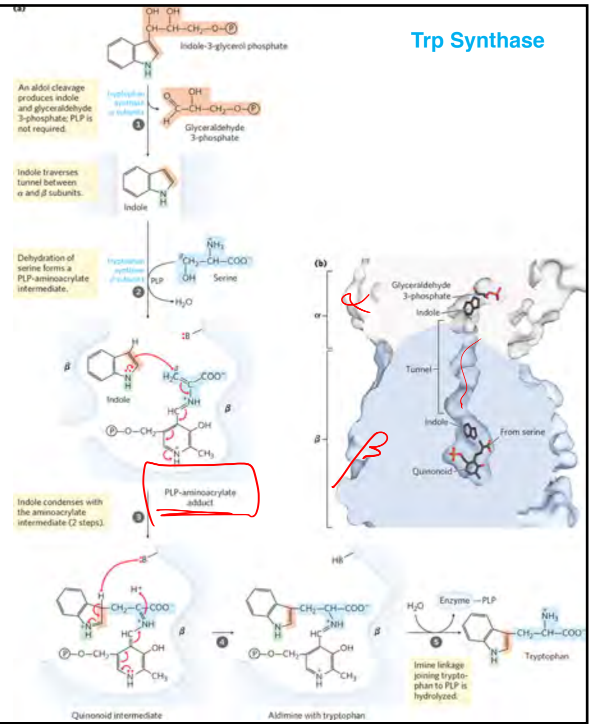
Aromatic Family: Phe, Trp



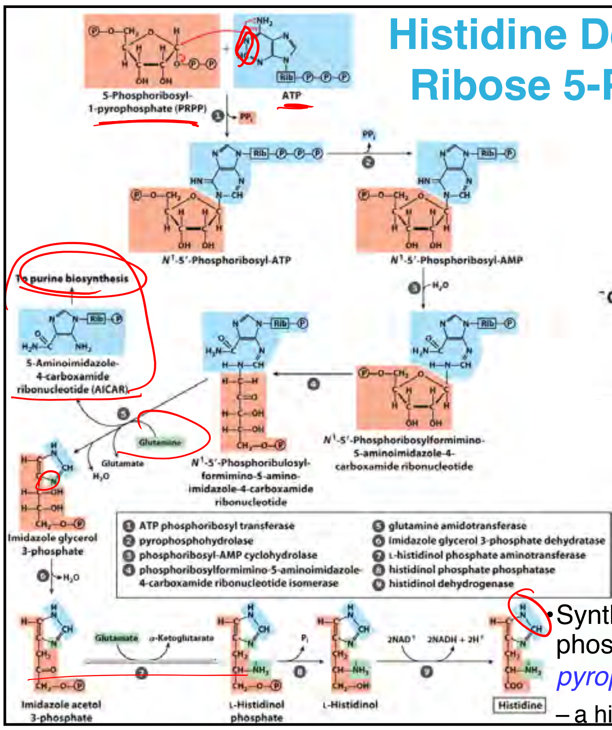
Biosynthesis Amino Acids & Nucleotides

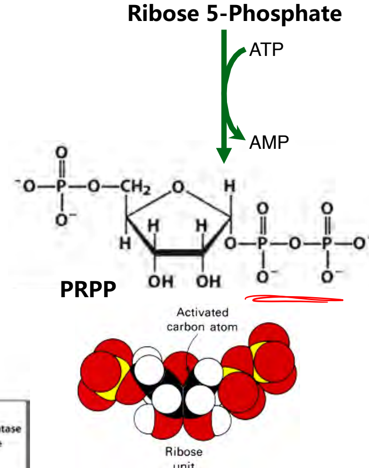
Trp Synthase





Histidine Derives from Ribose 5-Phosphate





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

Biosynthesis Amino Acids & Nucleotides

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Tyr	1	*	Phe

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 ← urea	Histidine
Asparagine	Cysteine ← Met	Isoleucine
Aspartate	→ Glutamine	Leucine
Glutamate	→ Glycine	Lysine
Serine	Proline ← Arg	Methionine
	Tyrosine ← Phe	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.

