MMG 301, Lecture 20 **Respiration**

Questions for today:

1. What is Respiration (and how does it differ from fermentation)?
2. What substrates are oxidized and which reduced (aerobic vs anaerobic respiration)?
3. How are electrons transferred from the electron donor to the electron acceptor?
5. How is ATP generated?
4. What are examples of respiration?
6. How do we calculate the energy available from respiration?

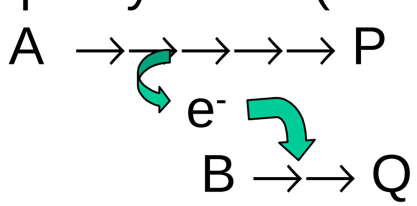
Overview of Respiration

-- Two substrates are required (A & B)

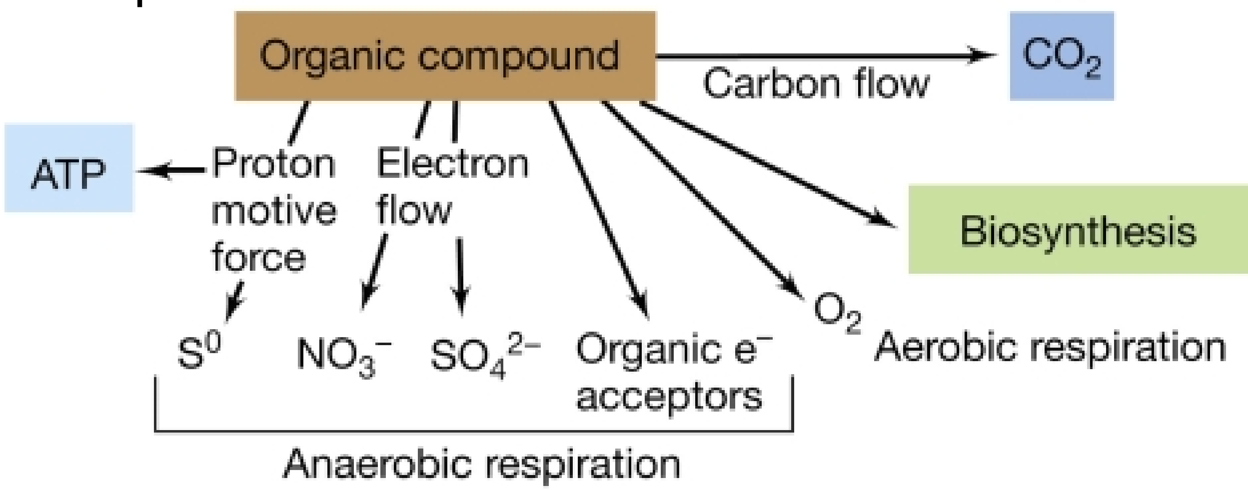
-- One substrate (A; organic or inorganic compound) is oxidized

-- The second substrate (B; O_2, nitrate, sulfate, or other compound) is reduced. This is referred to as the final electron acceptor

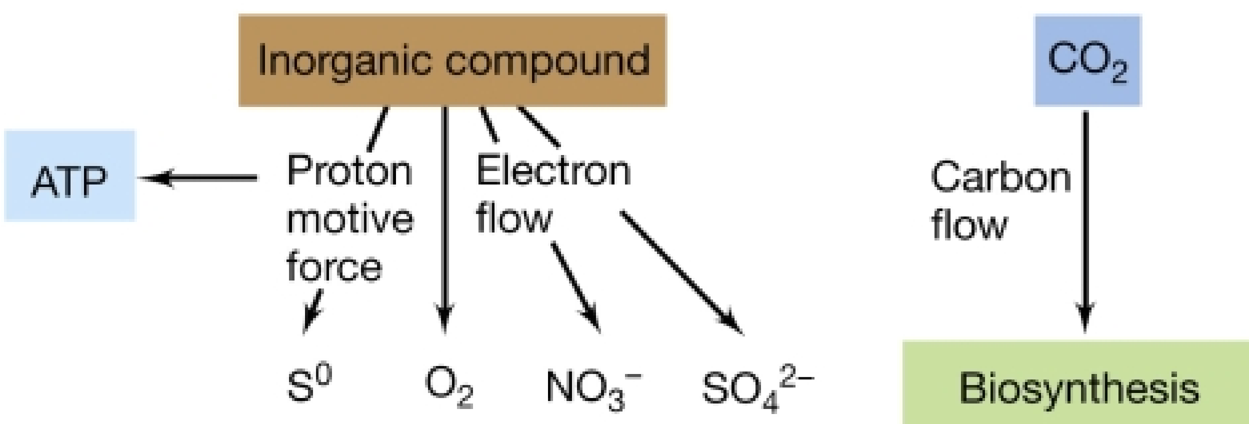
-- Electron transfer reactions drive ATP synthesis by “Electron Transport Phosphorylation” (ETP), little if any SLP



Chemoorganotrophic Respiration: an organic compound is oxidized (typically to CO_2) and the electrons transferred to oxygen or another acceptor



