OUTLINE: BB 422/622

Introduction and review
Transport
Glycogenolysis
Glycolysis
Other sugars
Pasteur: Anaerobic vs Aerobic
Fermentations

Exam-1 material

Pyruvate
Krebs' Cycle
Oxidative Phosphorylation
    Electron transport
    Chemiosmotic theory: Phosphorylation

Exam-2 material

Fat Catabolism
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

Exam-3 material

ANABOLISM I: PHOTOSYNTHESIS:
Overview and Key experiments:
Light Reactions
    energy in a photon/pigments
    Reaction center &Photosystems (PSII & PSI)
Proton Motive Force – ATP
Carbon Assimilation – Calvin Cycle
    Rubisco/Oxygenase (Glycolate cycle)
    remaking Ru $1,5P_2$
Overview and regulation
C4 versus C3 plants
Kornberg cycle - glyoxylate
Carbohydrate Biosynthesis in Animals
    precursors/Cori cycle
Gluconeogenesis
    reversible steps
    irreversible steps – four
Glycogen Synthesis
    UDP-Glc/Glycogen synthase/branching
Pentose-Phosphate Pathway
    oxidative-NADPH
    non-oxidative-Ribose 5-P
Regulation of Carbohydrate Metabolism
Anaplerotic reactions
Biosynthesis of Fatty Acids
    contrasts
    location & transport
    synthesis
        ACC
        FAS; ACP priming; 4 steps
        Control
Diversification of fatty acids
    elongation
    desaturation
    Eicosanoids
        Prostaglandins and Thromboxane

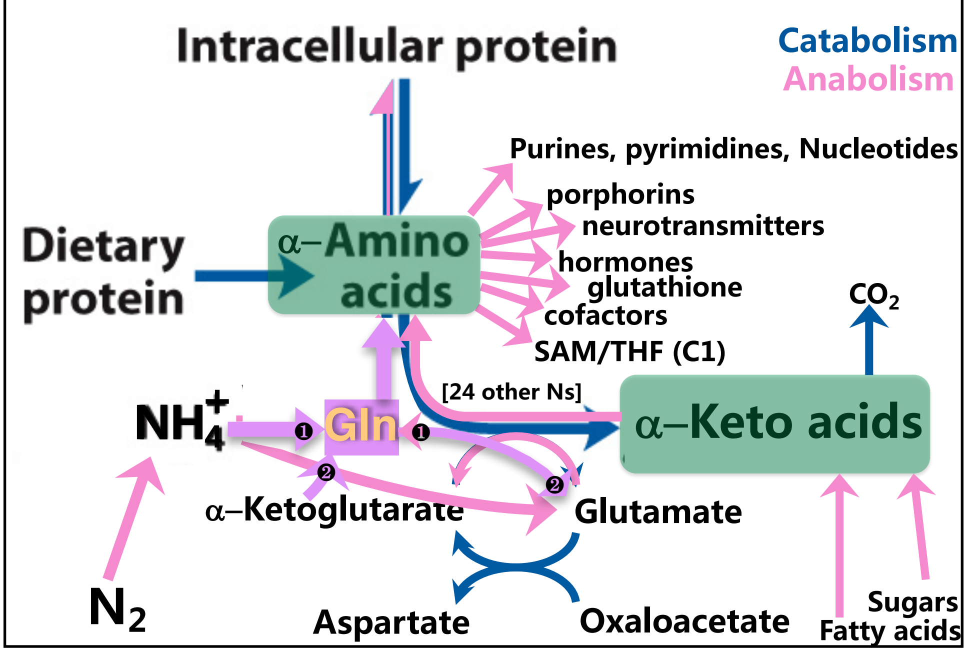
Exam-4 material

ANABOLISM II:
Biosynthesis of Fatty Acids and Lipids
- Fatty Acids
- Triacylglycerides
- Membrane lipids
    - Glycerophospholipids
    - Sphingolipids
    - Isoprene lipids: **Cholesterol**
        - Ketone body synthesis
        - Mevalonate
        - **Cholesterol**
            - bile acids
            - steroids
            - metabolism
            - control of cholesterol biosynthesis

