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 1,5P2

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

ANABOLISM III:

Biosynthesis Amino Acids & Nucleotides

Biosynthesis Amino Acids & Nucleotides

- Nitrogen (after H, O, and C) is a major element of living organisms
- Most nitrogen is inert in the atmosphere as dinitrogen
- Making dinitrogen useful is not easy

Atmosphere is 80% N_2 , but is chemically inert

need $N_2 + 3\ H_2 \rightarrow 2\ NH_3$

Even though $\Delta G'° = -33.5$ kJ/mol… ***breaking a triple bond has high activation energy (i.e., SLOW, kinetically stable),***

this can be accomplished using non-biological processes:

N_2 and $O_2 \rightarrow$ NO via lightning

N_2 and $H_2 \rightarrow NH_3$ via the industrial *Haber-Bosch process*

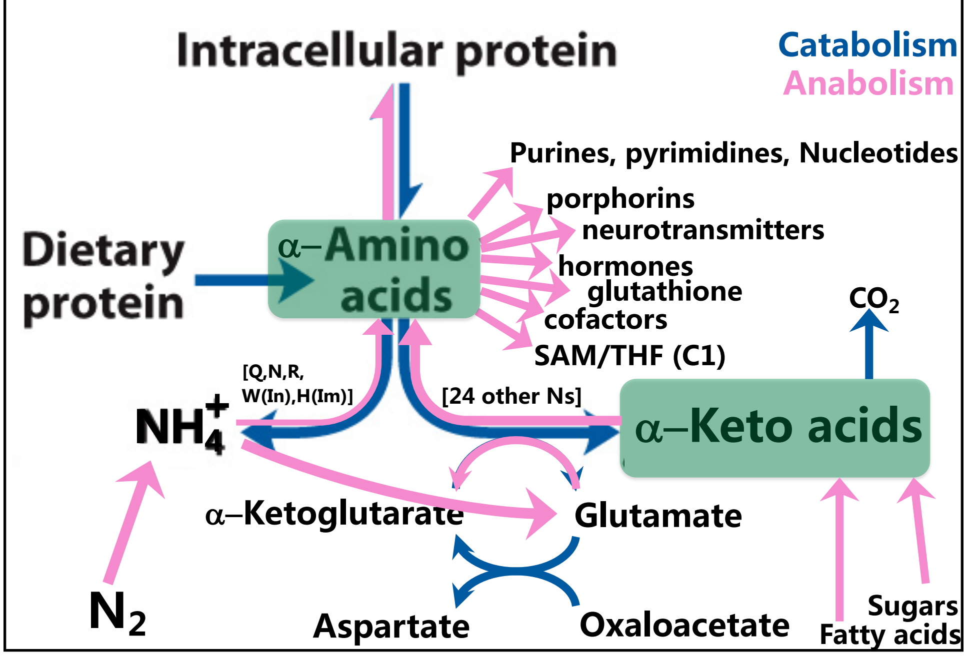
requires T>400 °C, P>300 atm

Industrial synthesis of NH_3 via the Haber process is one of mankind's most significant chemical processes.

Biosynthesis Amino Acids & Nucleotides

- Haber process

Biosynthesis Amino Acids & Nucleotides



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

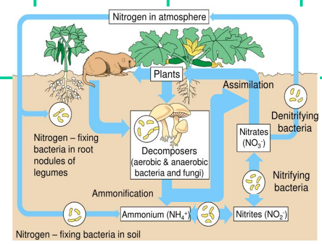
Biosynthesis Amino Acids & Nucleotides

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^{-6} • Nitrate • Also Nitric acid (HNO_3) and Dinitrogen pentoxide (N_2O_5) • "ate" is the higher oxidation state.	• NO_2 • N^{+4} O_2^{-4} • Nitrogen dioxide • gas	• NO_2^- • N^{+3} O_2^{-4} • Na-Nitrite • Also Nitrous acid (HONO) • "ite" is light on oxygen and oxidation state.	• NO • N^{+2} O^{-2} • Non-salt • Gas • Physio-logically important 2° messenger and paracrine signal	• N_2O • N^{+1} O^{-2} • Non-salt • gas	• N_2 • N^0 • Covalent triple bond • gas	• NH_3 • N^{-3} H_3^{+3} • NH_3: N has oxidation state of −3. • Ammonium cation salts gas

