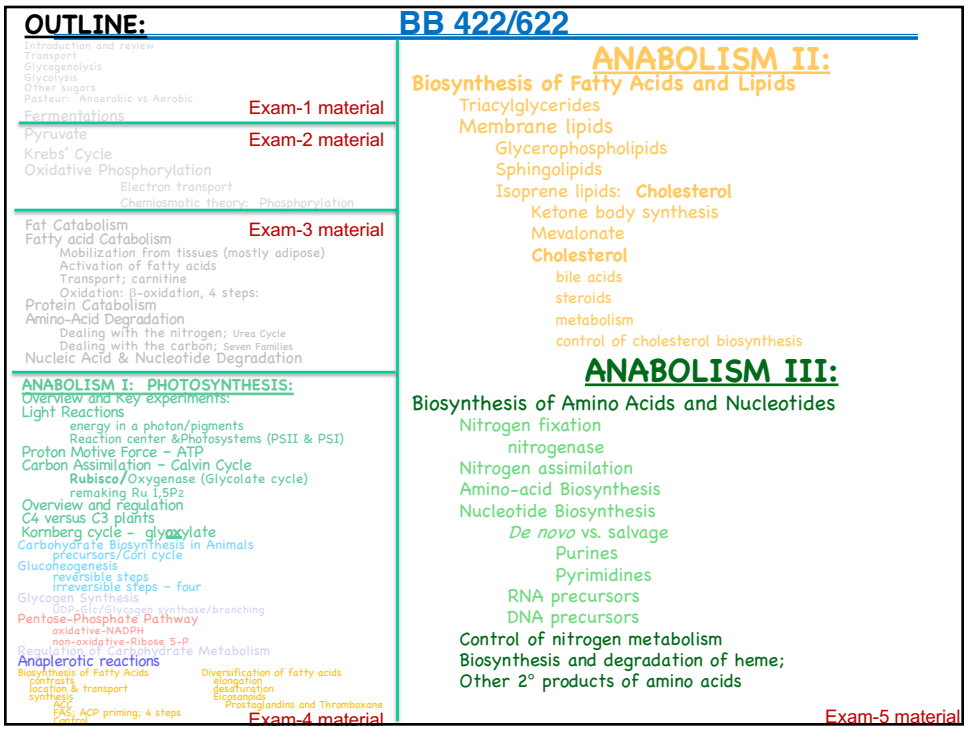


ANABOLISM III: Biosynthesis Amino Acids & Nucleotides

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 and deoxynucleotides
5) Control of nitrogen metabolism
6) Biosynthesis and degradation of heme; other 2° products of amino acids

Biosynthesis Amino Acids & Nucleotides

Regulation of Nucleotide Metabolism (Review):

Purines: Feedback inhibition by AMP, GMP, IMP at committed steps
Pyrimidines: Feedback inhibition by CTP at committed step
PRPP synthesis: Feedback inhibition by GDP, ADP, UMP, CTP
RNA/DNA precursors: GTP activates *CTP synthetase*; GTP used for AMP and ATP used for GMP; ***Ribonucleotide reductase***

Regulation of Overall Nitrogen Metabolism and Amino Acid Biosynthesis

Multilayered approach: Often, more than one mechanism of feedback regulation is utilized. Besides **Classic**, there are FOUR modes of Feedback control

Sequential – every committed product feeds back

Concerted – all end-products must be present to inhibit at all

Cummulative – all end-products inhibit the same percentage

Isozymes – use of isozymes for regulation of specific pathways; each end-product inhibits a specific isozyme