ANABOLISM III:
Biosynthesis of Amino Acids and Nucleotides
- Nitrogen fixation
    - nitrogenase
- Nitrogen assimilation
- Amino-acid Biosynthesis
- Nucleotide Biosynthesis
- Control of nitrogen metabolism
- Biosynthesis of secondary products of amino acids

Exam-5 material

Biosynthesis Amino Acids & Nucleotides



Biosynthesis Amino Acids & Nucleotides

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

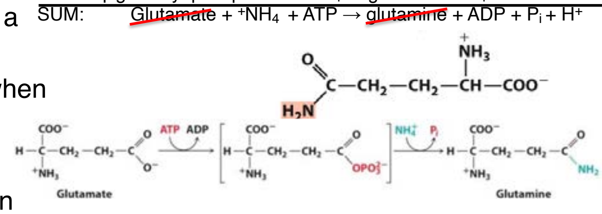
Ammonia is Incorporated into Biomolecules in <u>PLANTS & ANIMALS</u> Through Glu and Gln in 2 steps.

- 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!

First, let's discuss Glu-Synthetase….

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

Gln Amidotransferases

have similar mechanisms: GOGAT is like many others that transfer the amino group from the amide of Gln to keto functions.

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

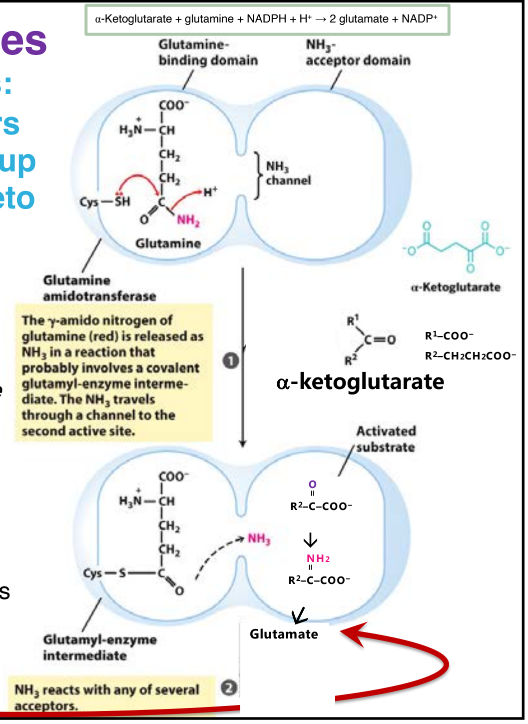
- There are two domains:
  - one binds Gln
  - other is amino group acceptor and binds substrate