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

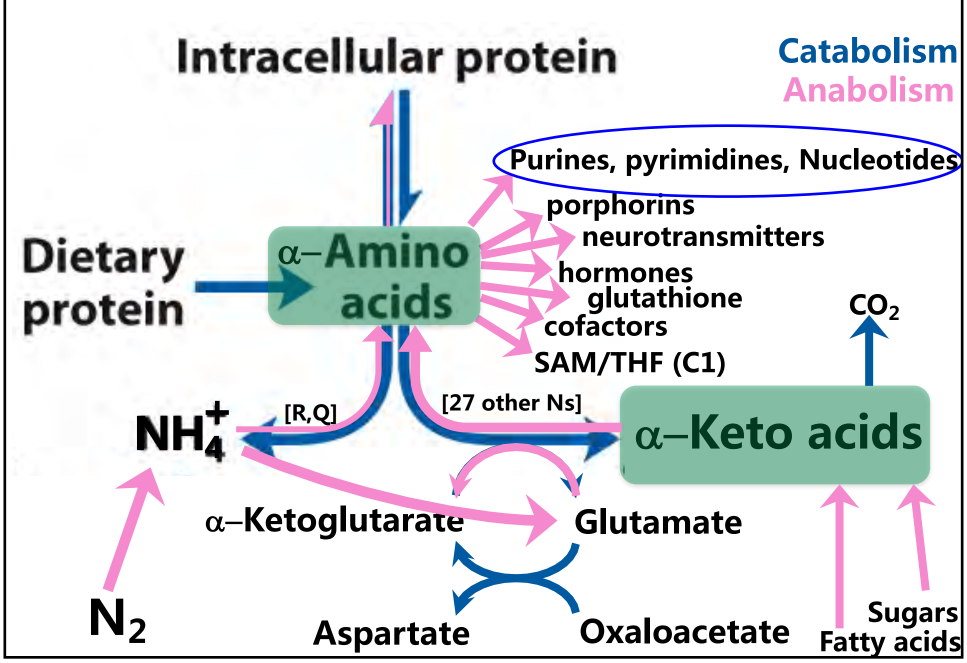
   b) essential

4) Biosynthesis of nucleotides

5) Control of nitrogen metabolism

6) Biosynthesis and degradation of heme; other 2° products of amino acids

Biosynthesis Amino Acids & Nucleotides



Biosynthesis Amino Acids & Nucleotides

Nucleotide Biosynthesis

- Bases
  - Purines (as nucleotides*)
  - Pyrimidines (as bases)
    - Adding the Ribose (PRPP)
- Making the deoxy-ribose
- Making the triphosphate precursors
- Regulating the levels for DNA synthesis

*Bases synthesized *while* attached to ribose; products are RMP

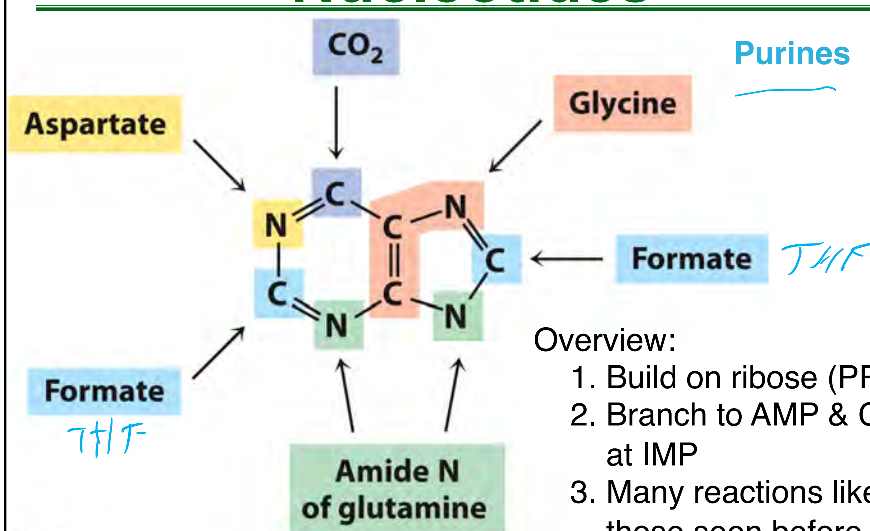
(R is one-letter code for purine, Y is one letter code for pyrimidine)

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.
   - 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

