BI/CH 422/622

OUTLINE:

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

Exam-1 material

Fermentations
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

PHOTOSYNTHESIS:
Overview of Photosynthesis
Key experiments:
Light Reactions
- energy in a photon
- pigments
- HOW
- Light absorbing complexes-"red-drop experiment"
  - Reaction center
  - Photosystems (PS)
    - PSII – oxygen from water splitting
    - PSI – NADPH

Proton Motive Force – ATP
Overview of light reactions

ANABOLISM I: Carbohydrates

Carbon Assimilation – Calvin Cycle
- Stage One – **Rubisco**
  - Carboxylase
  - Oxygenase
  - Glycolate cycle

Know mechanism

- Stage Two – making sugar
- Stage Three – remaking Ru $1,5P_2$

Overview and regulation
Calvin cycle connections to biosynthesis
C4 versus C3 plants
Kornberg cycle – gly**ox**ylate

Carbohydrate Biosynthesis in Animals
- precursors
- Cori cycle

Gluconeogenesis
- reversible steps
- irreversible steps – four
  - energetics
  - 2-steps to PEP in mitochondria: Pyr carboxylase-biotin & PEPCK
  - FBPase
  - G6Pase

Glycogen Synthesis
- UDP-Glc
- Glycogen synthase
- branching

Pentose-Phosphate Pathway
- oxidative-NADPH
- non-oxidative-Ribose 5-P

Regulation of Carbohydrate Metabolism
- Acetyl-CoA/Pyruvate
- Pyruvate/PEP
- F6P/FBP: Fru $2,6P_2$
- Glc/Glc6P: sequestration
- Glycogen: PKA/PP1
- Insulin signaling

Anaplerotic reactions

End of Exam-4 material

BI/CH 422/622

ANABOLISM OUTLINE:

Biosynthesis of Fatty Acids and Lipids

Fatty Acids
- contrasts
- location & transport
- Synthesis
  - acetyl-CoA carboxylase
  - fatty acid synthase
    - ACP priming
    - 4 steps
- Control of fatty acid metabolism
- Diversification of fatty acids
  - elongation
  - desaturation
  - Eicosanoids
    - Prostaglandins and Thromboxane

Triacylglycerides

Membrane lipids
- Glycerophospholipids
- ~~Sphingolipids~~
- Isoprene lipids: **Cholesterol**
  - Ketone body synthesis
  - Mevalonate
  - **Cholesterol**
    - bile acids
    - steroids
    - metabolism
    - control of cholesterol biosynthesis

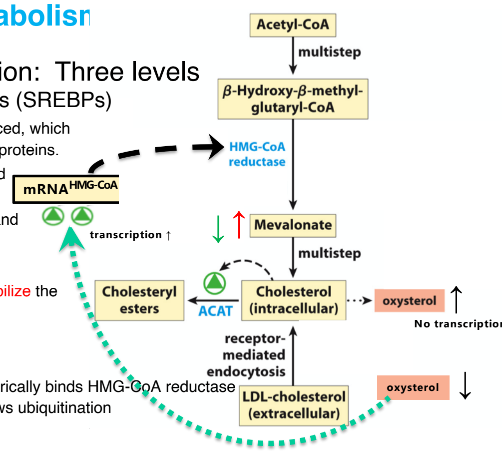
Biosynthesis of Amino Acids and Nucleotides

Nitrogen fixation
- nitrogenase

Cholesterol and Steroid Biosynthesis

Regulation of Cholesterol Metabolism

- Major regulation is by gene expression: Three levels
  - Sterol regulatory element-binding proteins (SREBPs)
    - When sterol levels are high, oxysterols are produced, which retain SREBPs in the ER membrane with other proteins.
    - When sterol levels fall, the complex is cleaved and moves to the nucleus.
    - It activates transcription of HMG-CoA reductase and LDL receptor, as well as other genes.
  - Translational control on mRNA stability
    - Increased mevalonate and other isoprenes destabilize the HMG-CoA mRNA
    - Decrease in mevalonate stabilizes the mRNA;
  - Post-translational control
    - High cholesterol produces oxysterol, which allosterically binds HMG-CoA reductase
    - Destabilizes by a conformational change that allows ubiquitination
    - Proteolytic degradation of HMG-CoA reductase
    - Also inhibits RME