Chemolithotrophic Respiration: an inorganic compound is oxidized and the electrons transferred to oxygen or another acceptor

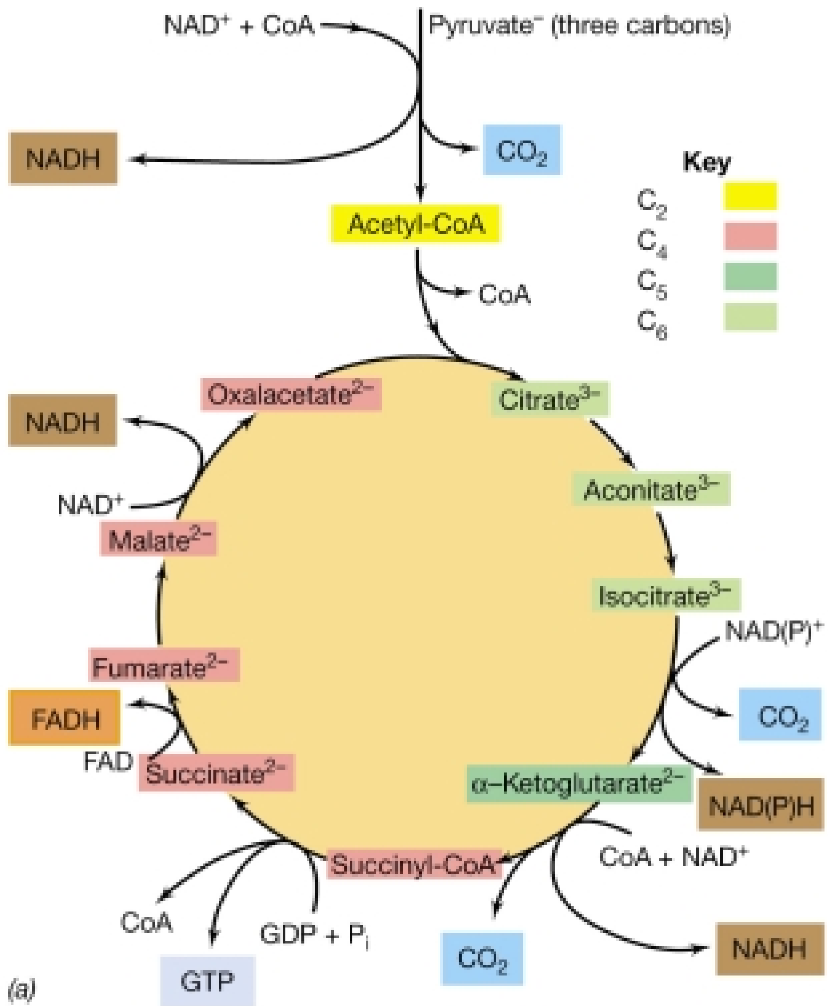


Note the *clear differences* from the situation in fermentation!

Examples of Substrate Oxidation

A. <u>Pyruvate</u> oxidation

Many organic compounds are converted to <u>pyruvate</u> (e.g., glycolysis). Each pyruvate is oxidized to 3 CO_2 by the <u>Citric Acid Cycle</u>



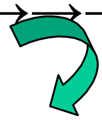
Electrons reduce 4 NAD + 1 FAD to form 4 NADH and 1 $FADH_2$ that are used for ETP
1 GTP made by SLP (used to form 1 ATP)

B. <u>Acetyl-CoA</u> oxidation

Various organic compounds (including fatty acids) are decomposed to → acetyl-CoA
Oxidized via <u>Citric Acid Cycle</u> with electrons going to 3 NAD and 1 FAD

C. <u>Non-Citric Acid Cycle</u> oxidation of <u>organic</u> compounds
Example: Respiration of single carbon compounds:

$CH_4 \rightarrow\rightarrow\rightarrow\rightarrow CO_2$ Methanotrophs



multiple electrons (used for ETP)

D. Oxidation of <u>Inorganic</u> substrates

$H_2 \rightarrow 2\ H^+ + 2\ e^-$ (used for ETP)

$H_2S \rightarrow 2\ H^+ + S^o + 2\ e^-$ (used for ETP)

$NO_2^- \rightarrow NO_3^- + e^-$ (used for ETP)

$Fe^{2+} \rightarrow Fe^{3+} + e^-$ (used for ETP)