Cys acts as nucleophile to cleave amide bond of Gln

→Forms glutamyl-enzyme intermediate

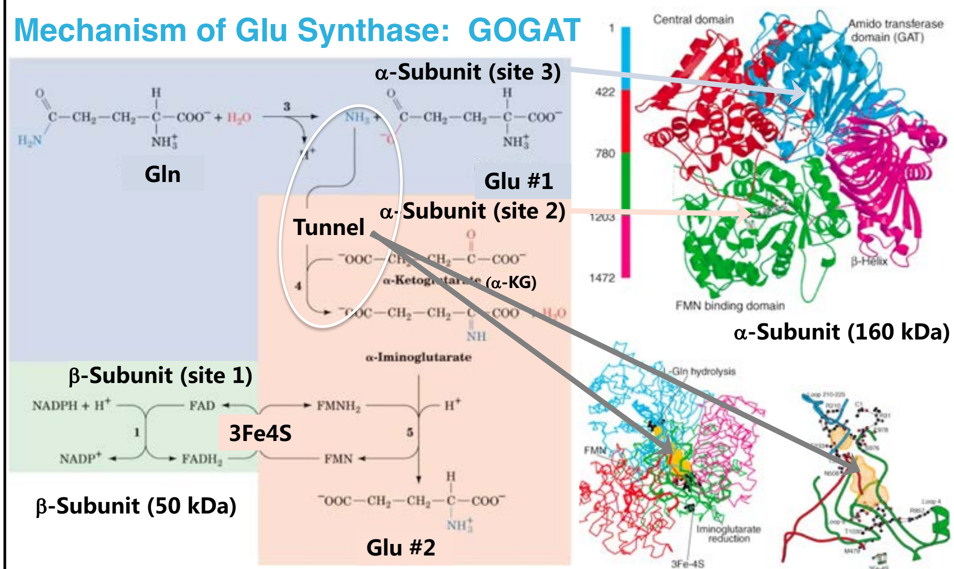
→NH_4 goes thru channel

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



Biosynthesis Amino Acids & Nucleotides

Mechanism of Glu Synthase: GOGAT



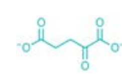
α-Ketoglutarate + glutamine + NADPH + H^+ → 2 glutamate + $NADP^+$

Gln Amidotransferases

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

have similar mechanisms: GOGAT is like many others that transfer the amino group from the amide of Gln to keto functions (usually).

For GOGAT:



α-ketoglutarate

R^1–C(=O)–R^2

R^1–COO^-

R^2–$CH_2CH_2COO^-$

"keto-containing"

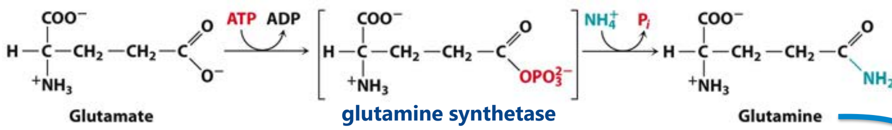
Substrate	Enzyme	Substituents
α-ketoglutarate	Glu synthase (GOGAT)	at C=O; R^1=COOH, R^2=propionate
Aspartate	Asn synthetase	at C=O; R^1 =OH, R^2 =2-aminopropionate
CO_2	Carbamoyl-P synthetase	at C=O; R^1 =OH, R^2 =O-phosphate
Chorismate	Anthranilate synthase	at C=C; R^1 =H, R^2 =Chorismate-aromatic
PRFI-AICAR	Gln amidotransferase	at C=O; R^1 =H, R^2 =1-amino-ribulosyl-5-P
PRPP	Gln-PRPP amidotransferase	at C–OPP_i; R^1 & R^2 =ribosyl-5-P
FGAR	Gln-FGAR amidotransferase	at C=O; R^1 =CH_2-NH-CHO, R^2 =N-ribosyl-P
XMP	Gln-XMP amidotransferase	at C=O; R^1 =N-XMP, R^2 =N-XMP
UTP	Cytidylate synthase	at C=O; R^1 =C-UTP, R^2 =N-UTP

Proposed mechanism for glutamine amidotransferases. Each enzyme has two domains. The glutamine-binding domain contains structural elements conserved among all of these enzymes, including a Cys residue required for activity. The NH_3-acceptor (second substrate) domain varies. These enzymes, which include carbamoyl-phosphate synthetase (initiates the urea cycle), are important in many amino-acid and nuceotide biosynthetic pathways.

