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

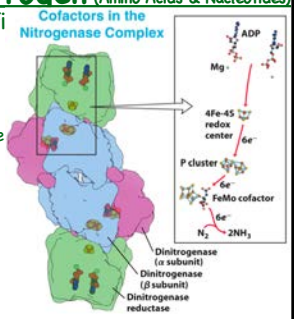
ANABOLISM I: Carbohydrates
PHOTOSYNTHESIS:
Exam-4 material
Overview;; Key experiments:
Light Reactions
Reaction center
Photosystems (PSII & PSI – NADPH)
Proton Motive Force – ATP
Carbon Assimilation – Calvin Cycle
Overview and regulation
C4 versus C3 plants
Krebs's cycle – glyoxylate
Carbohydrate Biosynthesis in Animals
Gluconeogenesis
Glycogen Synthesis
Pentose-Phosphate Pathway
oxidative-NADPH
non-oxidative-Ribose 5-P
Regulation of Carbohydrate Metabolism
Anaplerotic reactions

ANABOLISM II: Lipids

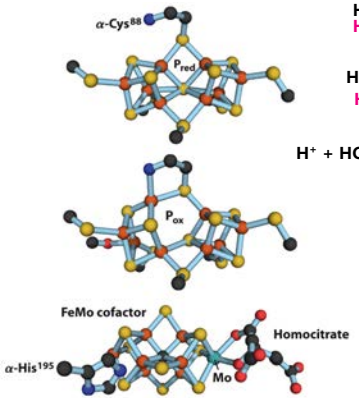
Fatty Acids
contrasts
location & transport
Synthesis: ACC & fatty acid synthase
Control of fatty acid metabolism
Diversification of fatty acids
elongation
desaturation
Eicosanoids
Prostaglandins and Thromboxane
Triacylglycerides
Membrane lipids
Glycerophospholipids
Isoprene lipids:
Ketone body synthesis
Cholesterol

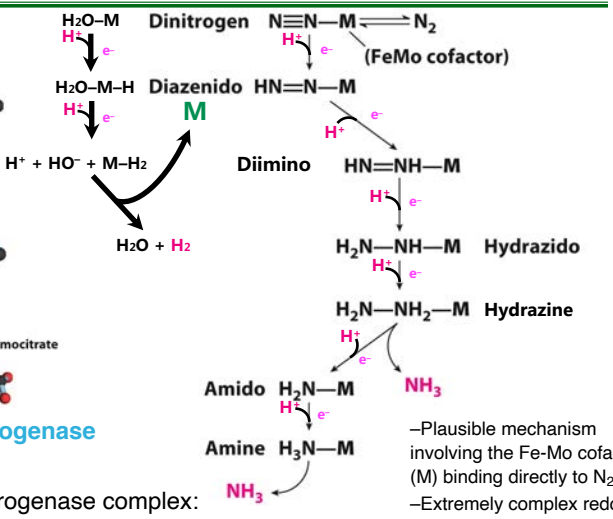
ANABOLISM III: Nitrogen (Amino Acids & Nucleotides)

Nitrogen cycle - Nitrogen fi
nitrogenase
Nitrogen assimilation
Plants
Nitrate/nitrite reductase
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





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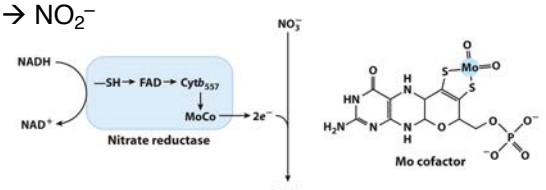
6) Biosynthesis and degradation of heme; other 2° products of amino acids

Biosynthesis Amino Acids & Nucleotides

Two Important Enzymes in Nitrogen Assimilation by PLANTS

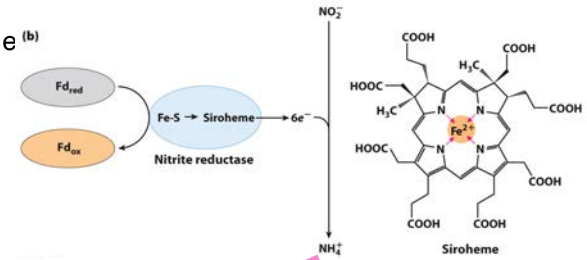
1. ***Nitrate reductase*** $NO_3^- + 2\ e^- \rightarrow NO_2^-$

