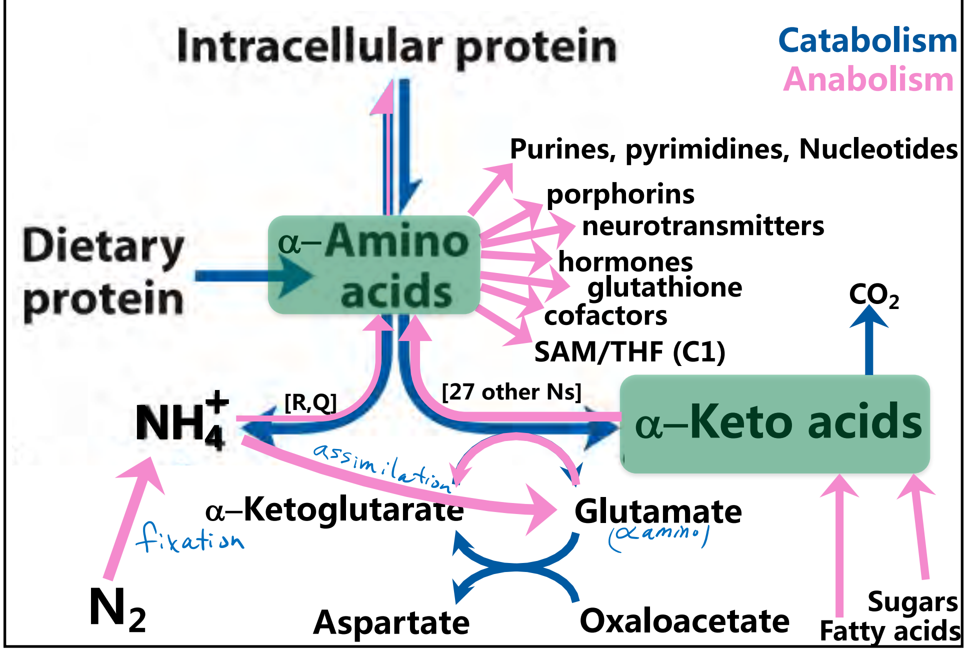
ANABOLISM III: Biosynthesis Amino Acids & Nucleotides

Biosynthesis Amino Acids & Nucleotides



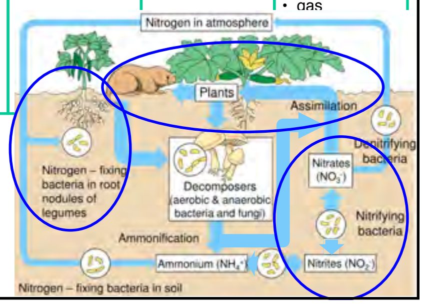
Biosynthesis Amino Acids & Nucleotides

Chemistry of Molecular Nitrogen

Review: Oxidation States of Nitrogen Compounds

Nitrate	Nitrogen(IV) dioxide	Nitrite	Nitric oxide (Nitrogen(II))	Nitrogen(I) oxide	Nitrogen	Ammonia
• NO_3^- • $N^{+5}O_3^-$ • Nitrate • Also Nitric acid (HNO_3) and Dinitrogen pentoxide (N_2O_5) • "ate" is the higher oxidation state.	• NO_2 • $N^{+4}O_2^-$ • Nitrogen dioxide • gas	• NO_2^- • $N^{+3}O_2^-$ • Na-Nitrite • Also Nitrous acid (HONO) • "ite" is light on oxygen and oxidation state.	• NO • N^{+2} • Non-salt • Gas • Physio-logically important 2° messenger and paracrine signal	• N_2O • N^{+1} • Non-salt • gas	• N_2 • N^0 • Covalent triple bond • gas	• NH_3 • $N^{-3}H_3^+$ • NH_3: N has oxidation state of –3. • gas