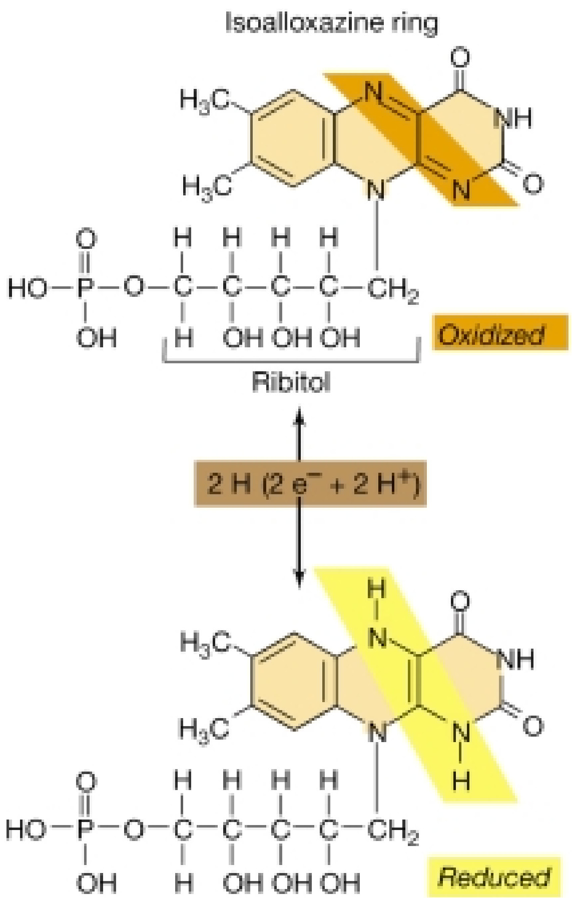
(*each pathway for C and D will require a separate series of enzymes for the specific oxidation reactions*)

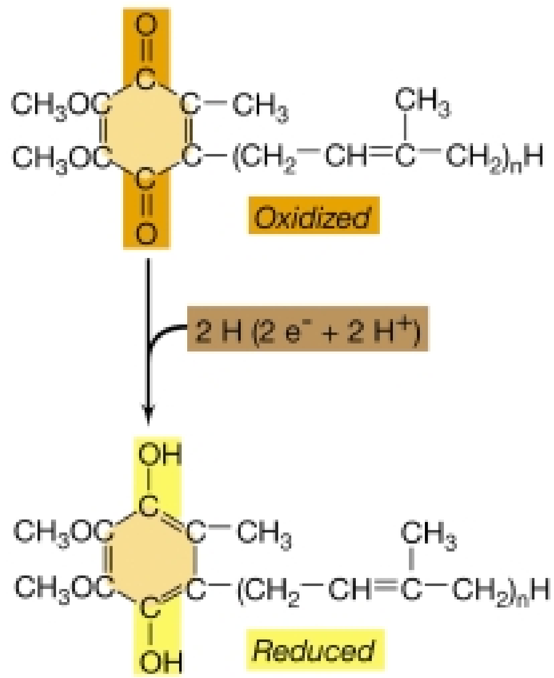
Electron Transport Chains

Electrons derived from substrate are handed off through a series of electron carriers:

NAD(P) -- shown last time; $2H^+$ and $2e^-$

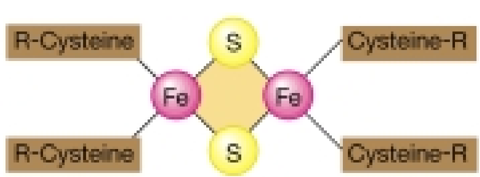
Flavins **Quinones (Q)**

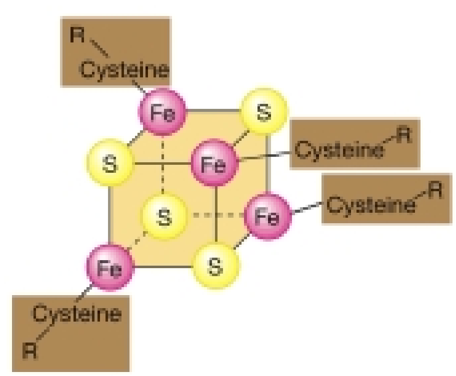




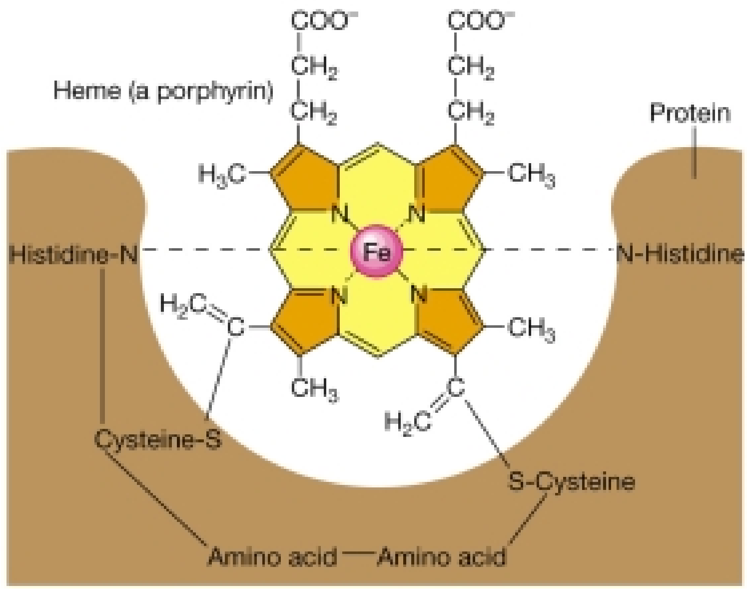
You should be able to recognize these structures!