- large, soluble protein
- contains novel Mo cofactor
- e^- from NADH



2. ***Nitrite reductase*** $NO_2^- + 6\ e^-$ [(b)]

- found in chloroplasts in plants: e^- comes from ferredoxin (F_d)
- in non-photosynthetic microbes: e^- comes from NADPH



Amino acids

Biosynthesis Amino Acids & Nucleotides

Problem: need to get new ammonia onto the α-carbon for transamination

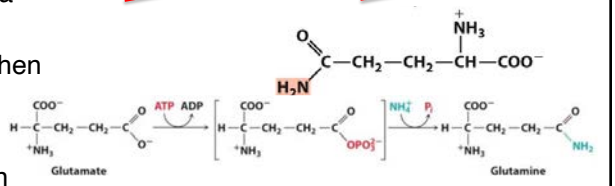
Ammonia is Incorporated into Biomolecules in PLANTS & ANIMALS Through Glu and Gln in 2 steps.

- Glutamine is made from Glu by glutamine synthetase in a two-step process (we discussed this previously when moving ammonia from extrahepatic tissues).
- Glutamate is made from Gln and α-Ketoglutarate by glutamate synthase (GOGAT) α-Ketoglutarate, an intermediate of the citric acid cycle, undergoes reductive amination with glutamine as nitrogen donor.

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^+



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

α-Ketoglutarate + $^{+}NH_4$ + ATP + NADPH + H^+ → glutamate + $NADP^+$ + ADP + P_i

Assimilation!

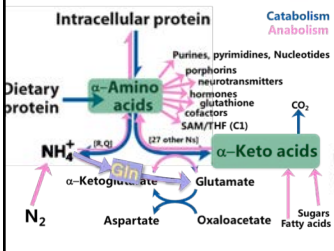
But, let's discuss GlySynthease….

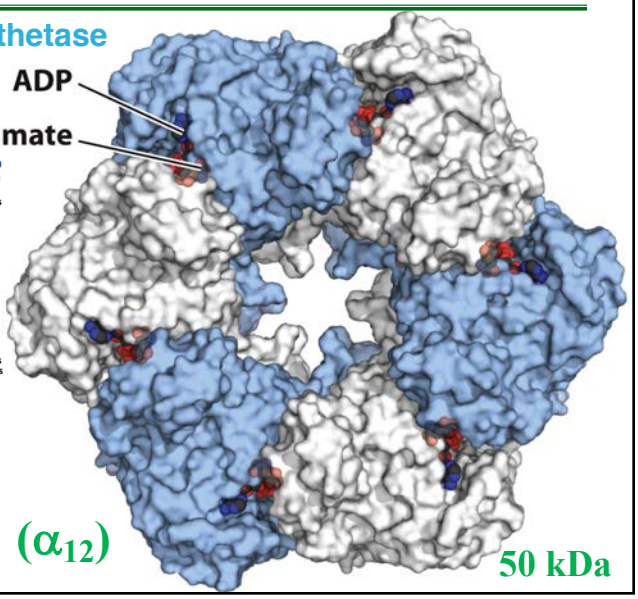
(An alternative name for this enzyme, glutamate:oxoglutarate aminotransferase, yields the acronym GOGAT, by which the enzyme also is known.)

Biosynthesis Amino Acids & Nucleotides

What about GOGAT….