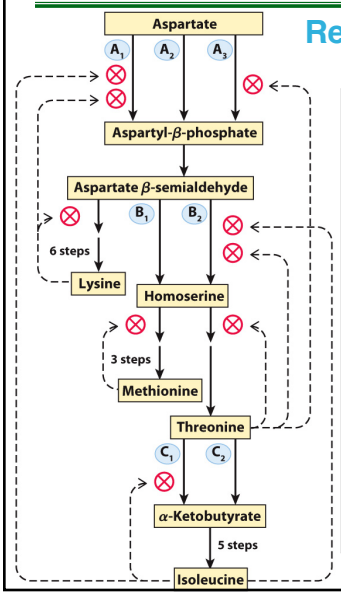
De novo purine-GMP

homoserine de-hydrogenase in Thr & Ile

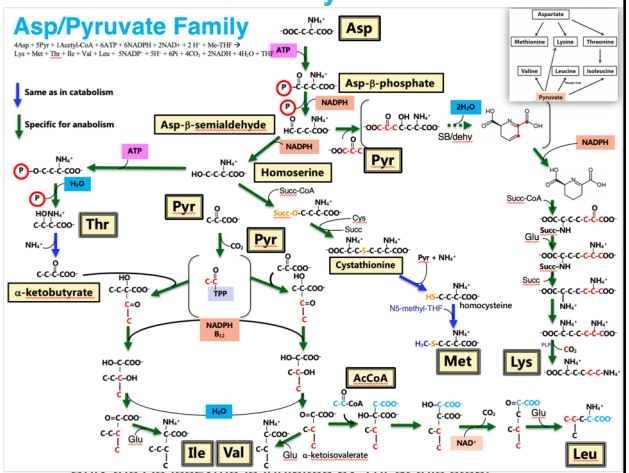
Glutamine Synthetase

Aromatic amino acids

Biosynthesis Amino Acids & Nucleotides

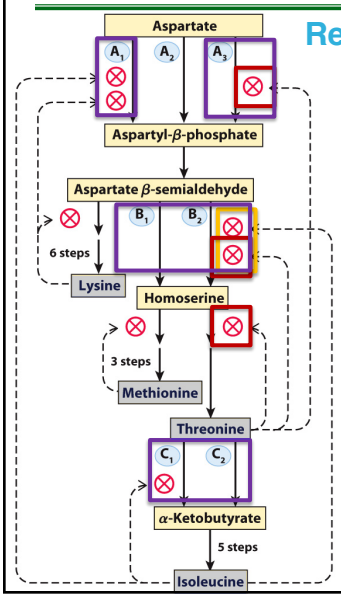


Regulation of Aspartate/Pyruvate-Derived Pathways



What is a good example of Cumulative feedback inhibition?

Biosynthesis Amino Acids & Nucleotides



Regulation of Aspartate/Pyruvate-Derived Pathways

Classic Feedback Inhibition in Lys, Met, Thr, and Ile Biosynthesis

Sequential Feedback Inhibition in Thr Biosynthesis

Concerted Feedback Inhibition of Homoserine Dehydrogenase in Ile and Thr Biosynthesis

Use of Isozymes Is Another Important Means of Regulation:

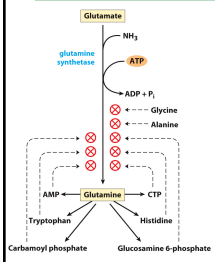
Homoserine dehydrogenase (B)
Serine/Threonine dehydratase (C)
Aspartate Kinase (A)

Asp Kinase initiates biosynthsis of Lys, Met, Thr, and Ile. Use of isozymes, all regulated by different effectors, allows *E. coli* to produce the amino acids when needed.

Example: *Asp Kinase* isozyme A1 is inhibited if Ile or Lys is high, but not if Met or Thr are high.
Only the A3 isozyme is inhibited by Thr at this step.

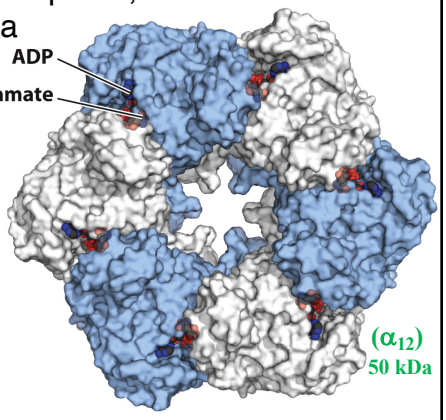
What is a good example of Cumulative feedback inhibition?