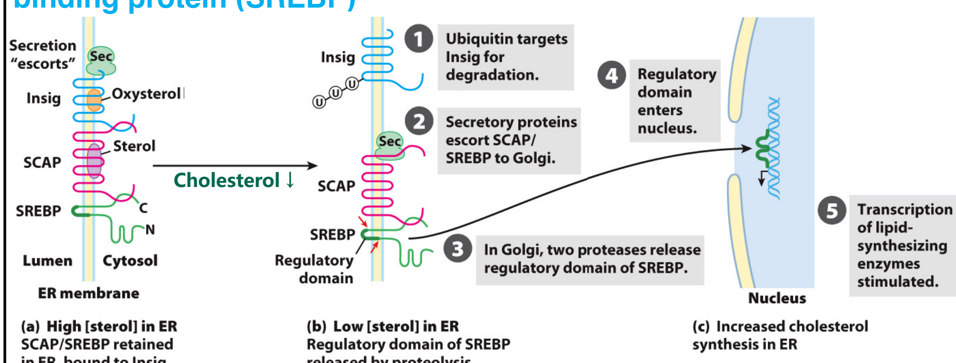


- Minor regulatory mechanisms: Phosphorylation/de-phosphorylation
  - Covalent modification provides short-term regulation
  - Glucagon, epinephrine
    - cascades lead to phosphorylation, ↓ activity
    - Also, AMP-dependent protein kinase
      when AMP rises, kinase phosphorylates the enzyme → activity ↓, cholesterol synthesis ↓
  - Insulin: cascades lead to dephosphorylation,↑ activity

Other effects of high cholesterol
Activation of ACAT, which increases esterification for storage
Transcriptional regulation of the LDL receptor gene activation

Cholesterol and Steroid Biosynthesis

Regulation of Cholesterol Synthesis by Sterol regulatory element-binding protein (SREBP)



(a) High [sterol] in ER
SCAP/SREBP retained in ER, bound to Insig

(b) Low [sterol] in ER
Regulatory domain of SREBP released by proteolysis

(c) Increased cholesterol synthesis in ER

HMG-CoA reductase↓

HMG-CoA reductase↑
LDL receptor↑

- Insig (***ins***ulin-***i***nduced ***g***ene ***p***rotein) senses cholesterol levels through oxysterol.
  - Also helps sequester SREBP in ER
  - triggers ubiquination of HMG-CoA reductase
  - targets the enzyme for degradation by proteasomes

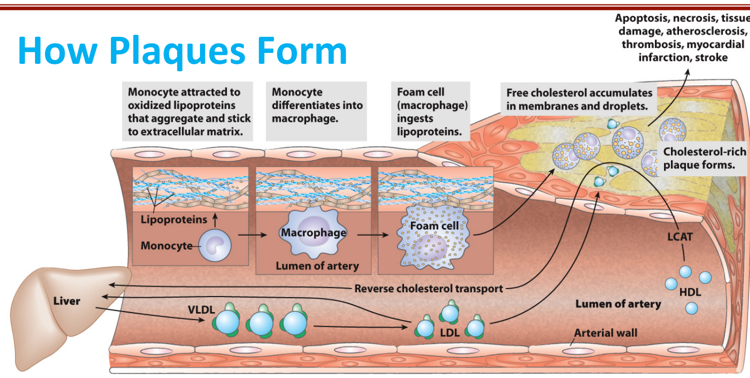
Cholesterol and Steroid Biosynthesis

Cardiovascular Disease (CVD) Is Multifactorial

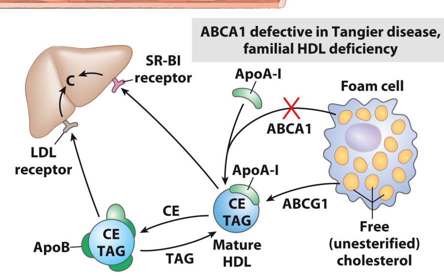
- **Very high LDL-cholesterol** levels tend to correlate with atherosclerosis.
  - although many heart attack victims have normal cholesterol, and many people with high cholesterol do not have heart attacks
- **Low HDL-cholesterol** levels are negatively associated with heart disease.
- Reverse Cholesterol Transport by HDL explains why HDL is cardioprotective