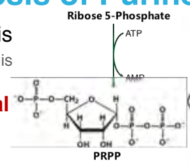


Overview:
1. Build on ribose (PRPP)
2. Branch to AMP & GMP at IMP
3. Many reactions like those seen before

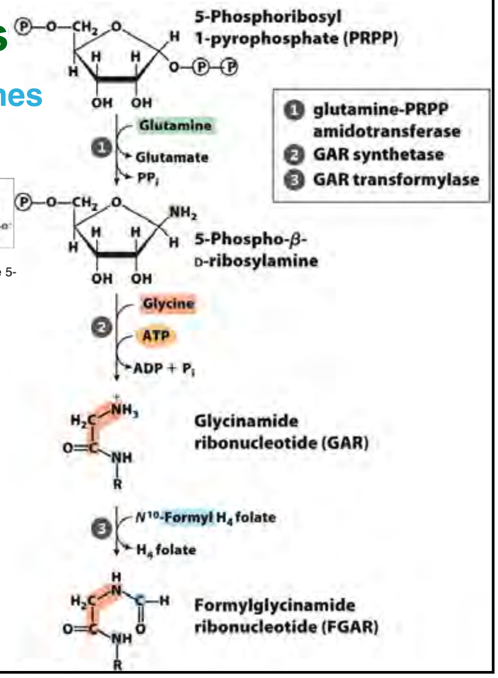
Biosynthesis Amino Acids & Nucleotides

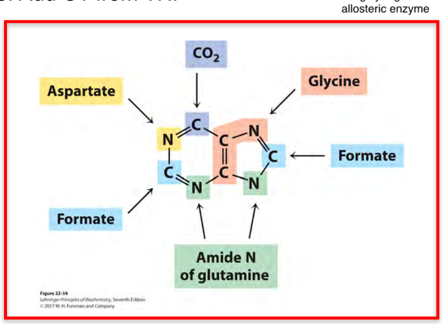
De Novo Biosynthesis of Purines

0. Begins with PRPP synthesis
1. PRPP reacts with Gln (like His with ATP NH2, or anthranilate in Trp synthesis-very common). **Committal Step**
2. Addition of three carbons from glycine by making amide (like Asn).
3. Add C1 from THF



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

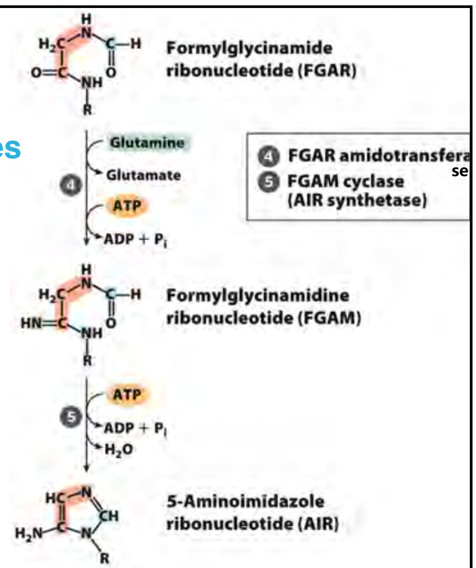


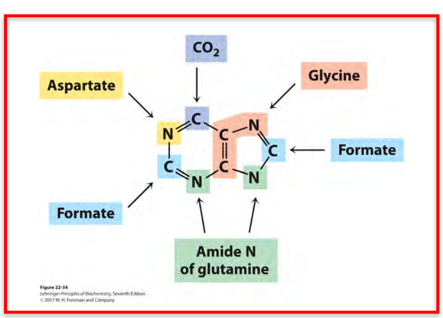


Biosynthesis Amino Acids & Nucleotides

De Novo Biosynthesis of Purines

4. FGAR reacts with Gln (just saw in Trp (chorismate) and first step here (aldehyde of ribose in PRPP).
5. Looks like Schiff base, but its an elimination after phosphorylation.