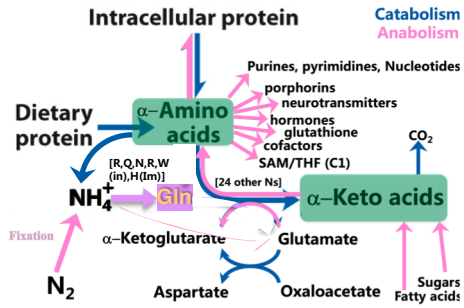
Biosynthesis Amino Acids & Nucleotides



Cumulative allosteric inhibition by:
CTP, AMP, His,
Trp, Carbamoyl-phosphate, Glc-amine-6-P, Gly, Ala

Glutamine Synthetase





Glutamate + NH_4^+ + ATP → glutamine + ADP + P_i + H^+

Biosynthesis Amino Acids & Nucleotides

Adenylylation of Glutamine Synthetase

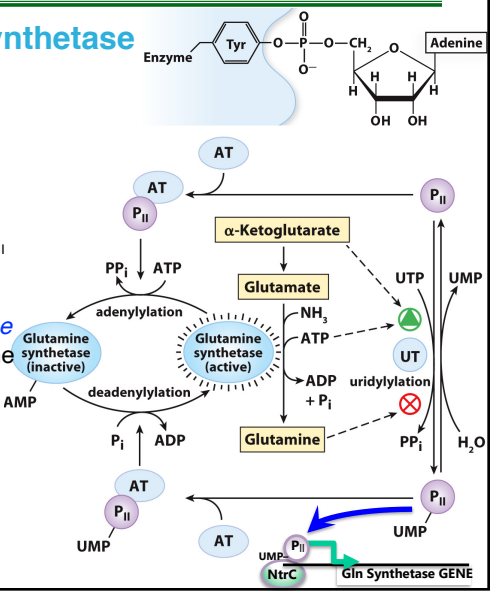
Adenylylation (attachment of AMP) to Tyr-397 assists in inhibition.

- Increases sensitivity to feedback inhibitors
- Part of complex cascade that is dependent on [Glu], [α-ketoglutarate], [ATP], and [P_i]
- Activity of *adenylyltransferase (AT)* regulated by binding to regulatory protein P_{II}
- ***Adenylylation*** inhibits *Gln synthetase*.

When P_{II} is uridylylated by *uridylyltransferase (UT)*, *adenylyltransferase* deadenylates Gln synthetase (increasing the latter's activity).

- Uridylyation controlled by Gln (inhibits) and α-KG & ATP (activates).

ALSO, uridylylated P_{II} upregulates transcription of Gln synthetase.



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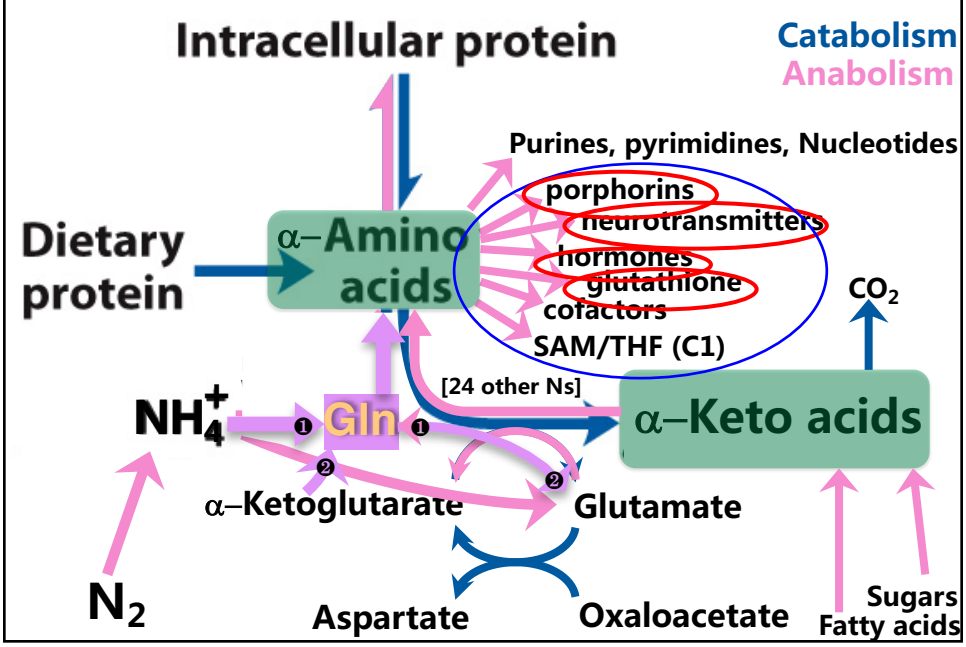
Biosynthesis Amino Acids & Nucleotides