Cholesterol and Steroid Biosynthesis

How Plaques Form



- HDL picks up cholesterol from non-liver tissues, including foam cells at growing plaques.
- HDL carries cholesterol back to the liver
- Participation of ABCA1and ABCG1 transporters and SR-B1 receptors

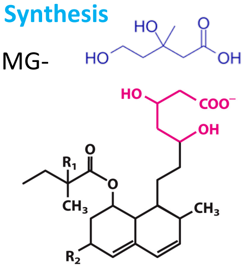


Cholesterol and Steroid Biosynthesis

Familial Hypercholesterolemia

- Due to genetic mutation in LDL receptor
- Impairs receptor-mediated uptake of cholesterol from LDL
- Cholesterol accumulates in the blood and in foam cells.
- Regulation mechanisms based on cholesterol sensing inside the cell don't work.
- Homozygous individuals can experience severe CVD as youths.

Statin Drugs Inhibit HMG-CoA Reductase to Lower Cholesterol Synthesis



- Statins resemble mevalonate → competitive inhibitors of HMG-CoA reductase
- First statin, lovastatin, found in fungi
  - lowers serum cholesterol by tens of percent
- Also reported to improve circulation, stabilize plaques by removing cholesterol from them, and reduce vascular inflammation

Cholesterol and Steroid Biosynthesis

We learned that:

Summary

- synthesis of fatty acids is a multistep process starting from acetyl-CoA and its carboxylated product, malonyl-CoA; it uses a poly-enzyme protein
- After synthesis of palmityl-CoA, fatty acids are elongated and desaturated; PUFAs are used for prostaglandin, thromboxane, and leukotriene synthesis
- phospholipids are a precursor to TAGs
- phospholipids and TAGs are built on a glycerol backbone that can be derived from dihydroxyacetone phosphate or glycerol
- head groups are attached using one of two methods, both use CDP carrier
- pathways to the synthesis of specific head groups vary by organism and may use salvage pathways
- Ketone-body synthesis goes through HMG-CoA
- cholesterol is derived from isoprene units, which comes from HMG-CoA
- production of isoprene for cholesterol biosynthesis occurs via the mevalonate pathway and starts with multiple acetyl-CoA
- cholesterol can be metabolized and modified in a variety of ways
- cholesterol and TAGs are trafficked in lipoproteins; classified by density
- Regulation of lipid biosynthesis....both fatty acids and cholesterol

ANABOLISM III: 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

- 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

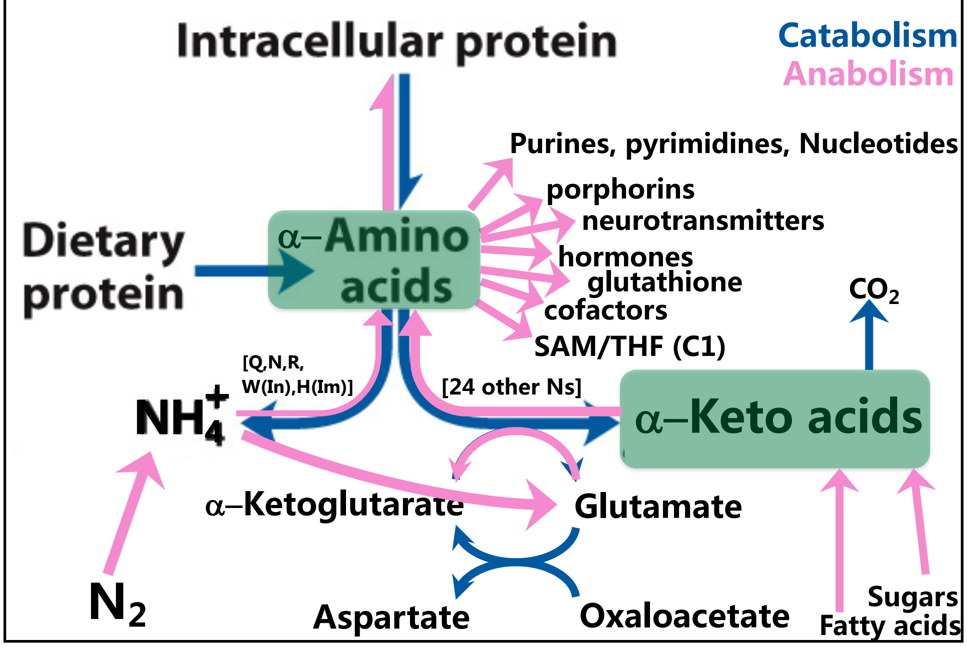
- Haber process

Haber Process

Dr. Kornberg:

Lecture 04.07.17 (4:03-6:25) 2 min

Biosynthesis Amino Acids & Nucleotides



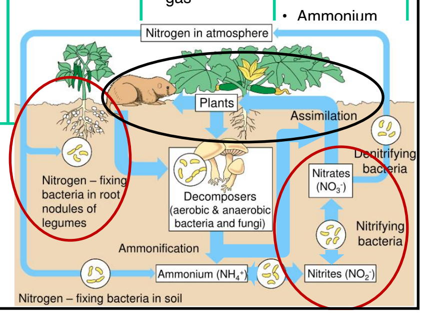
Biosynthesis Amino Acids & Nucleotides

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. • Ammonium

Biology of Molecular Nitrogen: The Nitrogen Cycle



Biosynthesis Amino Acids & Nucleotides

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

Both:

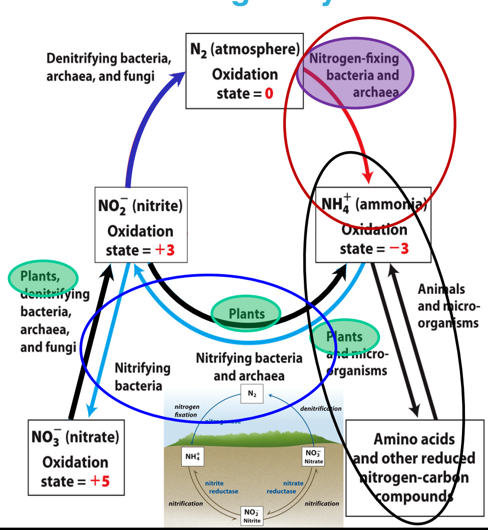
- Are electron transfer processes
- Use Mo cofactor
- Involve multiple redox cofactors, such as Fe-S, NADH, NADPH, ferrodoxin, flavodoxin, and so on

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.
   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

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

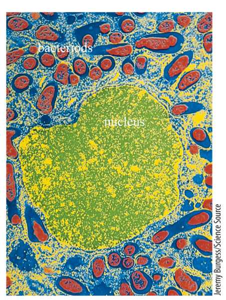
- Most are single-celled prokaryotes (archaea).
- Some live in symbiosis with *plants.*
  - (e.g., proteobacteria with legumes such as peanuts, beans)
- A few live in symbiosis with *animals.*
  - (e.g., spirochaete with termites)

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

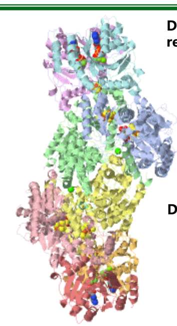
Biosynthesis Amino Acids & Nucleotides

Diazotrophs



Nitrogen-Fixing Nodules

–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 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

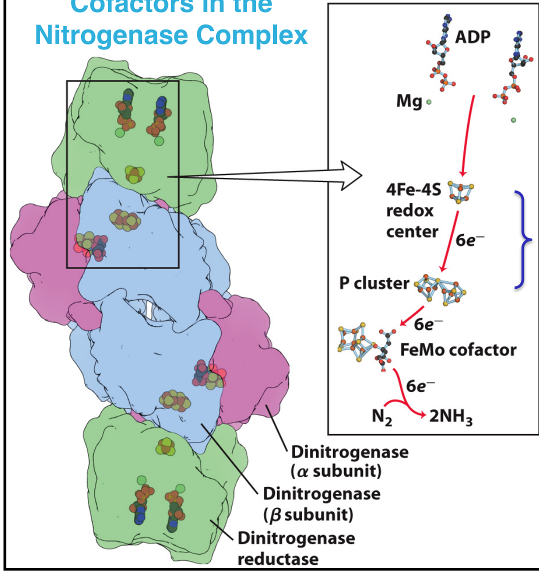
$$N_2 + 8\,H^+ + 8\,e^- + nATP \rightleftharpoons 2\,NH_3 + H_2 + nADP + nP_i$$

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

About 16 ATP molecules are consumed per one N_2.