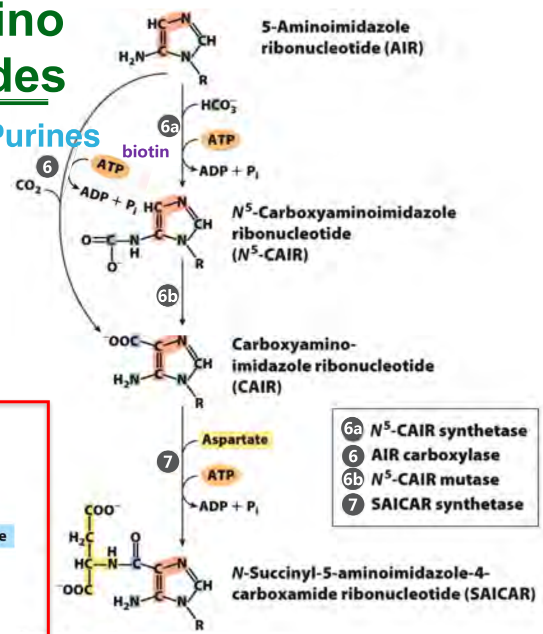


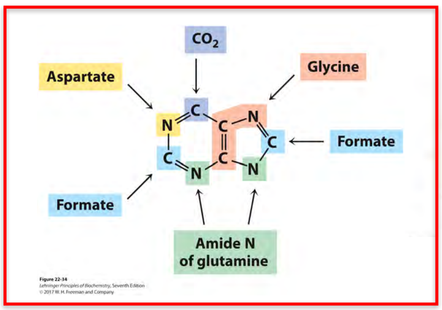


Biosynthesis Amino Acids & Nucleotides

De Novo Biosynthesis of Purines

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



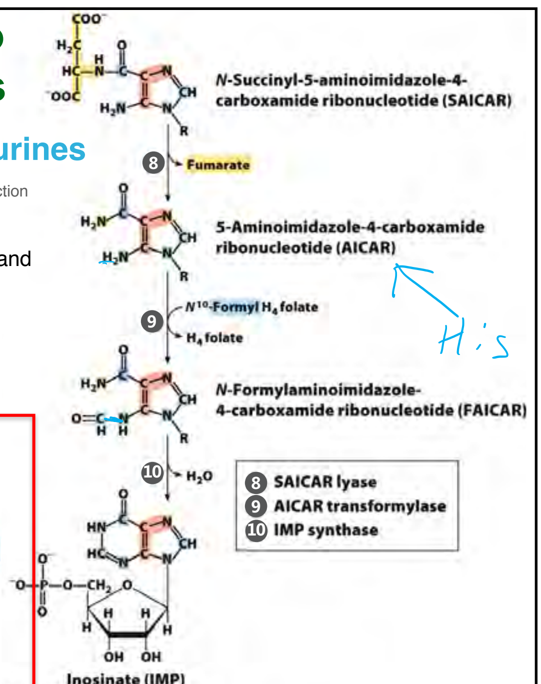


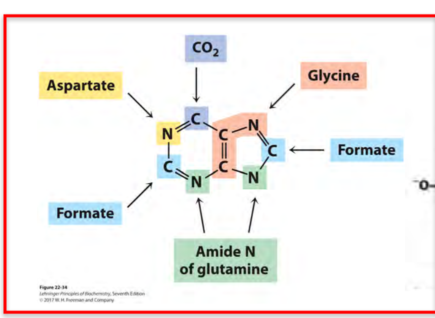
Biosynthesis Amino Acids & Nucleotides

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)

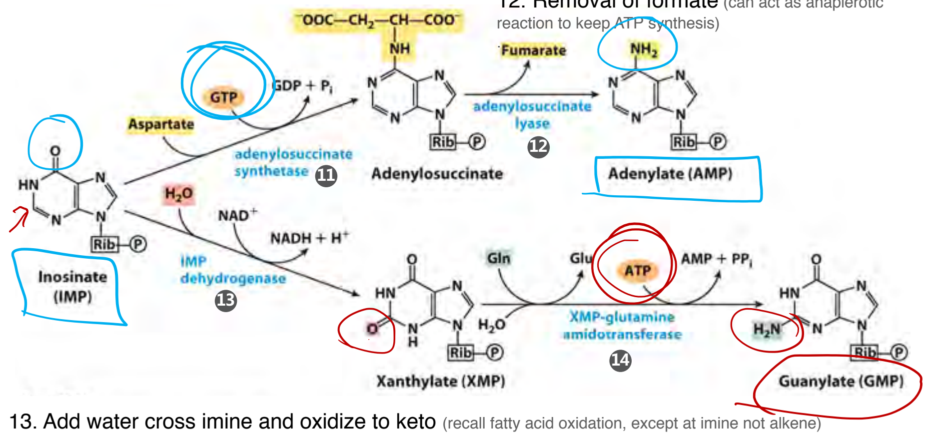




Biosynthesis Amino Acids & Nucleotides

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)