Biology of Molecular Nitrogen: The Nitrogen Cycle

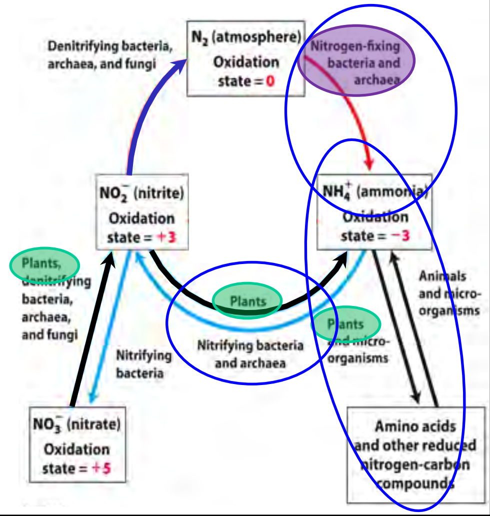


Biosynthesis Amino Acids & Nucleotides

Chemical transformations maintain a balance between N_2 and biologically useful forms of nitrogen.

1. ***Fixation.*** Bacteria reduce N_2 to NH_3/NH_4^+.
2. ***Nitrification.*** Bacteria oxidize ammonia into nitrite (NO_2^-) and nitrate (NO_3^-). Organisms die, returning NH_3 to soil. Nitrifying bacteria again convert NH_3 to nitrite and nitrate.
3. ***Assimilation.***
   a. Nitrate/nitrite: Plants and microorganisms reduce NO_2^- and NO_3^- to NH_3 via nitrite reductases and nitrate reductases.
   b. NH_3 is incorporated into amino acids.
4. ***Denitrification.*** Nitrate is reduced to N_2 under anaerobic conditions. NO_3^- is the ultimate electron acceptor instead of O_2.

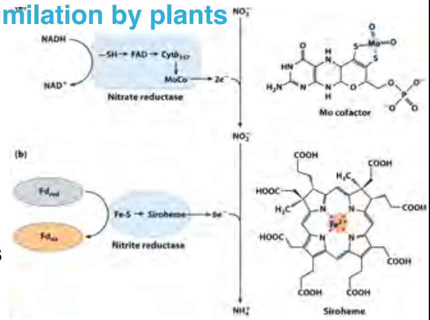
The Nitrogen Cycle



Biosynthesis Amino Acids & Nucleotides

Two Important Enzymes in Nitrogen Assimilation by plants

1. ***Nitrate reductase*** $NO_3^- + 2\ e^- \rightarrow NO_2^-$
    - large, soluble protein
    - contains novel Mo cofactor
    - *e*⁻ from NADH
2. ***Nitrite reductase*** $NO_2^- + 6\ e^- \rightarrow NH_4^+$
    - found in chloroplasts in plants: *e*⁻ comes from ferredoxin (F_d)
    - in non-photosynthetic microbes: *e*⁻ comes from NADPH



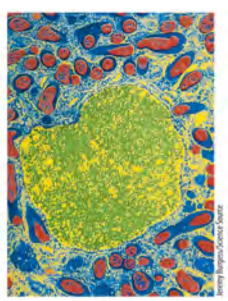
Only a Few Organisms Can "Fix" N_2 to Useful Forms

- Most are single-celled prokaryotes (archaea).
- Some live in symbiosis with *plants.*
    - (e.g., proteobacteria with legumes such as peanuts, beans)
- A few live in symbiosis with *animals.*
    - (e.g., spirochaete with termites)

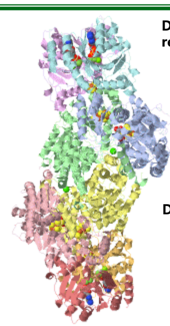
They have enzymes that overcome the high activation energy by binding and hydrolyzing ATP.

Biosynthesis Amino Acids & Nucleotides

Diazotrophs



–Takes care of energy requirement and O_2 lability.
–Bacteria have access to plant's carbohydrate and CAC intermediates for energy.
–Bacteria are covered with leghemoglobin to bind O_2 and prevent corruption of the catalyst in dinitrogenase.
–Can produce more NH_3 than plant needs; excess released to soil



Nitrogen-Fixing Nodules

Nitrogen Fixation Is Carried Out by the Nitrogenase Complex

Jmol

- $N_2 + 3H_2 \rightleftharpoons 2\ NH_3$
    - exergonic ($\Delta G°$ = –33.5 kJ/mol) but very **slow** due to the triple bond's high activation energy
- The nitrogenase complex (60+240 kDa) uses ATP to overcome the activation energy.
- Passes electrons to N_2 and catalyzes a step-wise reduction of N_2 to NH_3