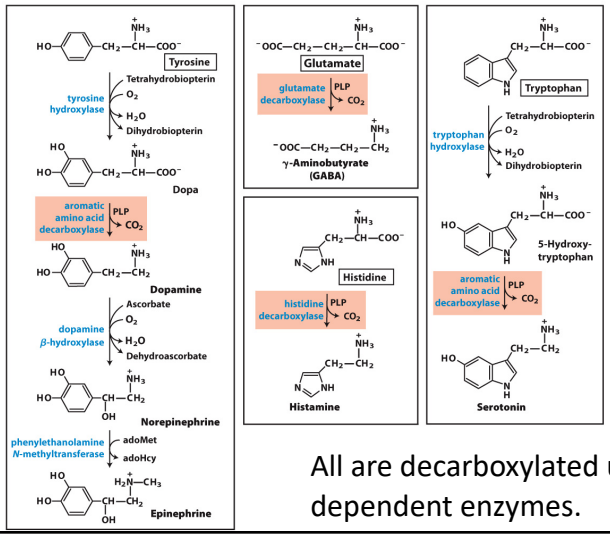
Biosynthesis Amino Acids & Nucleotides

Many Important Metabolites are Derived from Amino Acids

- Porphyrin rings (e.g., heme, cytochromes, chlorophylls, etc.)
- Phosphocreatine
- Glutathione
- Cofactors; niacin, biotin, folic acid
- Neurotransmitters (serotonin, GABA, adrenalin, DOPA, histamine)
- Signaling molecules
  - Hormones; melatonin, adrenaline
  - Paracrine signals; NO, leukotrienes
- Cell-wall constituents; Peptidoglycan, Lignin

Biosynthesis Amino Acids & Nucleotides

Some Neurotransmitters Are Derived from Amino Acids



All are decarboxylated using PLP dependent enzymes.

Biosynthesis Amino Acids & Nucleotides

Arg is the Precursor for Nitric Oxide (NO)

- Mid-80's discovery that pollutant NO played important role in blood pressure regulation, blood clotting, and so on
- Synthesized from Arg via *nitric oxide synthase* using NADPH
  - enzyme similar to cyt P450 reductase
  - stimulated by interaction with Ca^{2+} and calmodulin

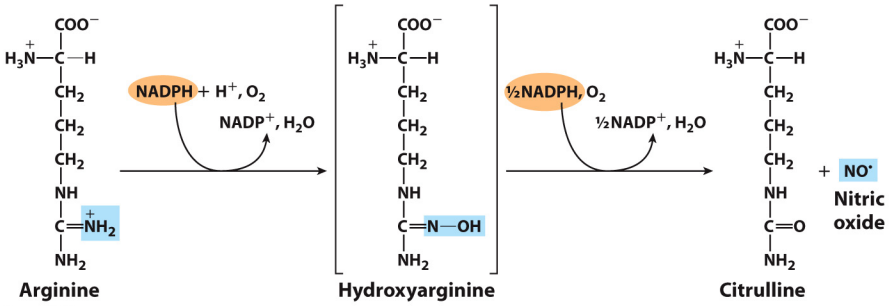


Figure 22-33

Biosynthesis Amino Acids & Nucleotides

Glutathione (GSH) Derives from Glu, Cys, and Gly

- GSH is present in most cells at high amounts.
- Reducing agent/antioxidant
  - keeps proteins, metal cations reduced
  - keeps redox enzymes in reduced state
  - removes toxic peroxides
- Oxidized to a dimer using disulfide bond (GSSG)