Structure of Gln Synthetase





(α_{12})

50 kDa

Biosynthesis Amino Acids & Nucleotides

GOGAT, and many other Gln Amidotransferases have similar mechanisms:

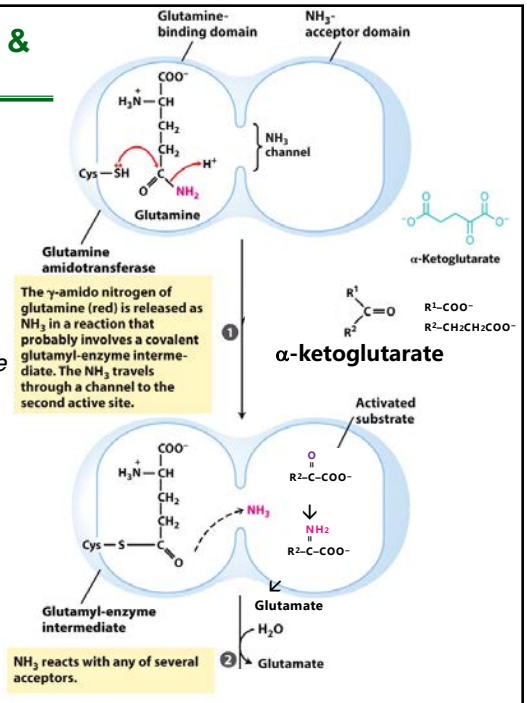
- There are two domains:
  - one binds Gln
  - other is amino group acceptor and binds substrate

Cys acts as nucleophile to cleave amide bond of Gln

→Forms glutamyl-enzyme intermediate

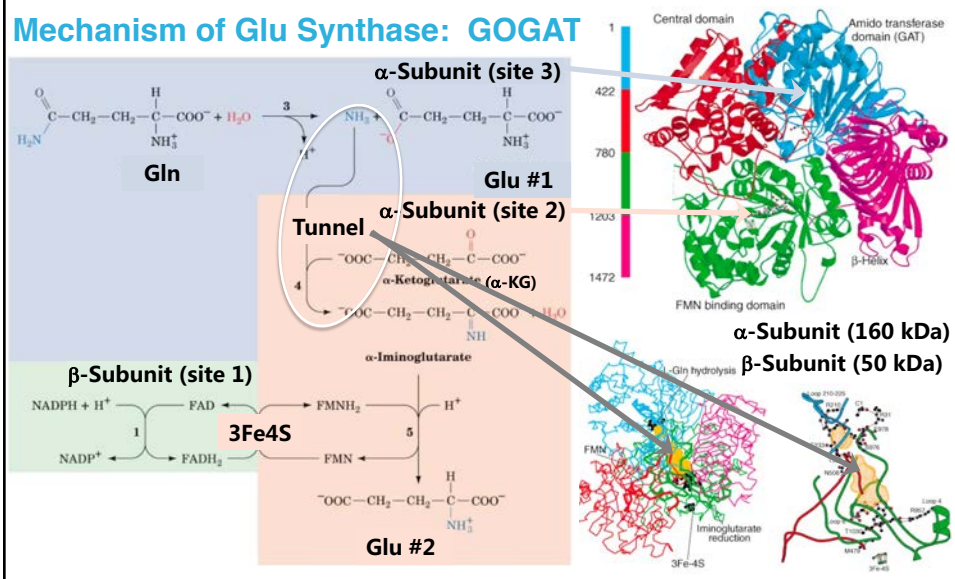
→NH_4 goes thru channel

- Then second substrate binds to accept NH_4
- In the case of GOGAT, there is another subunit to provide for the reduction



Biosynthesis Amino Acids & Nucleotides

Mechanism of Glu Synthase: GOGAT

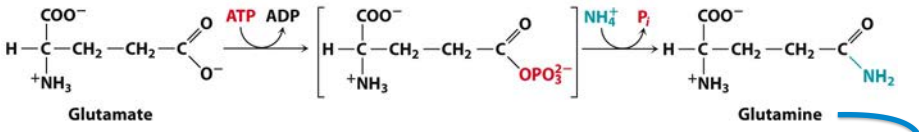


α-Ketoglutarate + glutamine + NADPH + H^+ → 2 glutamate + $NADP^+$

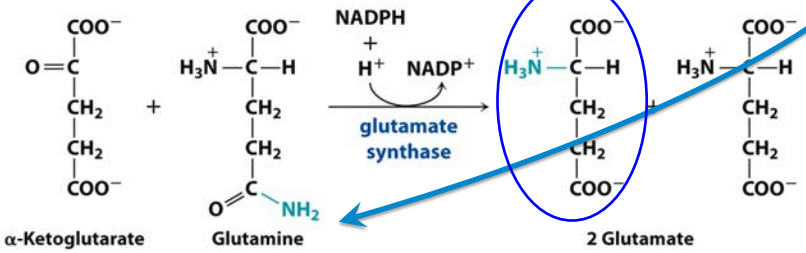
Biosynthesis Amino Acids & Nucleotides

Ammonia assimilation

- Glutamine Synth*et*ase



- Glutamate Synthase: GOGAT



Net: α-ketoglutarate + $^{+}NH_4$ → Glutamate (ATP and NADPH needed)