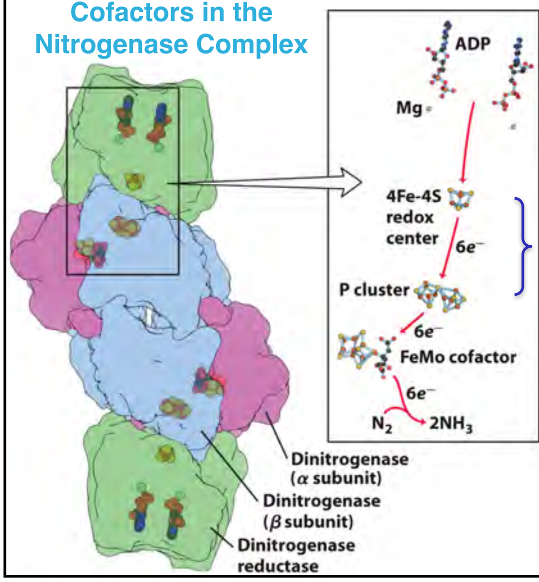
$$N_2 + 8\ H^+ + 8\ e^- + nATP \rightleftharpoons 2\ NH_3 + H_2 + nADP + nP_i$$

$$2\ NH_3 + 2\ H^+ \rightleftharpoons 2\ NH_4^+$$

About 16 ATP molecules are consumed per one N_2.

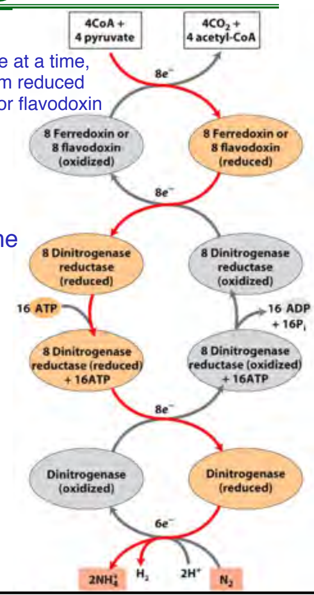
Biosynthesis Amino Acids & Nucleotides

Cofactors in the Nitrogenase Complex



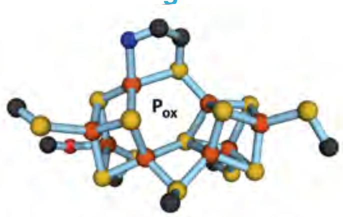
e– flow, one at a time, starting from reduced ferredoxin or flavodoxin

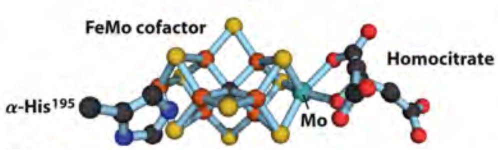
This is the critical step



Biosynthesis Amino Acids & Nucleotides

The Electron-Transfer Cofactors in Dinitrogenase





The Fe-S "P-cluster" at the interface of the α and β-Dinitrogenase Subunits

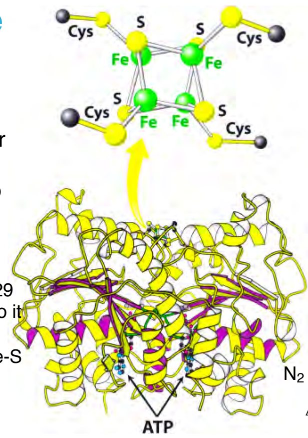
- Consists of:
  - 8 Fe atoms
  - 7 S atoms
  - Bound by 7 Cys
- Electrons are passed from the reductase Fe-S one at a time.
- Electrons are passed to the Fe-Mo cofactor.