Biology of Molecular Nitrogen: The Nitrogen Cycle



Biosynthesis Amino Acids & Nucleotides

Nitrogen Fixation *versus* Nitrogen Assimilation

Nitrogen Fixation	Nitrogen Assimilation
• Converts N_2 to NH_3 • Requires multiple ATPs • Uses electrons from pyruvate	• Converts NO_3, NO_2, and/or NH_3 to **amino acids** • Uses electrons from NADH, NADPH, or photosynthetic transfer from ferrodoxin

Both:

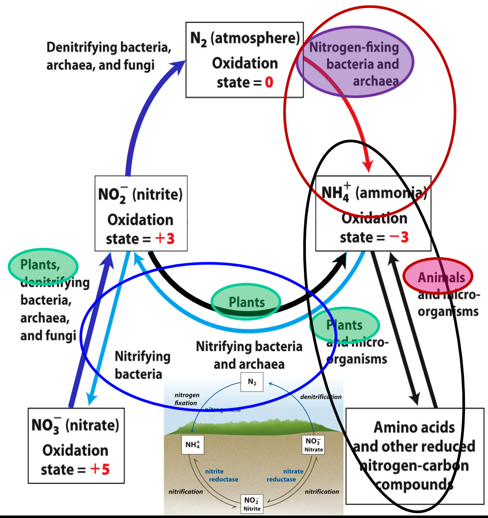
- Are electron transfer processes
- Use Mo cofactor
- Involve multiple redox cofactors, such as Fe-S, NADH, NADPH, ferrodoxin, flavodoxin, and so on

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.
   Animals can only do → 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

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

$$N_2 + 8\,H^+ + 8\,e^- + 16\,ATP + 16\,H_2O \rightarrow 2\,NH_3 + H_2 + 16\,ADP + 16\,P_i$$

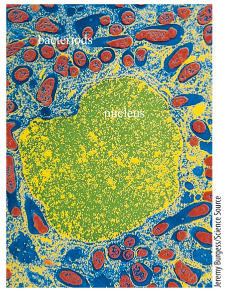
- Most are single-celled prokaryotes (archaea).
- Some live in symbiosis with *plants.*
  - (e.g., proteobacteria with legumes such as peanuts, beans)
- A few live in symbiosis with *animals.*
  - (e.g., spirochaete with termites)

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

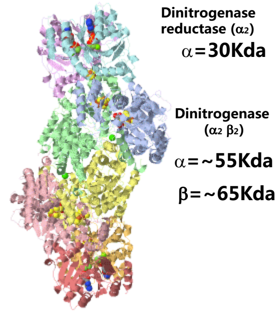
Like CO_2 fixation by Rubisco, oxygen can parasitize this process

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 the 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 $(60+240\ kDa)_2$ uses ATP to overcome the activation energy.
- Passes electrons to N_2 and catalyzes a step-wise reduction of N_2 to NH_3

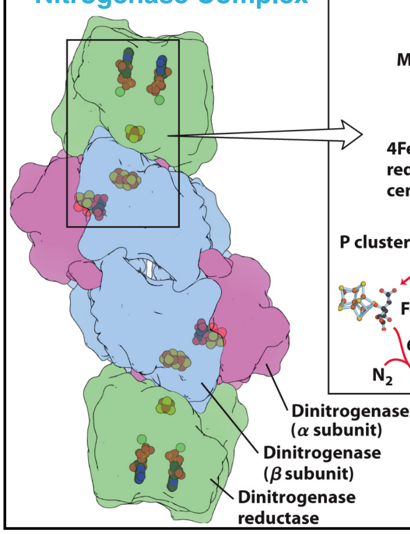
$$N_2 + 8\ H^+ + 8\ e^- + nATP \rightleftharpoons 2\ NH_3 + H_2 + nADP + nP_i$$

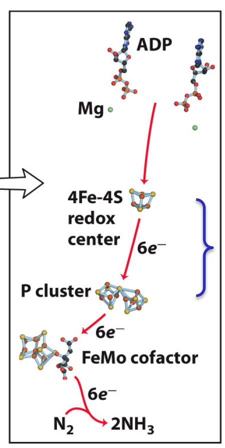
$$2\ NH_3 + 2\ H^+ \rightleftharpoons 2\ {}^+NH_4$$

About 16 ATP molecules are consumed per one N_2.