Iron-sulfur (Fe/S) clusters: only electron transfer!





Cytochromes (Cyt): only electron transfer!

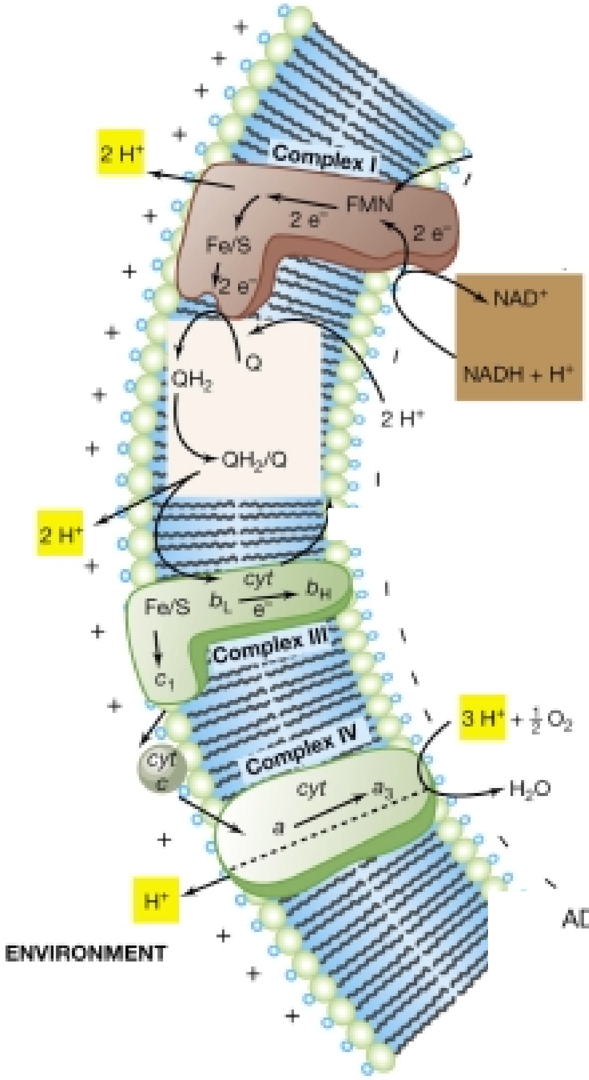


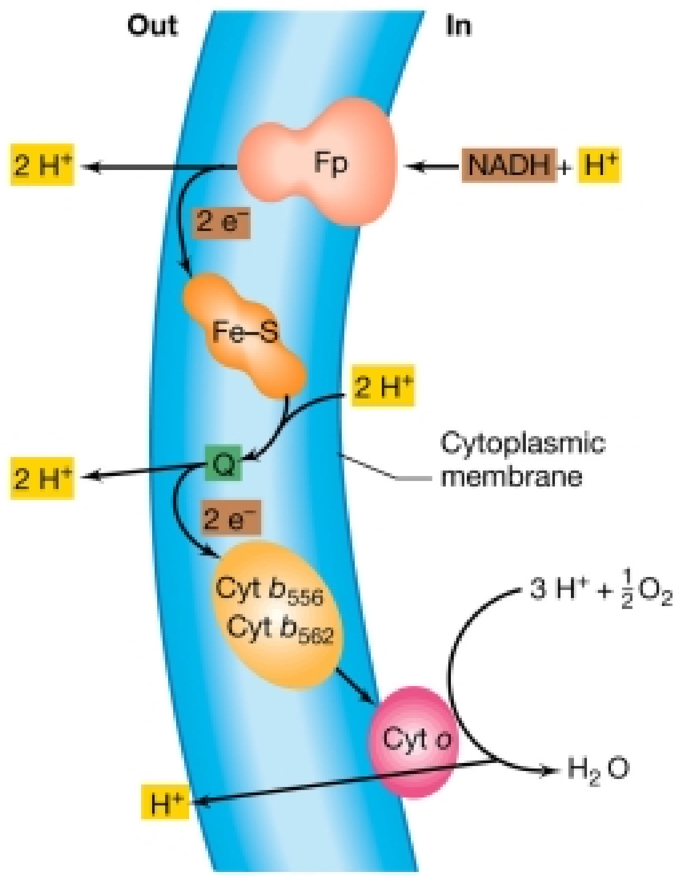
These cofactors (or proteins containing them) are positioned in or on the cytoplasmic membrane in a manner that allows for a "bucket-brigade" transfer of electrons while transferring protons across the membrane: **proton motive force (PMF)**