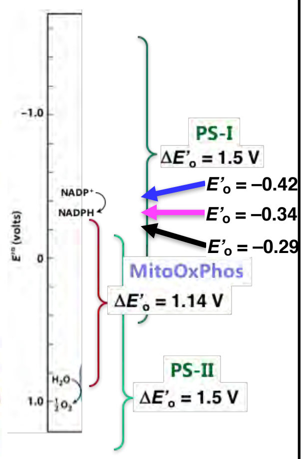
The Fe-Mo Cofactor in the Dinitrogenase β-Subunit

- Consists of:
  - 7 Fe atoms
  - 9 S atoms
  - 1 Mo atom
  - 1 bound homocitrate
- The nitrogen binds to the center of the Mo-FeS cage and is coordinated to the molybdenum atom.
- Electrons are passed to the molybdenum-bound nitrogen via the iron-sulfur complex.

Biosynthesis Amino Acids & Nucleotides

Dinitrogen Reductase





- Source of e^- varies between organisms.
  - often pyruvate → ferredoxin
1. Pyruvate passes e^- to ferredoxin or flavodoxin.
2. Ferredoxin or flavodoxin pass e^- to dinitrogenase reductase.
3. Dinitrogenase reductase:
   - Reduced by F_d
   - Binding of ATP changes E_0' from –0.29 to –0.42 and changes conformation so it can bind dintrogenase
   - transfer of e^- to dinitrogenase from Fe-S to P-cluster, now only 14Å away
   - hydrolysis of 2ATPs with release of proton to dinitrogenase
   - transfer of 8 e^- and 8 H^+ for one N_2

1. ATP hydrolysis *and* ATP *binding* help overcome the high activation energy.
2. Has regions homologous to GTP-binding proteins used in signaling (switch-1 and -2)
3. The reductase passes e^- to dinitrogenase.................physically!

$N_2 + 6\,H^+ + 6\,e^- \rightarrow 2\,NH_3$ $E^{o'} = -0.34$ v

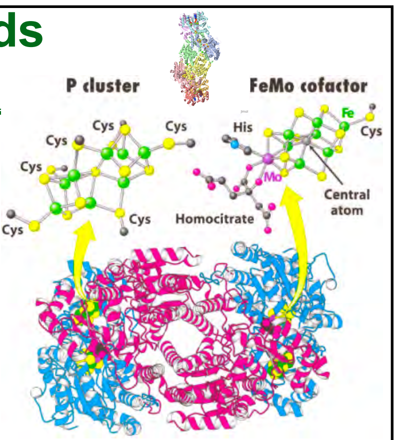
$\Delta E^{o'} = E^{o'}{}_{(reduction)} - E^{o'}{}_{(oxidation)}$

= –0.34 V – (–0.29 V)
= –0.05 V → +7 kcal/mole

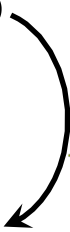
= –0.34 V – (–0.42 V)
= +0.08 V → –11 kcal/mole