ANABOLISM III: Biosynthesis Amino Acids & Nucleotides

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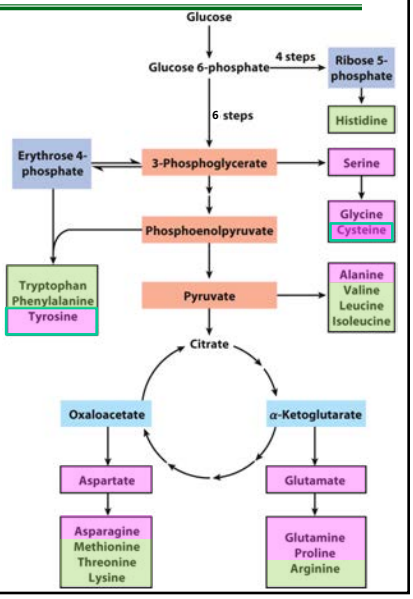
Biosynthesis Amino Acids & Nucleotides

Amino Acid Synthesis Overview

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



Biosynthesis Amino Acids & Nucleotides

Amino Acid Biosynthesis

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

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

Biosynthesis Amino Acids & Nucleotides

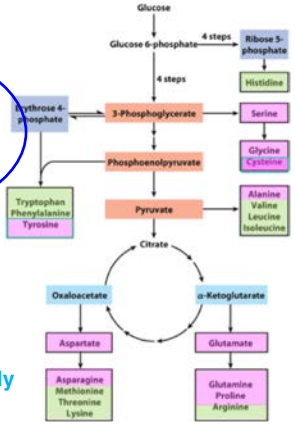
Non-essential Amino acids:

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

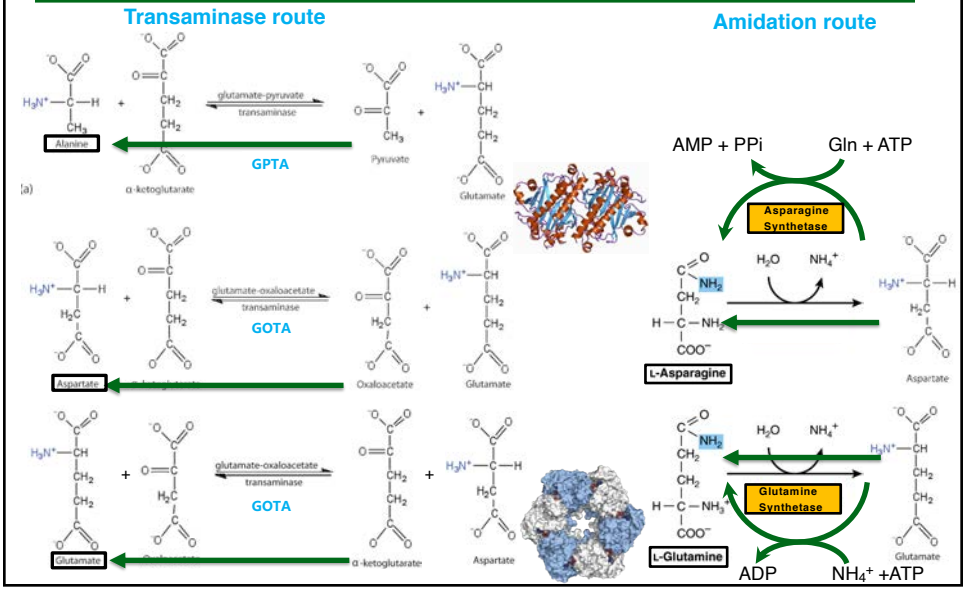
Arg-Val-His-Ile-Leu-Lys-Met-Phe Thr-Trp
Professor A.V.HILL M.P. was a Tea Totaller