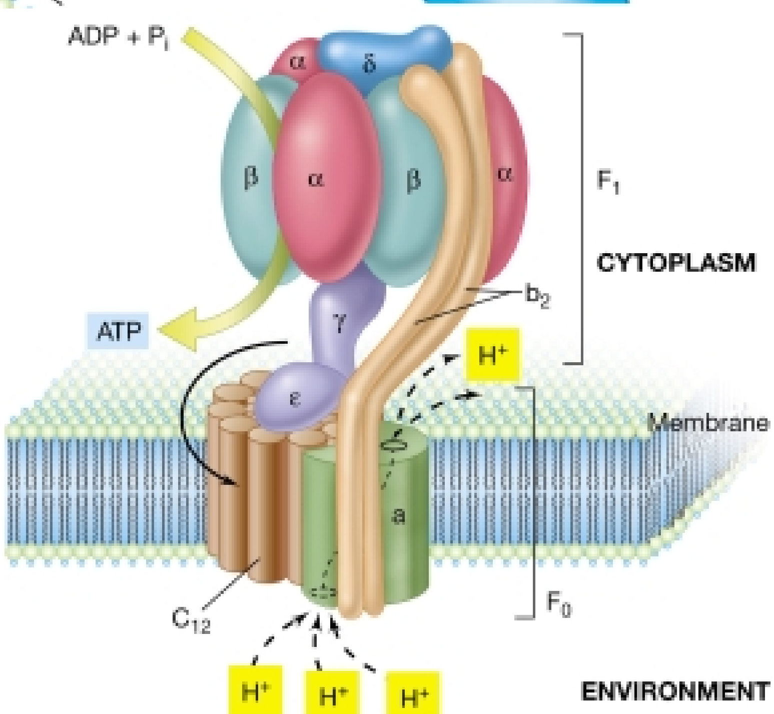
The electrons are finally used to reduce the final electron acceptor substrate: O_2 (aerobic respiration) or nitrate, nitrite, sulfate, fumarate, ferric ion, and other compounds (anaerobic respiration)

E*ach pathway requires a distinct series of electron transfer proteins and a unique oxidase*

Example: aerobic respiration in
Paracoccus denitrificans and *Escherichia coli*







Membranes allow buildup of PMF that drives ATP synthesis via ATP synthase

Putting it all together: examples of Respiration

Aerobic Respiration of Glucose

-- Glucose (A) is metabolized to pyruvate via glycolyis and oxidized to CO_2 by the citric acid cycle.

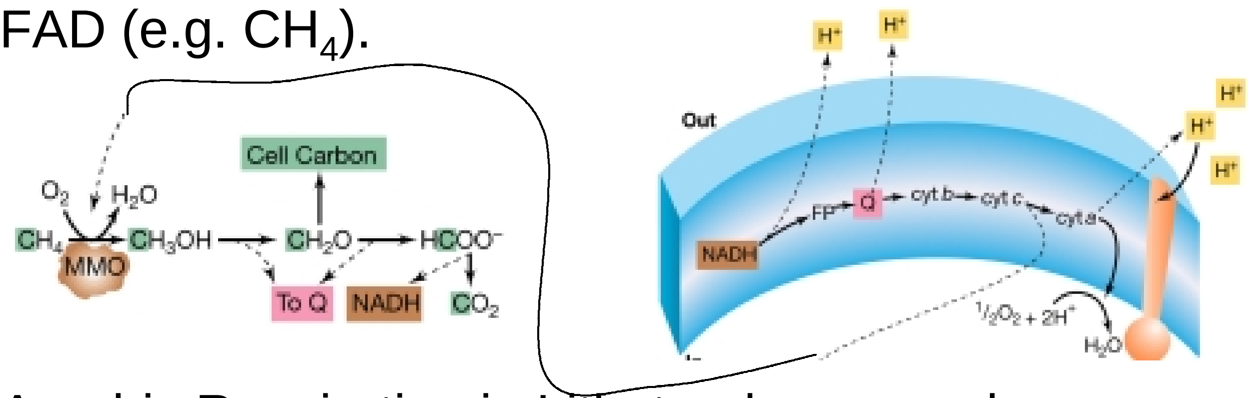
-- NADH and $FADH_2$ feed electrons into membrane bound electron carriers that result in protons being pumped.

-- Electrons oxidize oxygen (B) to produce water.

-- PMF drives ATP synthesis.

Aerobic respiration of other carbon compounds

-- Alternative pathways are used for oxidation of the carbon compound to provide reduced NAD and FAD (e.g. CH_4).



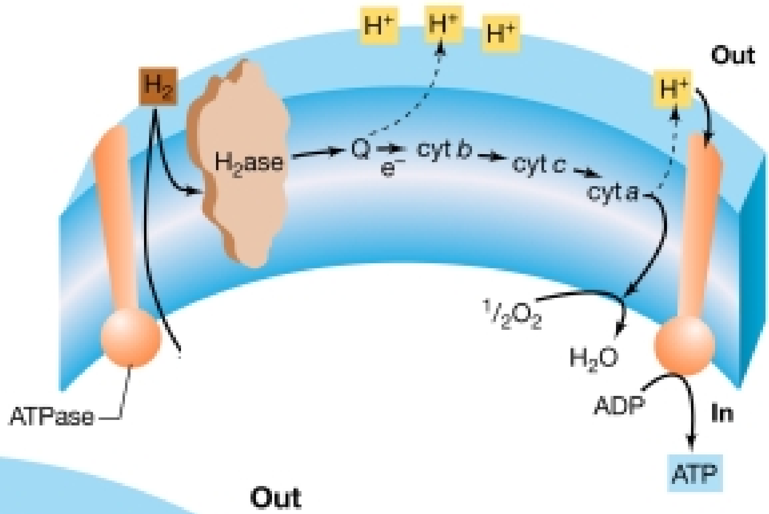
Aerobic Respiration in Lithotrophs occurs by an analogous system