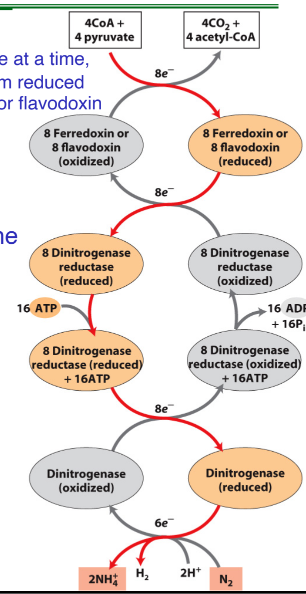
Biosynthesis Amino Acids & Nucleotides

Cofactors in the Nitrogenase Complex



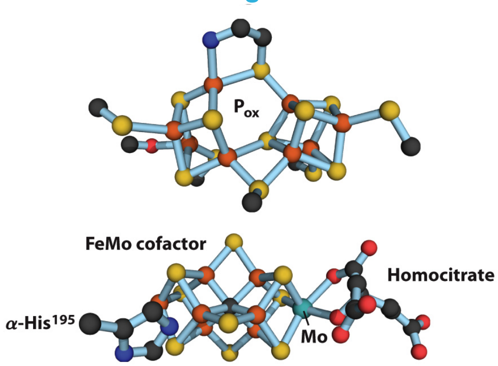
e– flow, one at a time, starting from reduced ferredoxin or flavodoxin

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

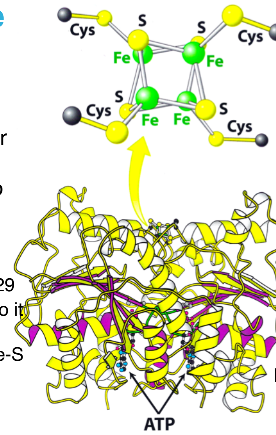
- Consists of:
  - 8 Fe atoms
  - 7 S atoms
  - Bound by 7 Cys
- Electrons are passed from the reductase Fe-S one at a time.
- Electrons are passed to the Fe-Mo cofactor.

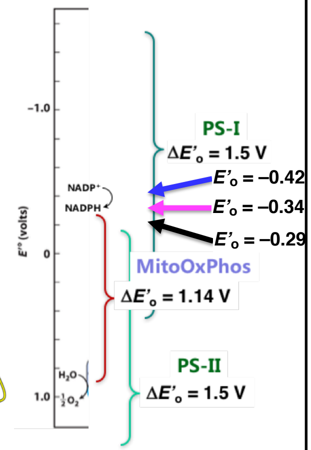
The Fe-Mo Cofactor in the Dinitrogenase β-Subunit

- 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

$N_2 + 6\,H^+ + 6\,e^- \rightarrow 2\,NH_3$ $E^{\circ\prime} = -0.34$ V

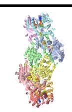
$\Delta E^{\circ\prime} = E^{\circ\prime}_{(reduction)} - E^{\circ\prime}_{(oxidation)}$
= –0.34 V – (–0.29 V)
= –0.05 V → +7 kcal/mole

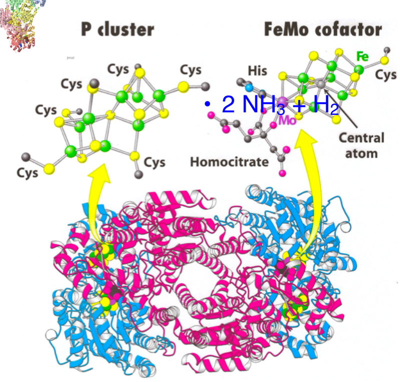
1. ATP hydrolysis *and* ATP *binding* help overcome the high activation energy.
2. Has regions homologous to GTP-binding proteins used in signaling (switch-1 and -2)
3. The reductase passes e^- to dinitrogenase.................physically!

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

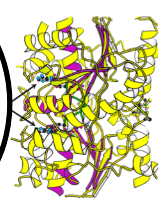
Biosynthesis Amino Acids & Nucleotides

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