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

Red=biosynthesis specific Green=essential

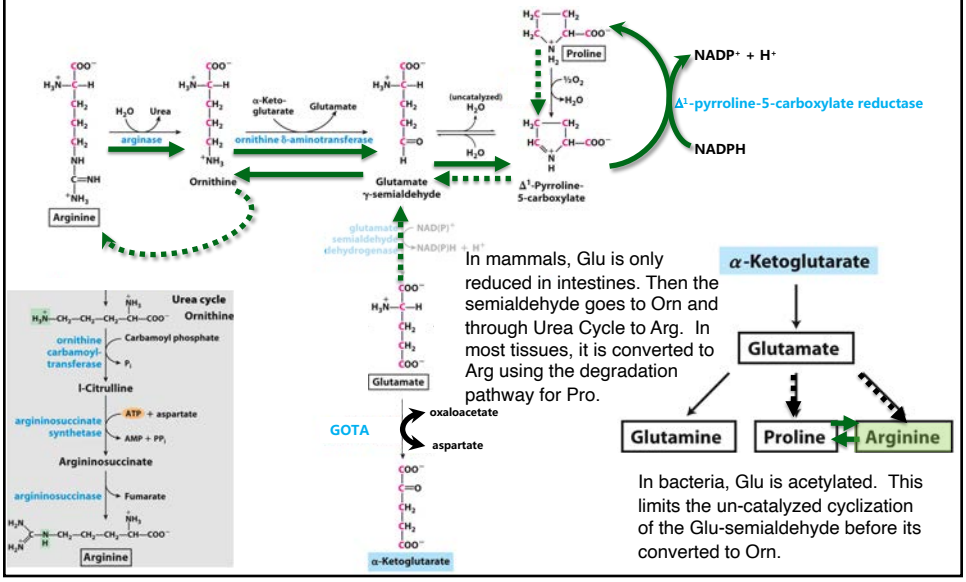


Biosynthesis Amino Acids & Nucleotides



Biosynthesis Amino Acids & Nucleotides

Glu Family: Pro (Arg)



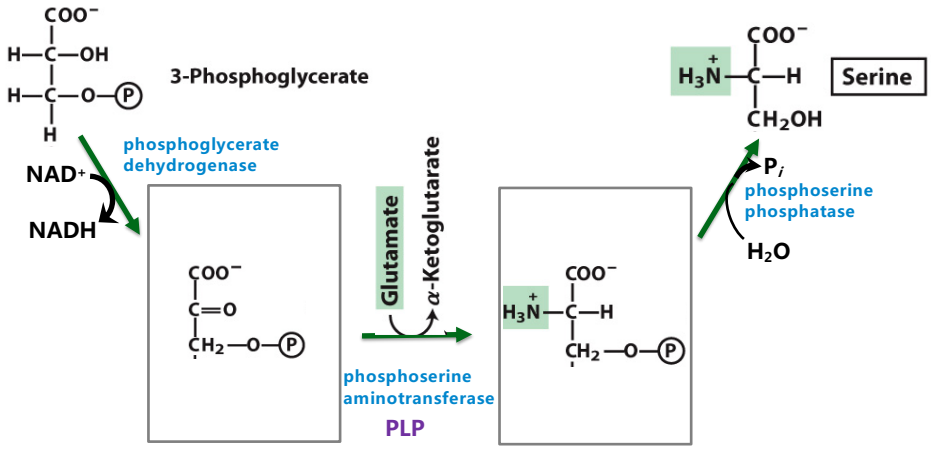
In mammals, Glu is only reduced in intestines. Then the semialdehyde goes to Orn and through Urea Cycle to Arg. In most tissues, it is converted to Arg using the degradation pathway for Pro.