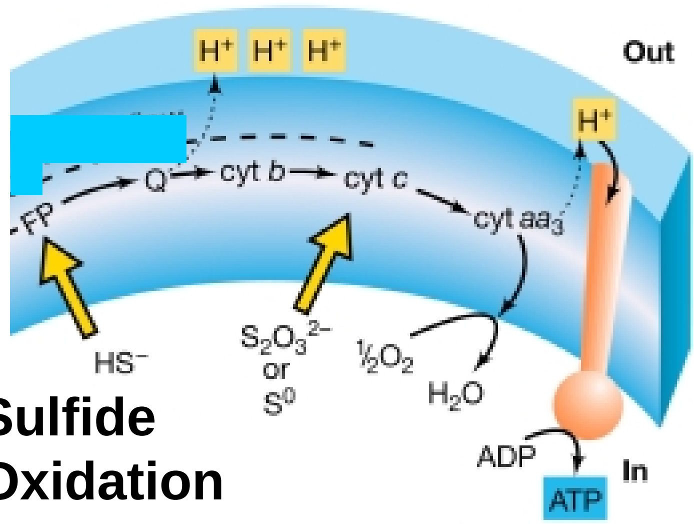
-- An inorganic substrate is oxidized (e.g., hydrogen, sulfide, nitrite, ferrous ion).

-- Electrons feed through an electron transport chain to generate PMF.

-- Oxygen is reduced to water.

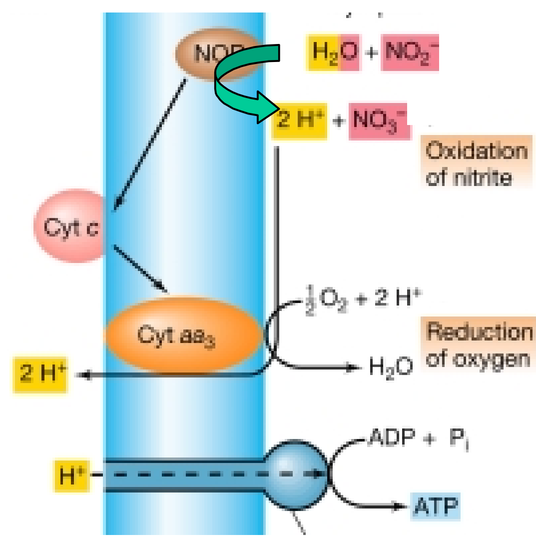
Hydrogen oxidation



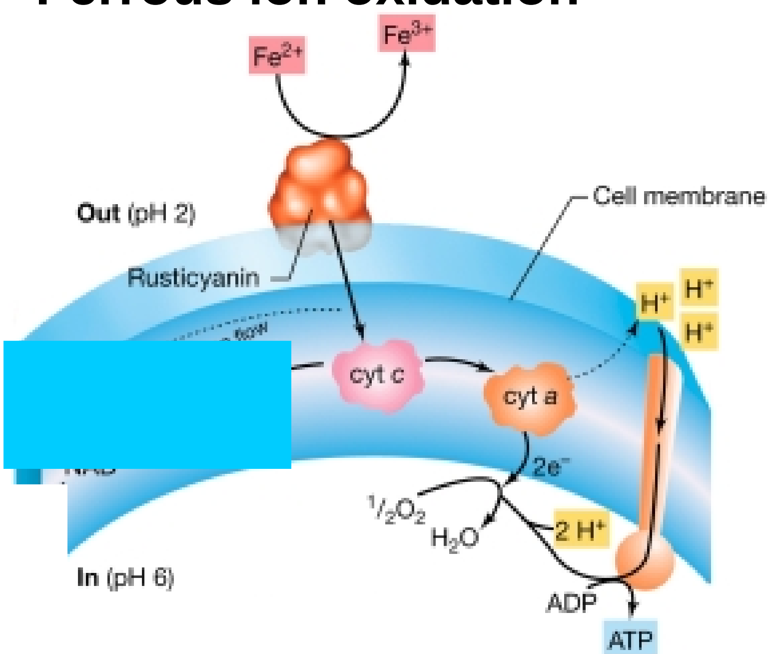


Sulfide Oxidation
(sulfide oxidase)

Nitrite oxidation
(nitrite oxidoreductase)