Biosynthesis Amino Acids & Nucleotides

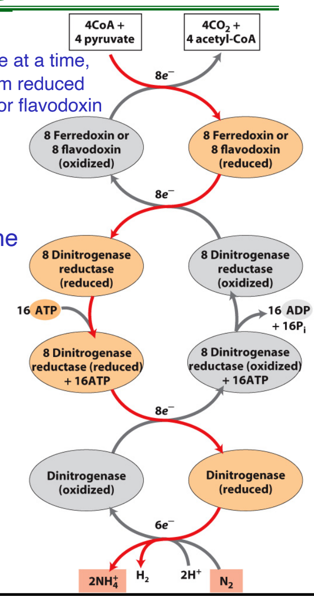
Cofactors in the Nitrogenase Complex





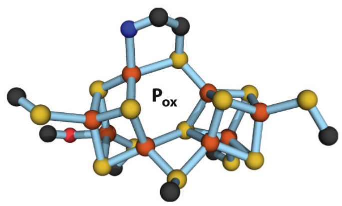
e– flow, one at a time, starting from reduced ferredoxin or flavodoxin

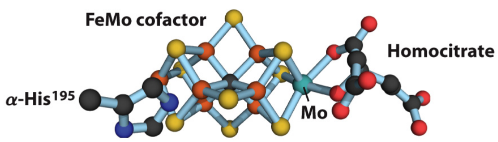
This is the critical step



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.

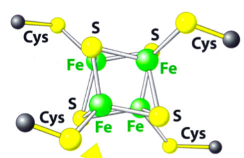
Biosynthesis Amino Acids & Nucleotides

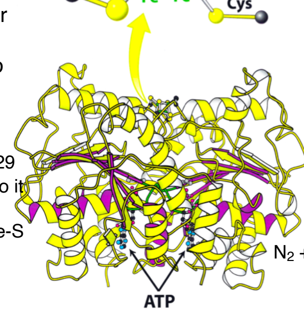
Dinitrogen Reductase

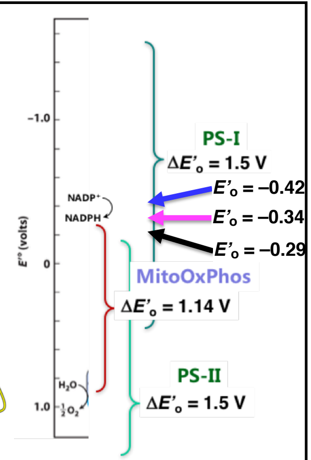
- Source of e^- varies between organisms.
  - often pyruvate → ferredoxin

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

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

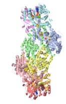
$= -0.34$ V – (–0.29 V)

$= -0.05$ V → +7 kcal/mole

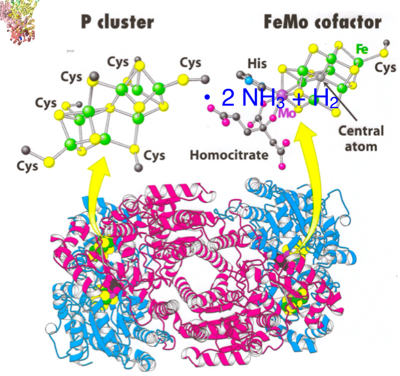
$= -0.34$ V – (–0.42 V)