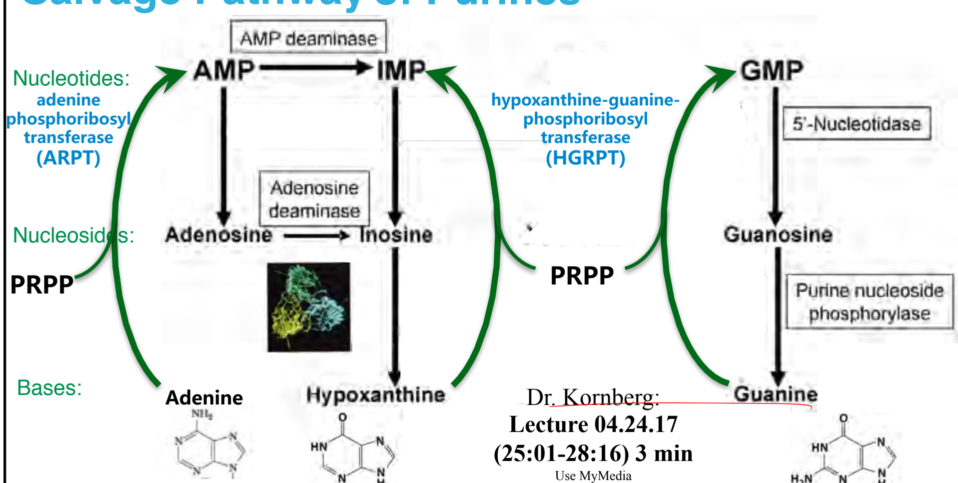


13. Add water cross imine and oxidize to keto (recall fatty acid oxidation, except at imine not alkene)
14. Add nitrogen from Gln (recall Asn Synthetase and 3rd time we saw use of Gln for this)

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

Biosynthesis Amino Acids & Nucleotides

Salvage Pathway of Purines



Dr. Kornberg:
Lecture 04.24.17
(25:01-28:16) 3 min
Use MyMedia

- 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.

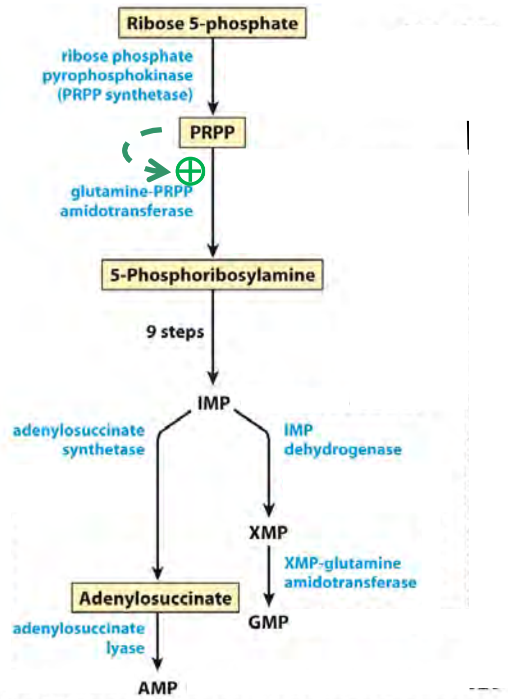
https://mymedia.bu.edu/channel/BI422/81224851