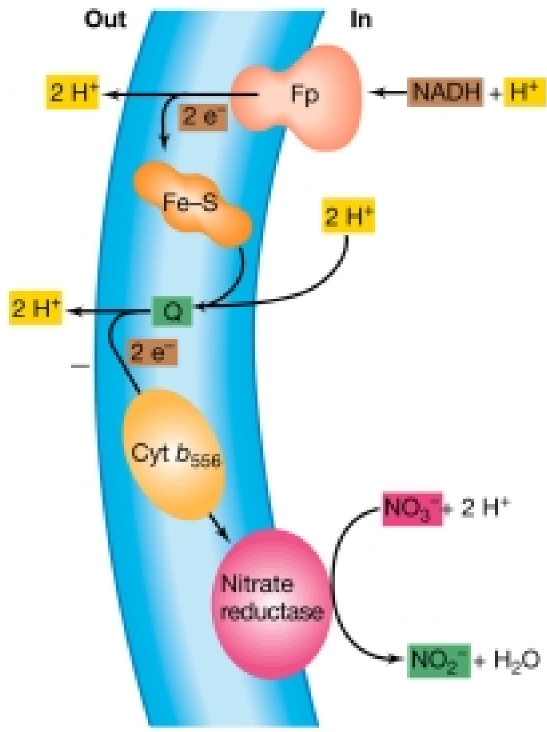
Ferrous ion oxidation

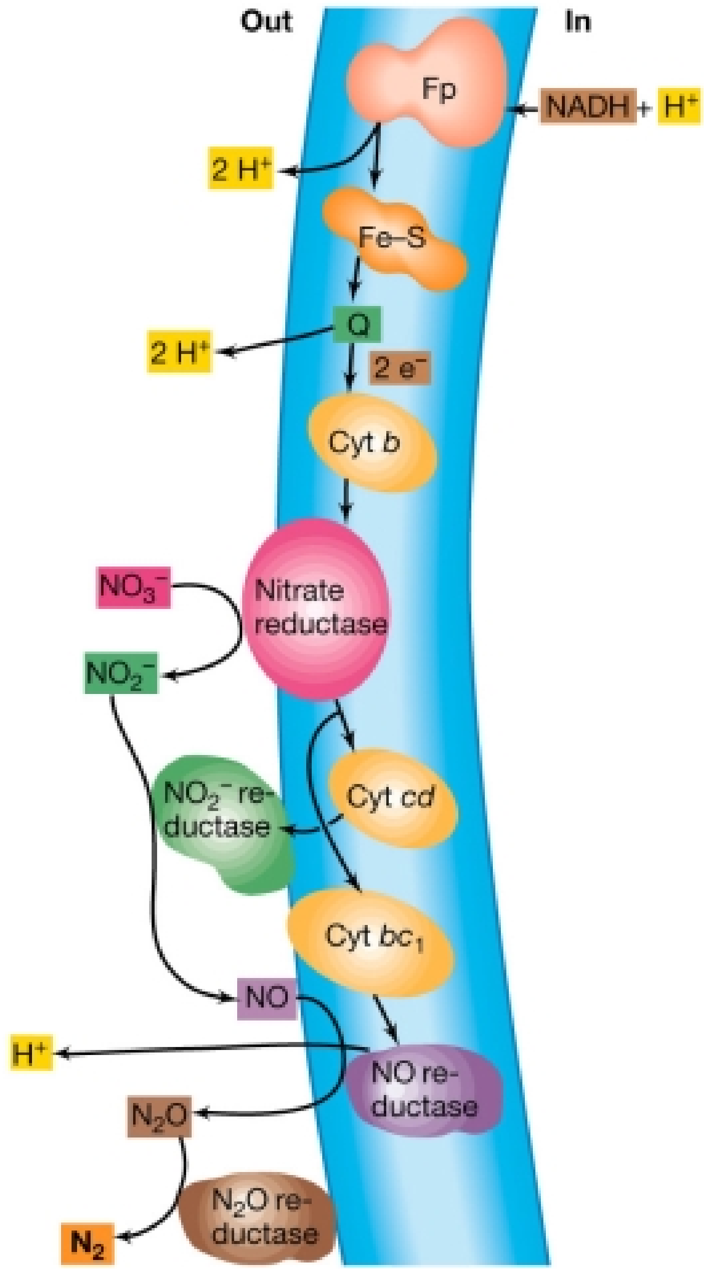


Understand the concept and recognize the key enzymes indicated, not the detailed pathways

Anaerobic Respiration: something other than oxygen is reduced (e.g., nitrate, sulfate, fumarate, etc.)