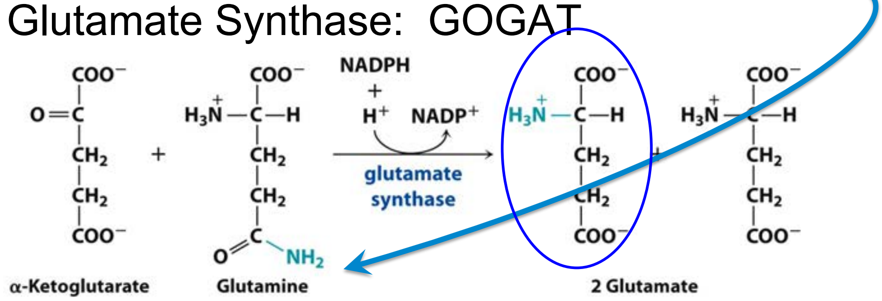
Biosynthesis Amino Acids & Nucleotides

Ammonia assimilation

- Glutamine Synth*et*ase



- Glutamate Synthase: GOGAT



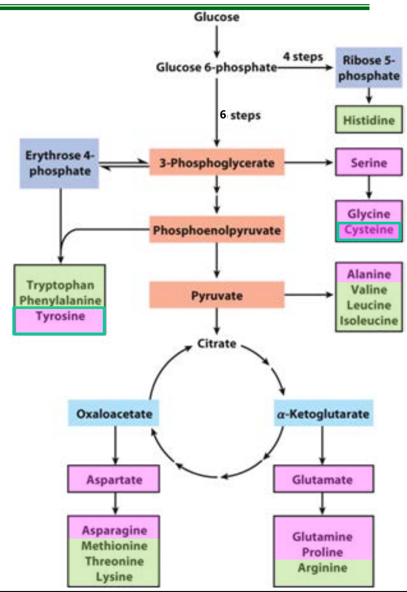
Net: α-ketoglutarate + $^{+}NH_4$ → Glutamate (ATP and NADPH needed)

ANABOLISM III: Biosynthesis Amino Acids & Nucleotides

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

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 Total-er

Biosynthesis Amino Acids & Nucleotides

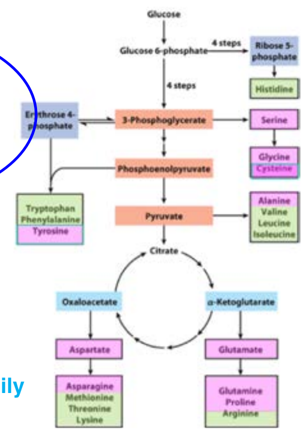
Non-essential Amino acids:

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
[Arg	3(1)	(✓)	Pro]	
Ser	3	–	3PGA	3-PGA Family
Gly	1	✓	Ser	
Cys	2	✓	Ser/Met	From Essential Family
Tyr	1	✓	Phe	

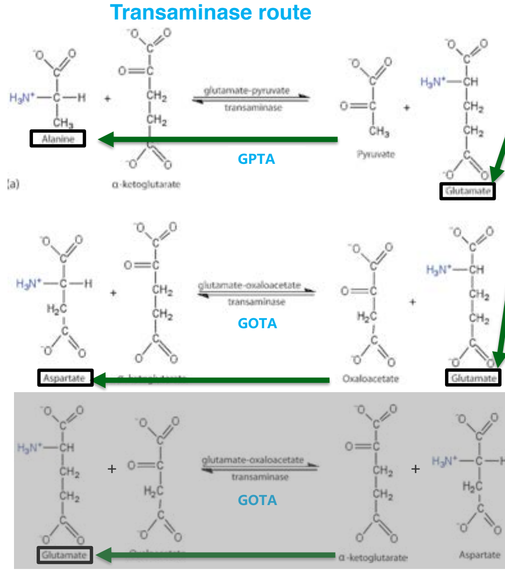
Red=biosynthesis specific Green=essential