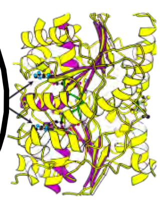
Biosynthesis Amino Acids & Nucleotides

Nitrogen Fixation by Dinitrogenase



F_d (Fe^{2+}) + 2 ATP

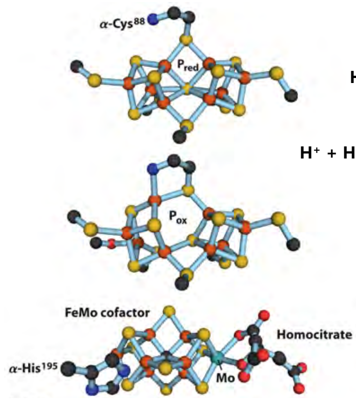


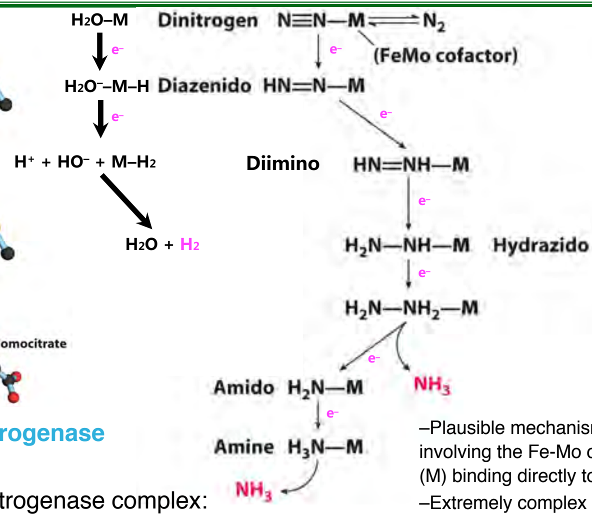


F_d (Fe^{3+}) +2 ADP+P_i

- Dinitrogenase catalyzes:
  - transfer of 6 e^- and 6 H^+ to nitrogen: formation of NH_3
  - transfer of 2 e^- to 2 protons: formation of H_2
  - ONE AT A TIME
- Has novel FeMo cofactor (or V in some organisms)
- The reduction of N_2 and protons occurs at FeMo cofactor.
- Formation of H_2 appears an obligatory side reaction.

Biosynthesis Amino Acids & Nucleotides





Redox Reactions in Dinitrogenase

- The net reaction of the nitrogenase complex:
  $N_2 + 8\ H^+ + 8\ e^- + 16\ ATP = 2\ NH_3 + H_2 + 16\ ADP + 16\ P_i$
- Mechanism of dinitrogenase is poorly understood.

–Plausible mechanism involving the Fe-Mo cofactor (M) binding directly to N_2.
–Extremely complex redox reaction that involves several metal atoms as cofactors and/or electron transporters

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

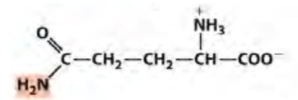
Ammonia is Incorporated into Biomolecules Through Glu and Gln

- Glutamine is made from Glu by glutamine synthetase in a two-step process (we discussed this previously when moving ammonia from extrahepatic tissues).

Glutamate + ATP → γ-glutamyl-phosphate + ADP

γ-glutamyl-phosphate + NH^{+4} → glutamine + P_i + H^+

SUM: Glutamate + NH^{+4} + ATP → glutamine + ADP + P_i + H^+



- Glutamate is made from Gln and α-Ketoglutarate by glutamate synthase. α-Ketoglutarate, an intermediate of the citric acid cycle, undergoes reductive amination with glutamine as nitrogen donor.

α-Ketoglutarate + glutamine + NADPH + H^+ → 2 glutamate + $NADP^+$

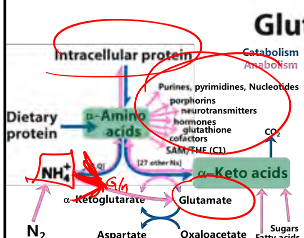
α-Ketoglutarate + NH^{+4} + ATP + NADPH + H^+ → glutamate + $NADP^+$ + ADP + P_i

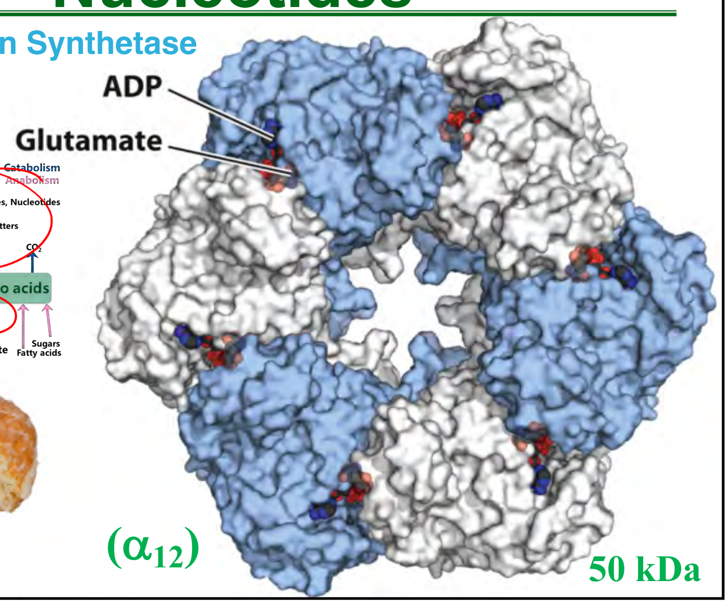
Assimilation!

(An alternative name for this enzyme, glutamate:oxoglutarate aminotransferase, yields the acronym GOGAT, by which the enzyme also is known.)