$= +0.08$ V → –11 kcal/mole

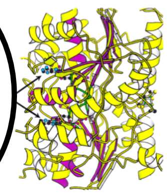
Biosynthesis Amino Acids & Nucleotides



Nitrogen Fixation by Dinitrogenase



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

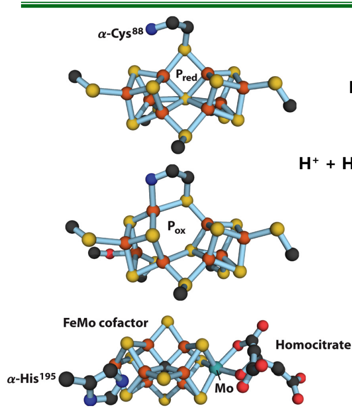


F_d (Fe^{3+})
+2 ADP+P_i

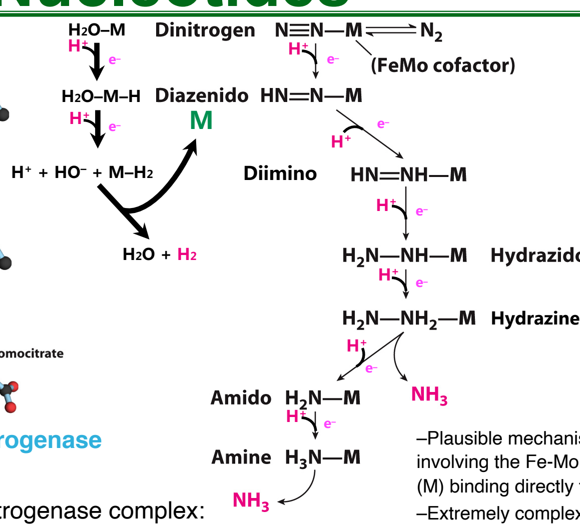
- Dinitrogenase catalyzes:
  - transfer of 6 e^- and 6 H^+ to nitrogen: formation of $2NH_3$ from N_2
  - transfer of 2 e^- to 2 protons: formation of H_2
  - ONE AT A TIME
- Has novel FeMo cofactor (or V in some organisms)
- The reduction of N_2 and protons occurs at FeMo cofactor.
- Formation of H_2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

It does this 8 times for completion of one N_2 reduction

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: $N_2 \rightarrow {}^{+}NH_4$

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3) Biosynthesis of amino acids
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5) Control of nitrogen metabolism

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Biosynthesis Amino Acids & Nucleotides

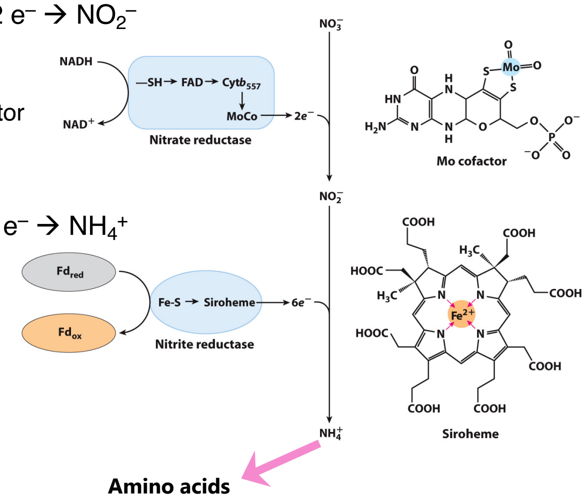
Two Important Enzymes in Nitrogen Assimilation by PLANTS

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



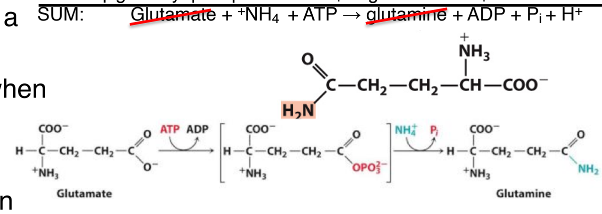
Biosynthesis Amino Acids & Nucleotides

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!

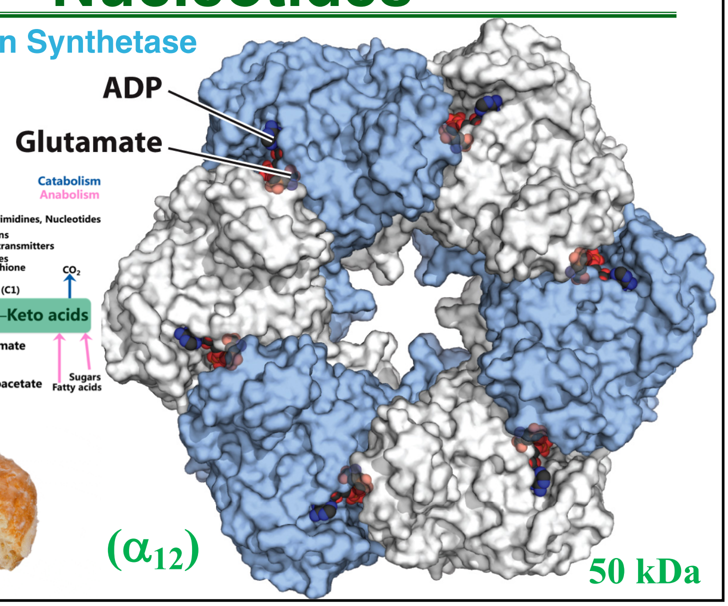
First, let's discuss Glu-Synthetase....