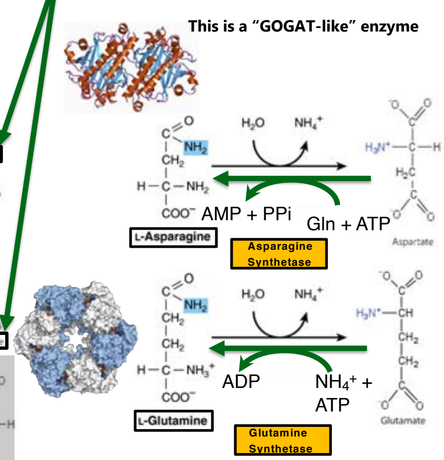


Biosynthesis Amino Acids & Nucleotides

Ala, Asp, Glu, Gln, Asn

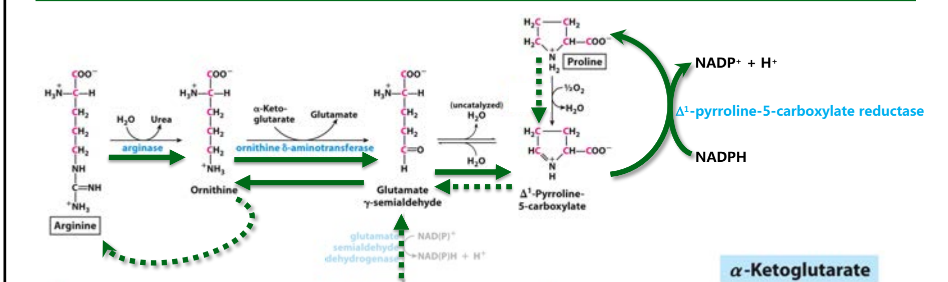
Transaminase route GOGAT Amidation route

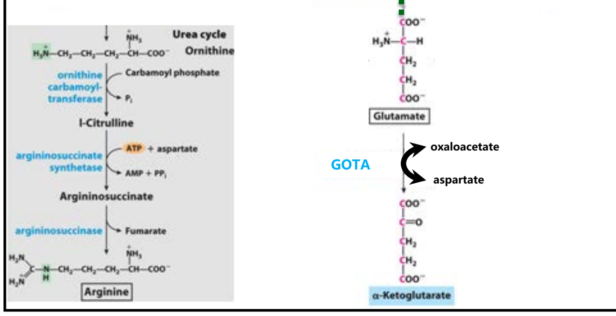


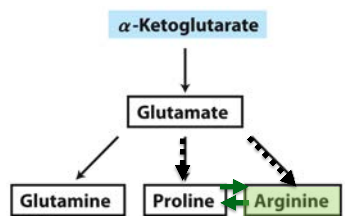


Biosynthesis Amino Acids & Nucleotides

Glu Family: Pro (Arg)



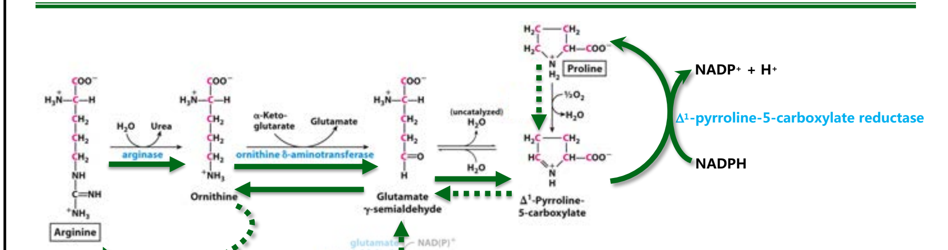


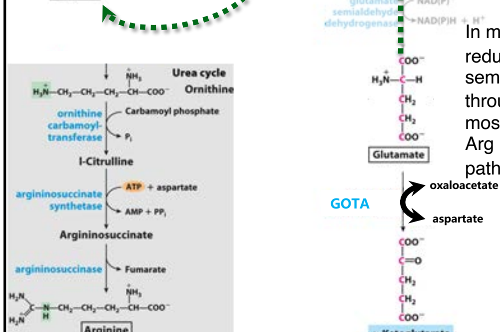


In bacteria, Glu is acetylated. This limits the un-catalyzed cyclization of the Glu-semialdehyde before its converted to Orn.

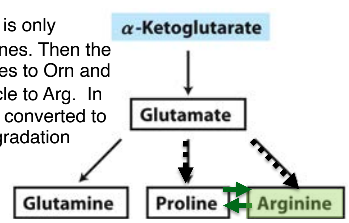
Biosynthesis Amino Acids & Nucleotides

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.