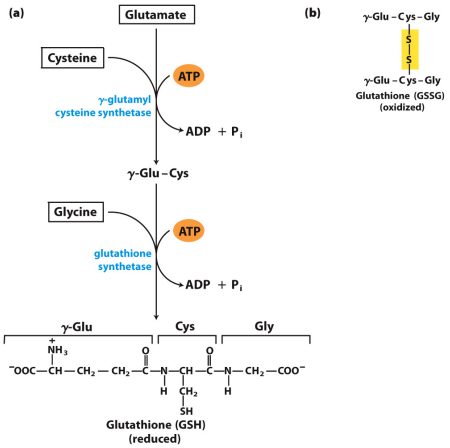


Figure 22-29
Lehninger Principles of Biochemistry, Seventh Edition
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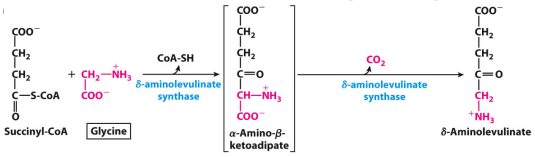
Biosynthesis Amino Acids & Nucleotides

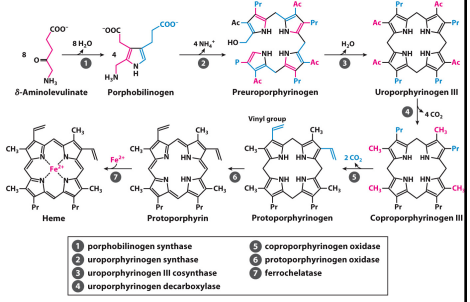
Porphyrin Biosynthesis

Glycine is the Precursor to Porphyrins; synthesis of δ-Aminolevulinate



- Porphyrin makes up the heme of hemoglobin, cytochromes, myoglobin.
- In higher animals, porphyrin arises from reaction of glycine with succinyl-CoA.
  - In plants and bacteria, glutamate is the precursor.
- The pathway generates two molecules of the important intermediate *δ-aminevulinate.*
- *Porphobilinogen* is another important intermediate.

Synthesis of Heme from δ-Aminolevulinate

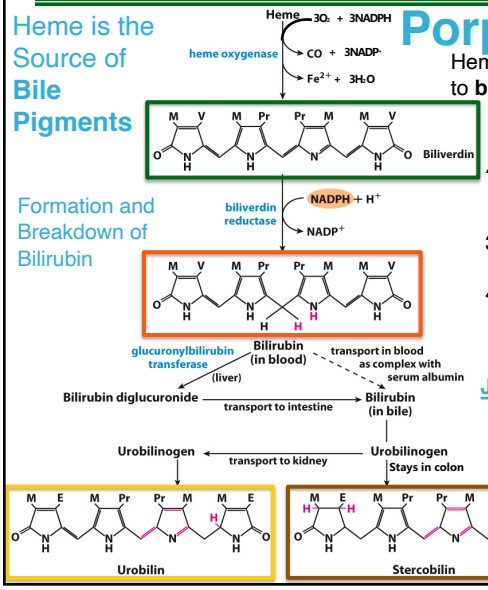


1. Two molecules of δ-aminolevulinate condense to form porphobilinogen.
2. Four molecules of porphobilinogen combine to form protoporphyrin.
3. Fe ion is inserted into protoporphyrin with the enzyme *ferrochelatase.*

Biosynthesis Amino Acids & Nucleotides

Heme is the Source of **Bile Pigments**

Porphyrin Degradation



Heme, from degradation of erythrocytes, is converted to **bilirubin** in two steps:

1. *Heme oxygenase* linearizes heme to create **biliverdin**, a green compound (bruises).
2. *Biliverdin reductase* converts biliverdin to **bilirubin,** a red-orange compound that travels bound to serum albumin in the bloodstream.
3. Bilirubin is oxidized in the kidney to **urobilin**, the major pigment of urine.
4. Urobiligen not taken up by the kidney stays in the colon and is reduced by intestinal microbiota to **stercobilin.**