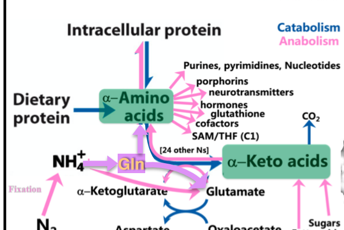
(An alternative name for this enzyme, glutamate:oxoglutarate aminotransferase, yields the acronym GOGAT, by which the enzyme also is known.)

Biosynthesis Amino Acids & Nucleotides

What about GOGAT....

Structure of Gln Synthetase



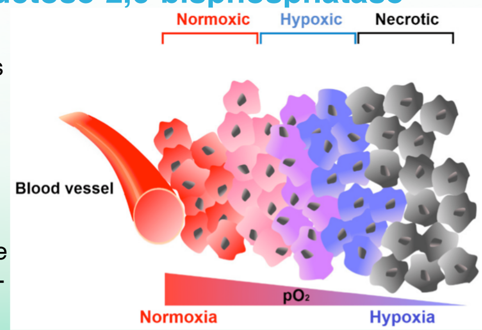


(α_{12})

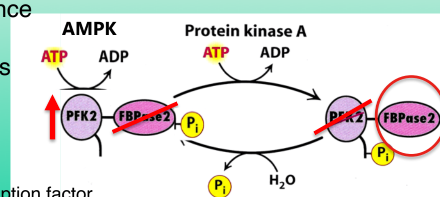
50 kDa

Clinical Correlations

6-phosphofructo-2-kinase/Fructose-2,6-bisphosphatase (PFK2/FBPase2) in Cancer



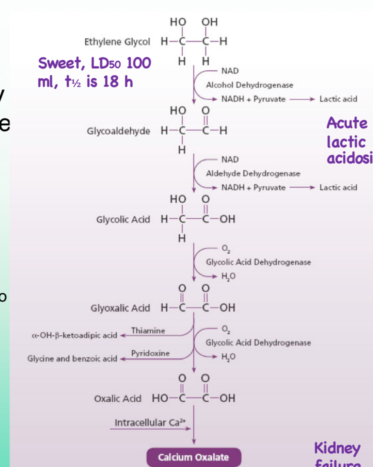
- Cancer cells learn how to avoid necrosis and apoptosis by fighting hypoxia.
- The generate most of their ATP via glycolysis: called the **Warburg Effect**
- How they increase glycolysis is due the the induction of a different isozyme of PFK2/FBPase2 that can favor the kinase when FBPase2 phosphorylated by AMP-dependent protein kinase (AMPK).
- So, in oxidative stress cancer cells experience hypoxia, which activates AMPK, and phosphorylation of PFK2/FBPase2 activates the kinase making much more Fru2,6P_2 for activating glycolysis.



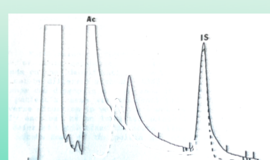
- The body fights cancer by making tumor- suppressors
- TIGAR is induced by p53, the tumor suppressor transcription factor.
- It evolved from the FBPase2 domain, and possesses activity that shuts down glycolysis in cancer cells
- Recently discovered activation of KHK-A, which acts as a protein kinase leading to activation of ROS fighting genes like superoxide dismutase and catalase

Clinical Correlations

Murder or Misdiagnosis?



- Poisoning with ethylene glycol: in 1937 a company Massingill made a elixir of sulfa-drug using ethylene glycol as an emulsifier
- 107 people died
- Prompted the Federal Government to for the Food and Drug Administration (FDA)



- In 1979, Ryan Stallings was brought to ER with vomiting, lethargy, and hyperventilation.
- Blood work revealed lactic acidosis and bicarb treatment saved him.
- But, lab tests revealed unusual component, that was identified as ethylene glycol. Ryan was put in protective custody.

- Foster parents brought Ryan back to ER 2 months later with same thing. Ryan died.
- Mother, Patricia, was arrested for 1st degree murder.
- While in custody, David and Patricia had another child, David Jr.
- David brought to another hospital with same symptoms.
- Lab tests revealed propionic acid, not ethylene glycol.
- Eventually Patricia was released at new trial.
- Children had Methylmalonyl Aciduria