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

Regulation of Purine Biosynthesis

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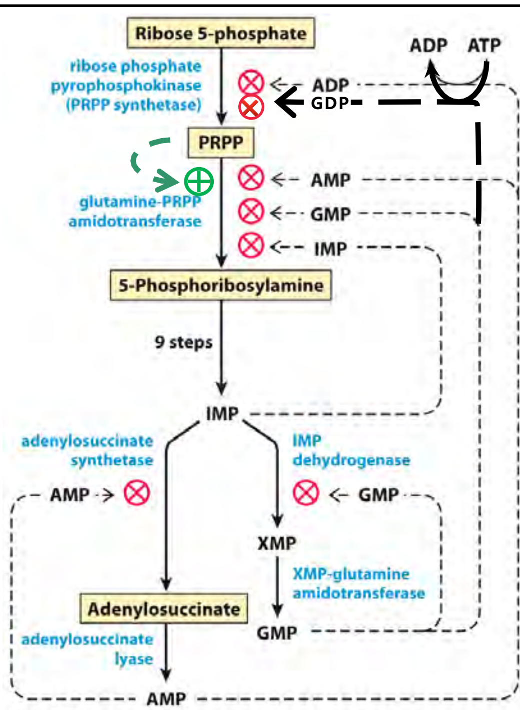
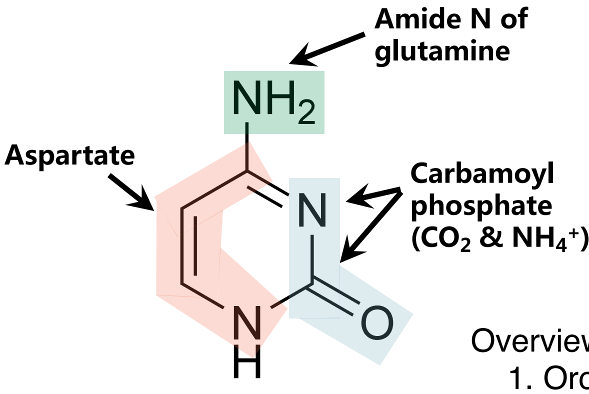


Biosynthesis Amino Acids & Nucleotides

Pyrimidines



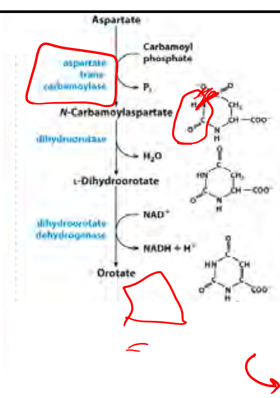
Overview:

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.



Biosynthesis Amino Acids & Nucleotides

De Novo Biosynthesis of Pyrimidines

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- Aspartate and carbamoyl phosphate provide all the atoms for the heterocycle or pyrimidine. The first pyrimidine is Orotate.
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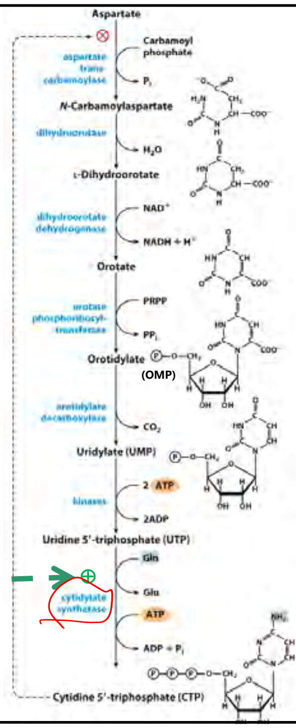
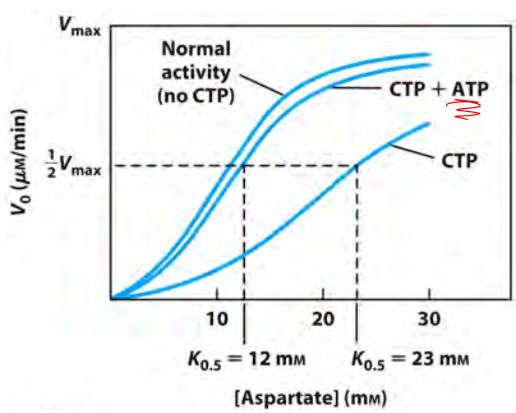
- UTP is converted to CTP by amination using Gln similar to making AMP from XMP.
- The biosynthesis of CTP is the CLASSIC feedback inhibition by the allosteric negative effector (CTP) on ATCase. Also, activation by GTP

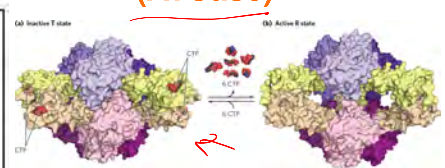


Biosynthesis Amino Acids & Nucleotides

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

Dr. Kornberg:
Lecture 04.26.17
(0:30-5:06) 4.5 min

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*

RNA

Very unique enzyme in all of biochemistry - use of free radicals

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.