Jaundice is Caused by Bilirubin Accumulation

Jaundice (yellowish pigmentation of skin, whites of eyes, etc.) can result from:

- impaired liver (in liver cancer, hepatitis)
- blocked bile secretion (due to gallstones, pancreatic cancer)
- insufficient *glucouronyl bilirubin transferase* to process bilirubin (occurs in infants)
  - treated with UV to cause photochemical breakdown of bilirubin

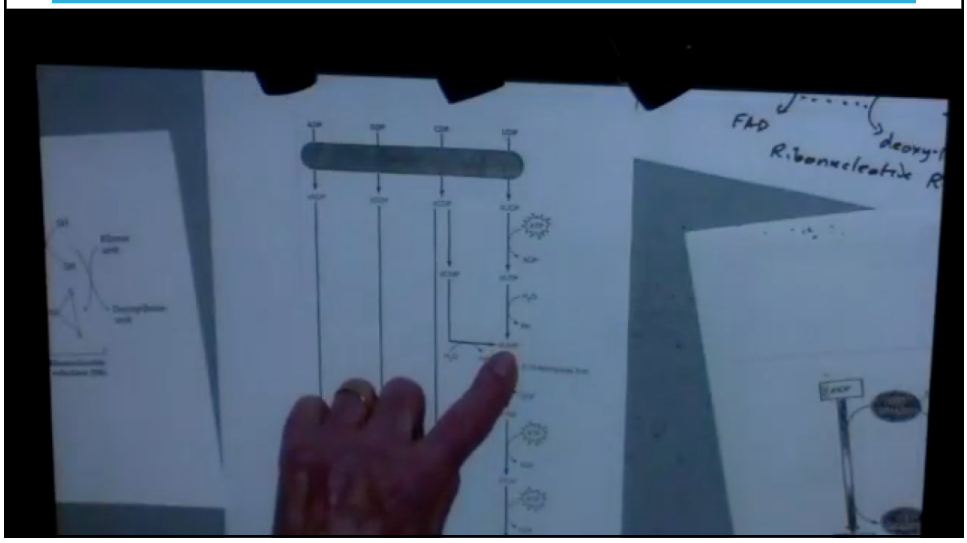
ANABOLISM III: Biosynthesis Amino Acids & Nucleotides

Summary

What we learned:

- Methods for fixation of molecular nitrogen to nitrates, nitrates, and **ammonia**
- Gln serves as the primary entry point of assimilation of ammonia via *Gln Synthetase* in animals; but made useful by *Glu Synthase* to make net Glu.
- The 20 common amino acids are synthesized from α-ketoglutarate, 3-phosphoglycerate, oxaloacetate, pyruvate, phosphoenolpyruvate, erythrose 4-phosphate, and ribose-5-phosphate (through phosphoribosyl pyrophosphate (PRPP).
- About half are non-essential in humans and are made much like they are degraded
- About half are essential and are made through extensive and inter-related paths
- Nucleotides can be synthesized either *de novo* from simple precursors, or reassembled from the salvage pathway using PRPP.
- *De novo* purines are synthesized on the ribose starting with PRPP., while pyrimidine rings are assembled prior to attachment to ribose using PRPP.
- Ribonucleotides(NDP) are converted to deoxyribonucleotides(dNTP) by *ribonucleotide reductase*, which is regulated in ways to ensure equal amounts of A:T & G:C.
- Regulation of amino-acid biosynthesis, as well as nucleotide synthesis, is by various types of feedback inhibition; in particular, the cumulative type at Gln synthetase
- Many examples of 2° products of amino acids, including Porphyrin biosynthesis and degradation, neurotransmitters, glutathione, and even NO gas.

Farewell to Biochemistry II (BB 422/622)



Farewell to Biochemistry II (BB 422/622)

This course is Dedicated to the memory of Sir Hans Kornberg

January 14, 1928 ~ December 16, 2019

I want to thank Dr. Kornberg for all his help this semester and the inspiration!

End of Material for Exam 5 & End of Biochemistry II

Please take a few minutes to do the course evaluation!

https://www.bu.edu/courseeval/

AND…….

God Bless You!