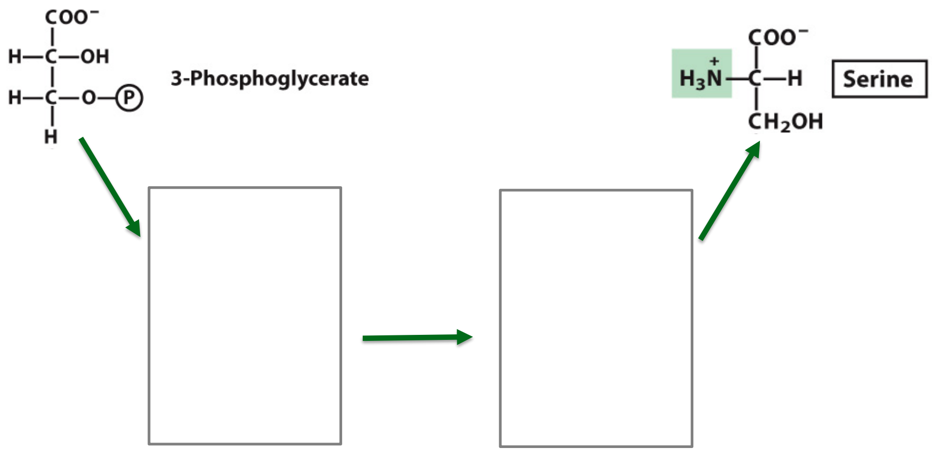


In bacteria, Glu is acetylated. This limits the un-catalyzed cyclization of the Glu-semialdehyde before its converted to Orn.

Biosynthesis Amino Acids & Nucleotides

3-PGA Family: Ser

Serine Derives from 3-Phosphoglycerate of Glycolysis

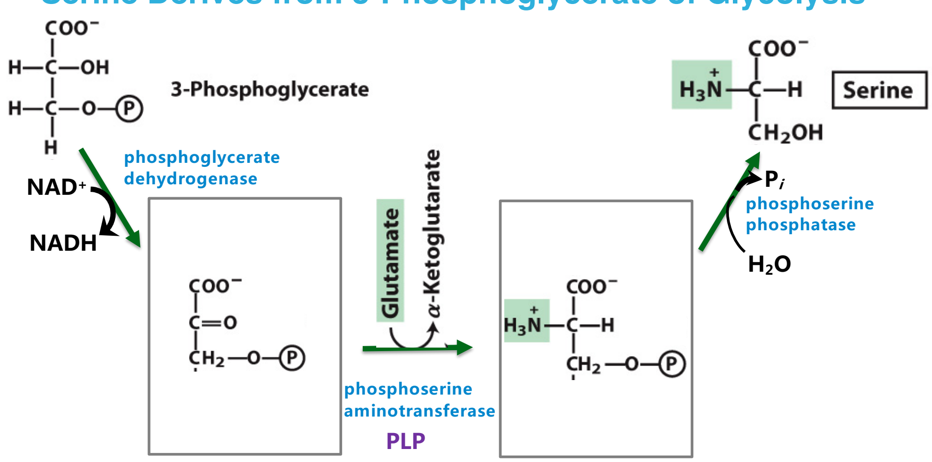


- Oxidation → transamination → dephosphorylation
  to yield serine

Biosynthesis Amino Acids & Nucleotides

3-PGA Family: Ser

Serine Derives from 3-Phosphoglycerate of Glycolysis



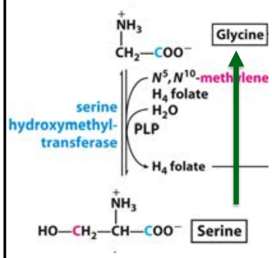
- Oxidation → transamination → dephosphorylation
  to yield serine