Biosynthesis Amino Acids & Nucleotides

Structure of Gln Synthetase



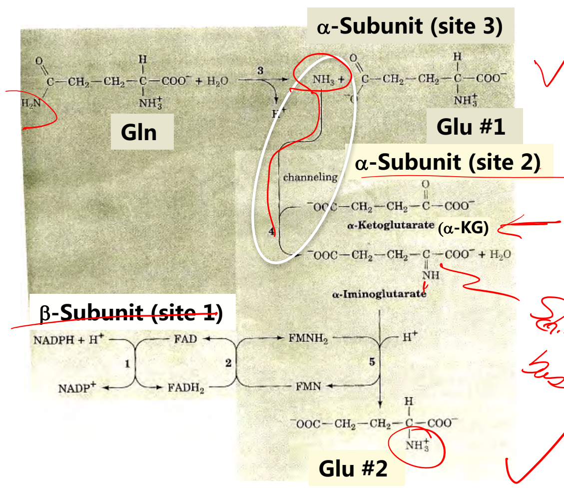


(α_{12})

50 kDa

Biosynthesis Amino Acids & Nucleotides

Mechanism of Glu Synthase



α-Ketoglutarate + glutamine + NADPH + H^+ → 2 glutamate + $NADP^+$

ANABOLISM III: Biosynthesis Amino Acids & Nucleotides

1) Nitrogen fixation: $N_2 \rightarrow NH_4$
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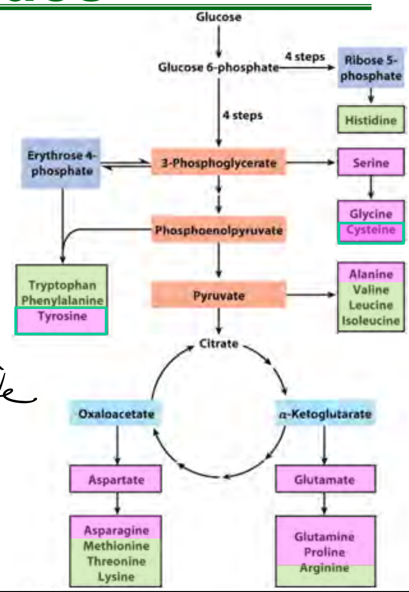
Biosynthesis Amino Acids & Nucleotides

Amino Acid Synthesis Overview

- Source of N is Glu, Gln, or NH_4^+
- Derive from intermediates of:
  - glycolysis
  - citric acid cycle
  - pentose phosphate pathway /Calvin cycle
- Bacteria can synthesize all 20.
- Mammals require some in diet:
  - –Essential
  - –Non-essential



Dr. Kornberg:
Lecture 04.07.17
(34:14-37:58) 4 min

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

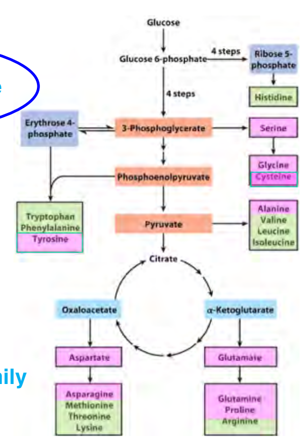
Biosynthesis Amino Acids & Nucleotides

<u>**Non-essential Amino acids**</u>:

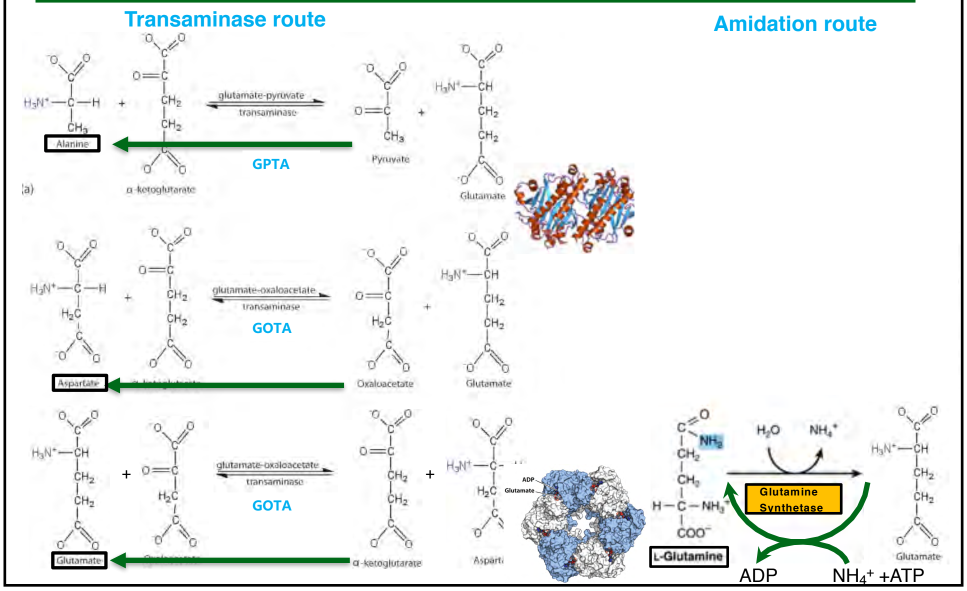
These are very few steps and often the same enzyme(s) used for degradation.

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

*reverse of degradation

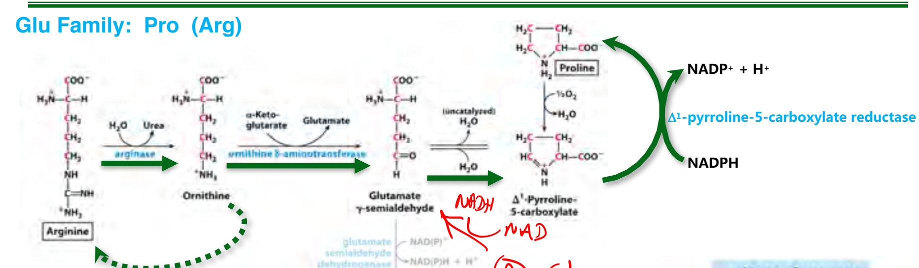


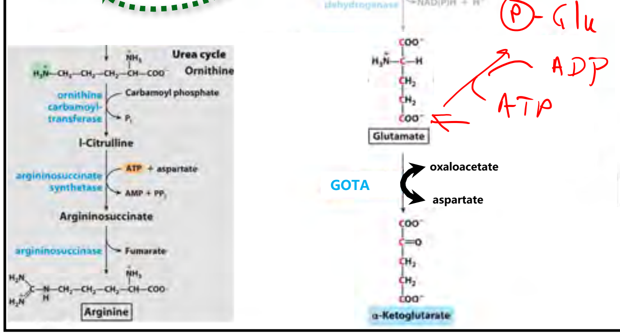
Biosynthesis Amino Acids & Nucleotides

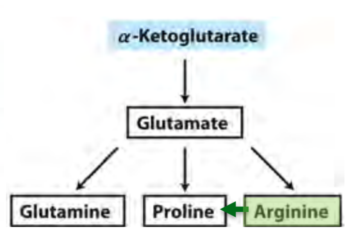


Biosynthesis Amino Acids & Nucleotides

Glu Family: Pro (Arg)





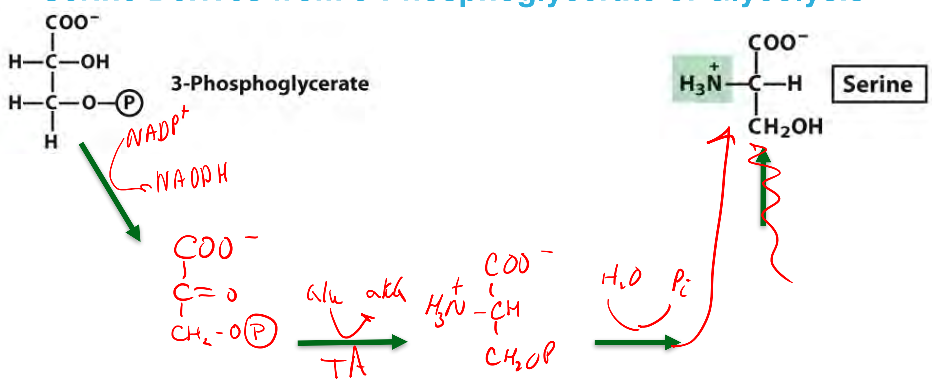


In bacteria, Glu is acetylated. This limits the un-catalyzed cyclization of the Glu-semialdehyde before its converted to Orn.