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



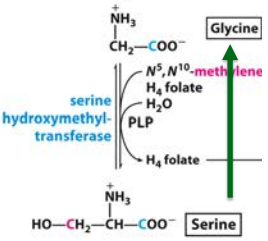
• Oxidation → transamination → to yield serine → dephosphorylation

Biosynthesis Amino Acids & Nucleotides

3-PGA Family +
From Essential Family: Gly, Cys

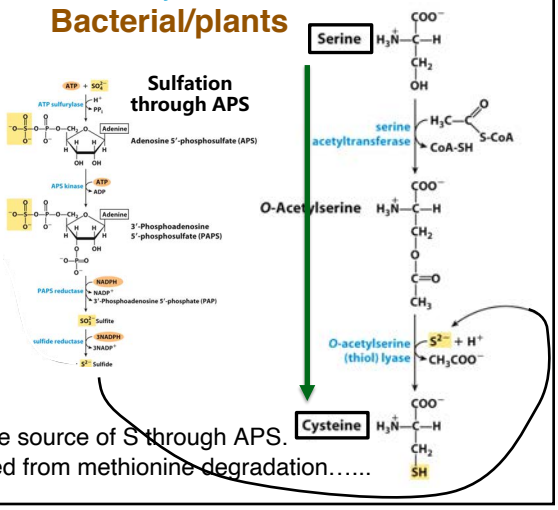
Serine contributes to Glycine and Cysteine

Glycine is reverse of degradation



Cysteine carbons are from Ser

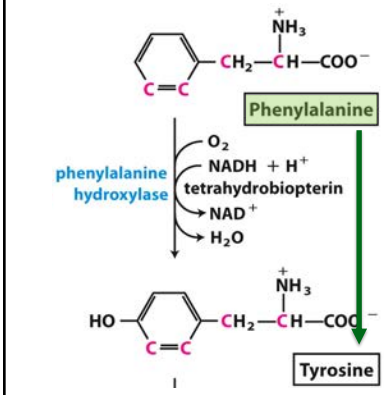
Bacterial/plants

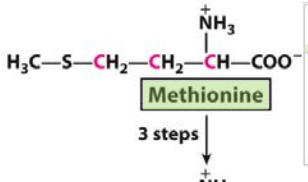


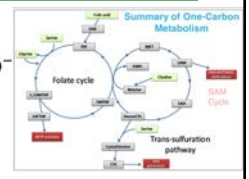
In bacteria and plants, sulfates are the source of S through APS.
In mammals, the Cys-sulfur is recycled from methionine degradation……

Biosynthesis Amino Acids & Nucleotides

3-PGA Family +
From Essential Family: Cys, Tyr







Biosynthesis of Cys from Homocysteine and Ser in **Mammals**

The sulfur for Cysteine and nearly all of Tyrosine come from Essential Amino Acids

via same enzymes used in degradation

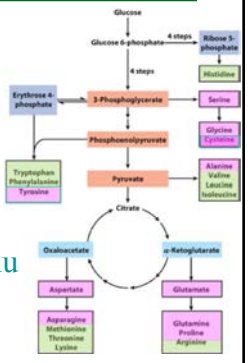
Biosynthesis Amino Acids & Nucleotides

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:

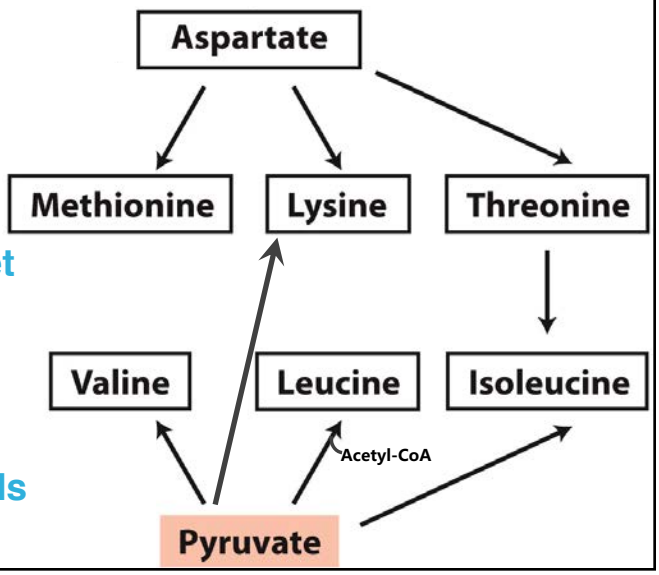
These require many steps and unique to those used for degradation.