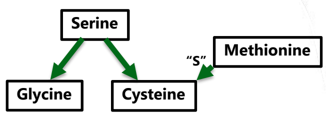
Biosynthesis Amino Acids & Nucleotides

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

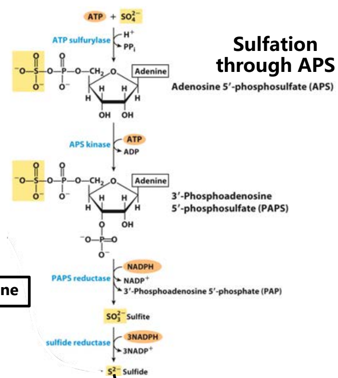
Serine contributes to Glycine and Cysteine

Glycine is reverse of degradation

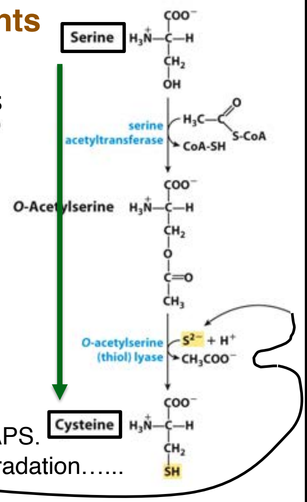




Bacterial/plants



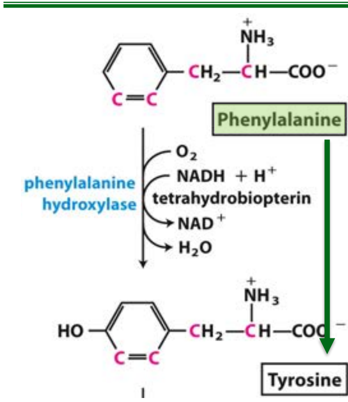
Cysteine carbons are from Ser

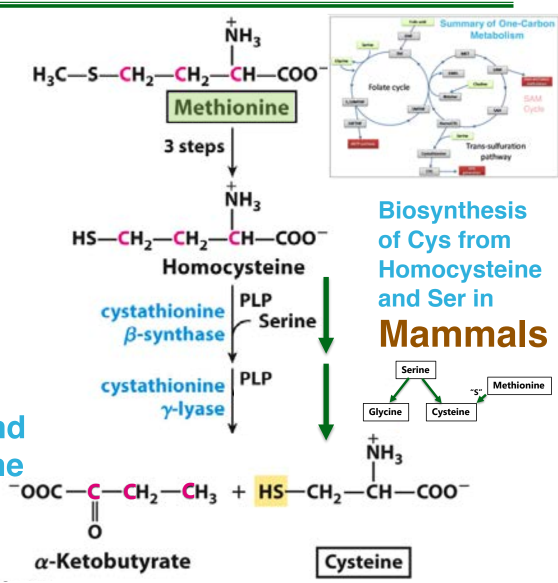


In bacteria and plants, sulfates are the source of S through APS.
In mammals, the Cys-sulfur is recycled from methionine degradation……

Biosynthesis Amino Acids & Nucleotides

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





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

via same enzymes used in degradati

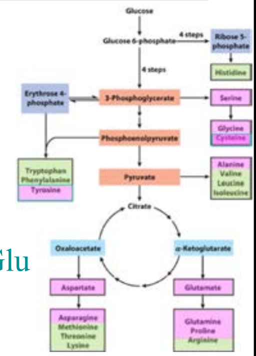
Biosynthesis Amino Acids & Nucleotides

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.