Biosynthesis Amino Acids & Nucleotides

3-PGA Family: Ser

Serine Derives from 3-Phosphoglycerate of Glycolysis



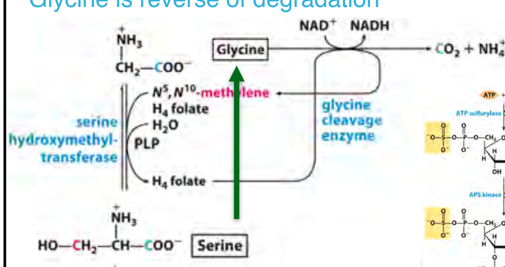
- Oxidation → transamination → dephosphorylation to yield serine

Biosynthesis Amino Acids & Nucleotides

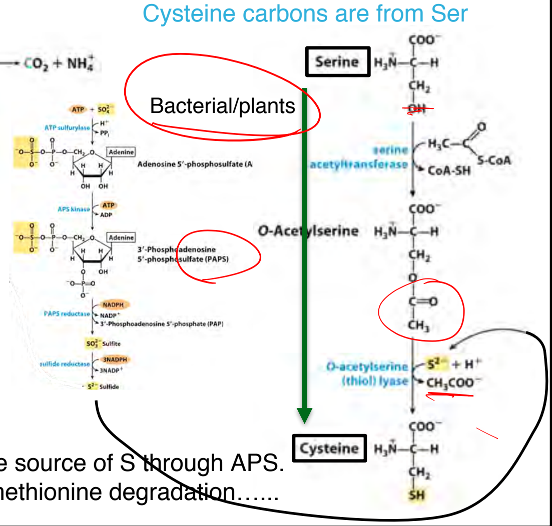
3-PGA Family
From Essential Family

Serine contributes to Glycine and Cysteine

Glycine is reverse of degradation



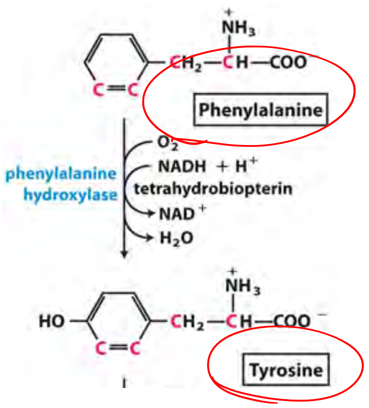
Cysteine carbons are from Ser

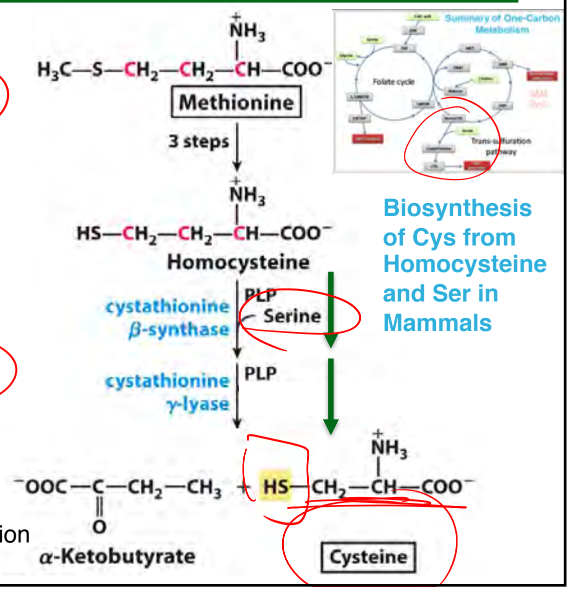


In bacteria and plants, sulfates are the source of S through APS.
In mammals, sulfur is recycled from methionine degradation……

Biosynthesis Amino Acids & Nucleotides

From Essential Family





Biosynthesis of Cys from Homocysteine and Ser in Mammals

The sulfur for Cysteine and Tyrosine come from Essential Amino Acids

via same enzymes used in degradation