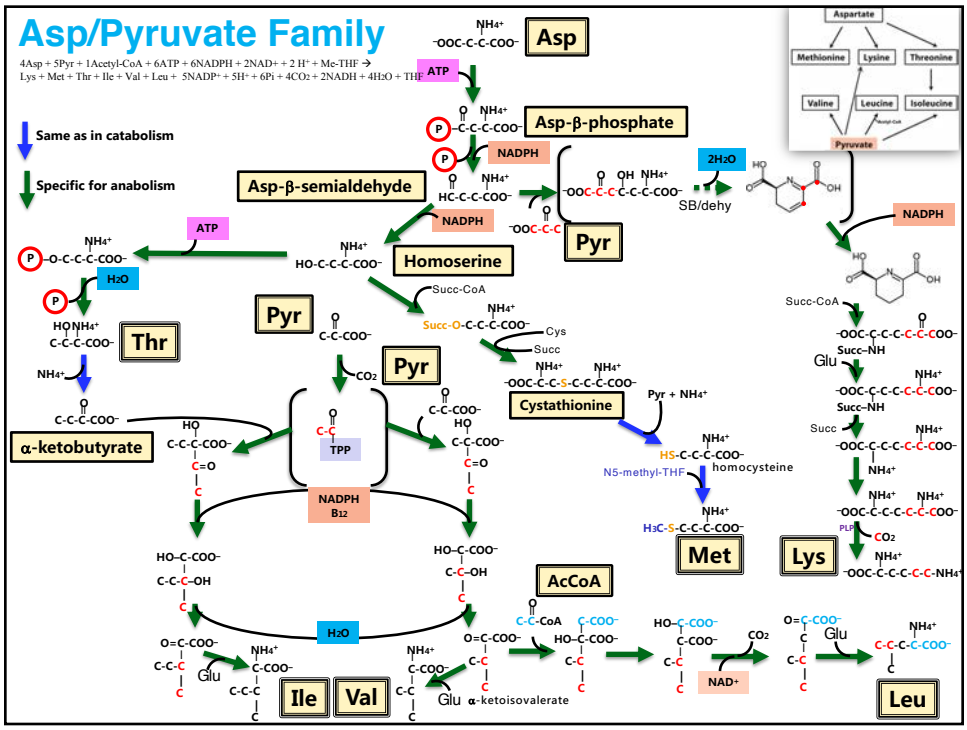
Asp/Pyruvate Family	Met	7	–Asp/Cys/THF/Glu
	Thr	5	–Asp/Glu
	Lys	9	–Asp/Pyr/Glu
	Ile	10	–Asp(Thr)/Pyr/Glu
	Val	4	–Pyr/Glu
	Leu	7	–Pyr/AcCoA/Glu
Aromatic Family	Phe	10	–E4P/PEP/Glu
	Trp	12	–E4P/PEP/Gln/R5P/Ser
Histidine	His	10	–R5P/ATP/Gln/Glu

Biosynthesis Amino Acids & Nucleotides

Asp/Pyruvate Family: Lys, Met, Thr, Ile, Val, Leu



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


Asp/Pyruvate Family
4Asp + 5Pyr + 1Acetyl-CoA + 6ATP + 6NADPH + 2NAD+ + 2 H+ + Me-THF →
Lys + Met + Thr + Ile + Val + Leu + 5NADP+ + 5H+ + 6Pi + 4CO2 + 2NADH + 4H2O + THF
Same as in catabolism
Specific for anabolism
Asp
ATP
Asp-β-phosphate
NADPH
Asp-β-semialdehyde
NADPH
Homoserine
ATP
H2O
Thr
α-ketobutyrate
Pyr
Pyr
TPP
NADPH
B12
Succ-CoA
Cystathionine
Cys
Succ
Pyr + NH4+
N5-methyl-THF
homocysteine
Met
2H2O
SB/dehy
Pyr
NADPH
Succ-CoA
Glu
Succ
Lys
PLP
CO2
AcCoA
H2O
Ile
Val
Glu
α-ketoisovalerate
NAD+
Glu
Leu
Aspartate
Methionine
Lysine
Threonine
Valine
Leucine
Isoleucine
Pyruvate