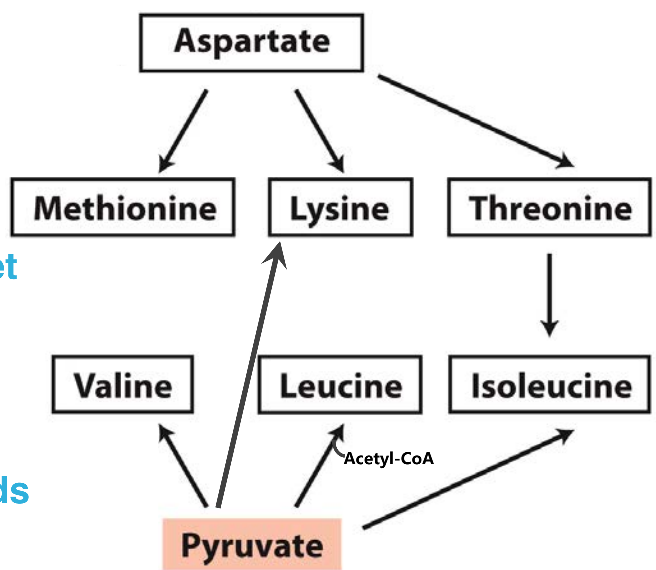


Family	Amino acid	Steps	Precursors
Asp/Pyruvate Family	Met	7	–Asp/Cys/THF/Glu
	Thr	5	–Asp/Glu
	Lys	9	–Asp/Pyr/Glu
	Ile	10	–Asp(Thr)/Pyr/Glu
	Val	4	–Pyr/Glu
	Leu	7	–Pyr/AcCoA/Glu
Aromatic Family	Phe	10	–E4P/PEP/Glu
	Trp	12	–E4P/PEP/Gln/R5P/Ser
Histidine	His	10	–R5P/ATP/Gln/Glu

Biosynthesis Amino Acids & Nucleotides

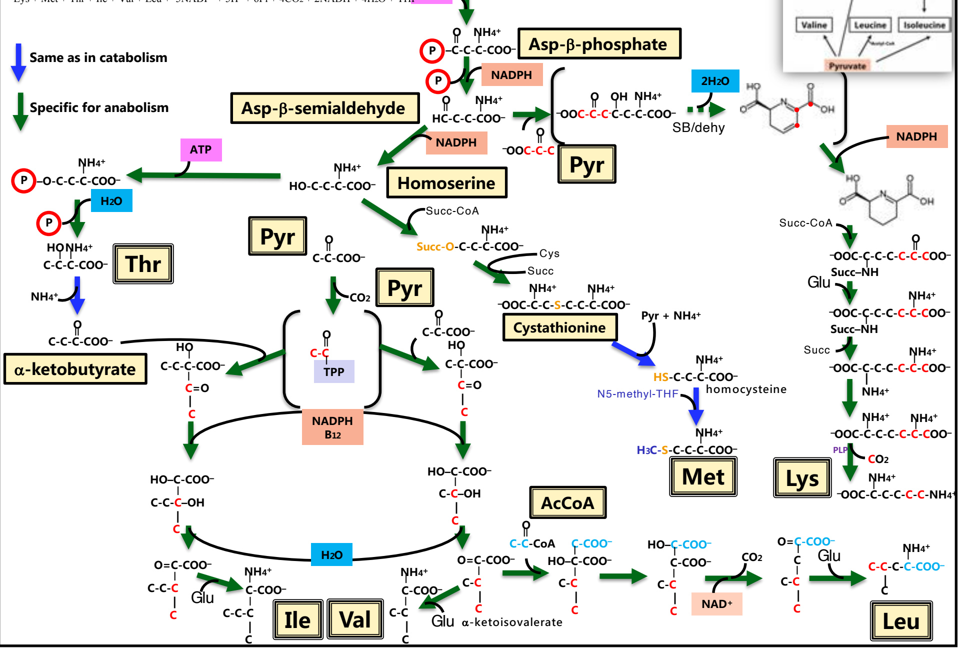
Asp/Pyruvate Family:
Lys, Met, Thr, Ile, Val, Leu

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



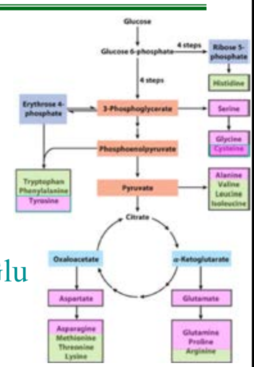
Biosynthesis Amino Acids & Nucleotides

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

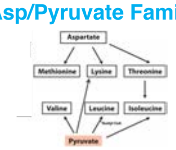
Red=biosynthesis specific *reverse of degradation

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.