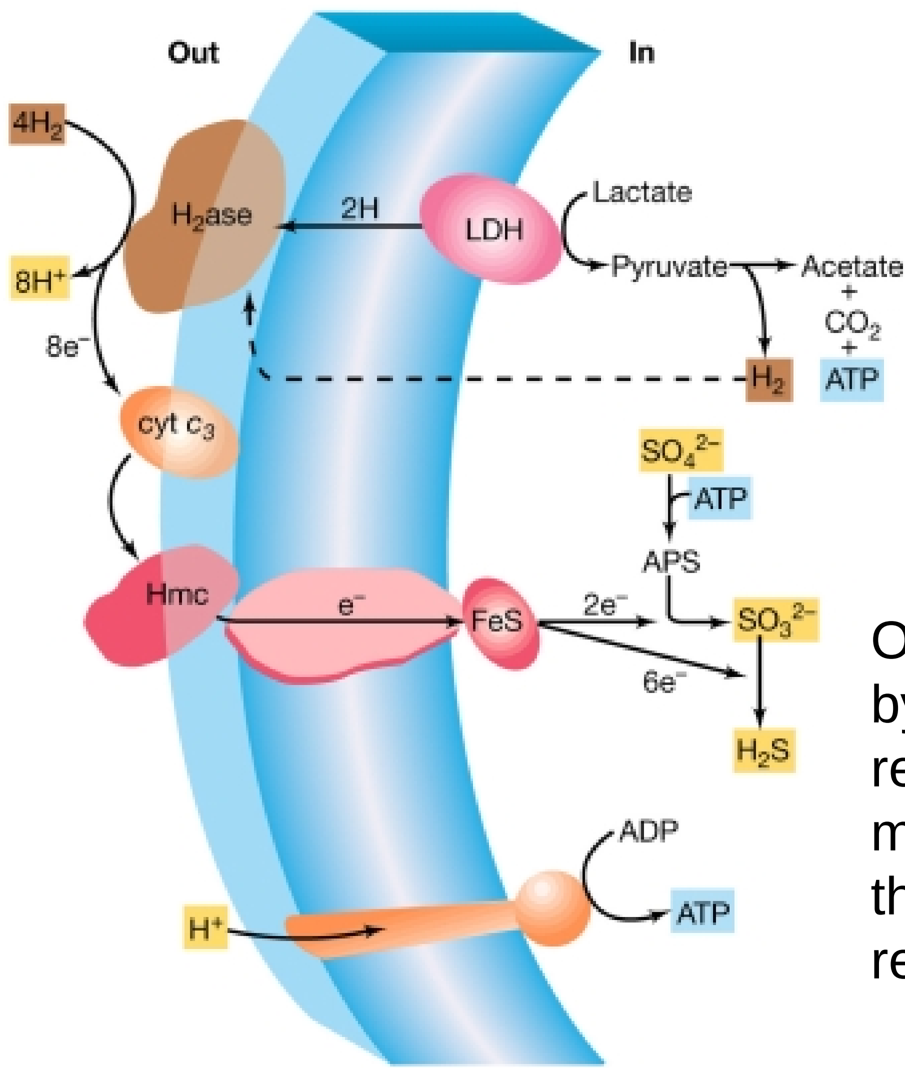
Denitrification: Energy-generating nitrate reduction in *E. coli* vs. *Pseudomonas*





Note that some portions of the electron transport chain may be identical to that used for aerobic respiration

Dissimilative sulfate reduction: Energy generation by anaerobic respiration of sulfate



Lactic acid or other carbon compound is oxidized with the electrons reducing sulfate to form sulfide

Only carried out by one closely related group of microorganisms: the sulfate reducers

Other electron acceptors used for anaerobic respiration:

fumarate + $2H^+ + 2e^- \rightarrow$ succinate

dimethylsulfoxide + $2H^+ + 2e^- \rightarrow$ dimethylsulfide

ferric ion + $e^- \rightarrow$ ferrous ion

(many others)

Measurement of Energy

Direct calculation of $\Delta G^{o\prime}$

$$\text{Glucose} + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O$$

(-917 kJ/mol) (0) (-394 kJ/mol)(-237 kJ/mol)

$\Delta G^{o\prime}$ = [6(-394)+6(-237)]-(-917) = -2869 kJ/mol

10-fold that available via fermentation!

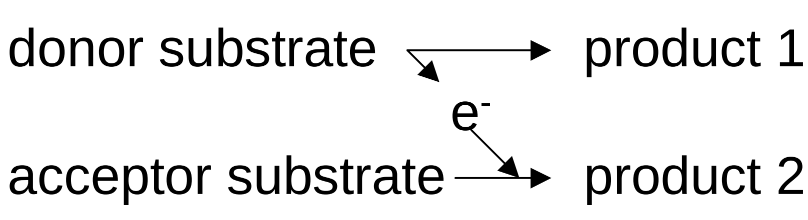
Find ~38 ATP are made (2 during glycolysis, 2 GTP from citric acid cycle, and the rest via ETP)

Efficiency = 100 [38*(-32 kJ/mol)/-2869 kJ/mol) = 42%

This approach also works when using different electron acceptors (I.e., types of anaerobic respiration) and when using different substrates to be oxidized (e.g., H_2, H_2S, NO_3^-, etc.). However, the values of free energies are not always readily available (see table A1.1). ***Is there an easier way to calculate energy for these reactions?***

Calculation of energy by $\Delta E_o{}'$ (measured in V)

These reactions all involve two half-reactions



E_o' is the tendency of a compound to become reduced, in units of V

$\Delta E_o' = E_o'$ (electron acceptor) - E_o' (electron donor)

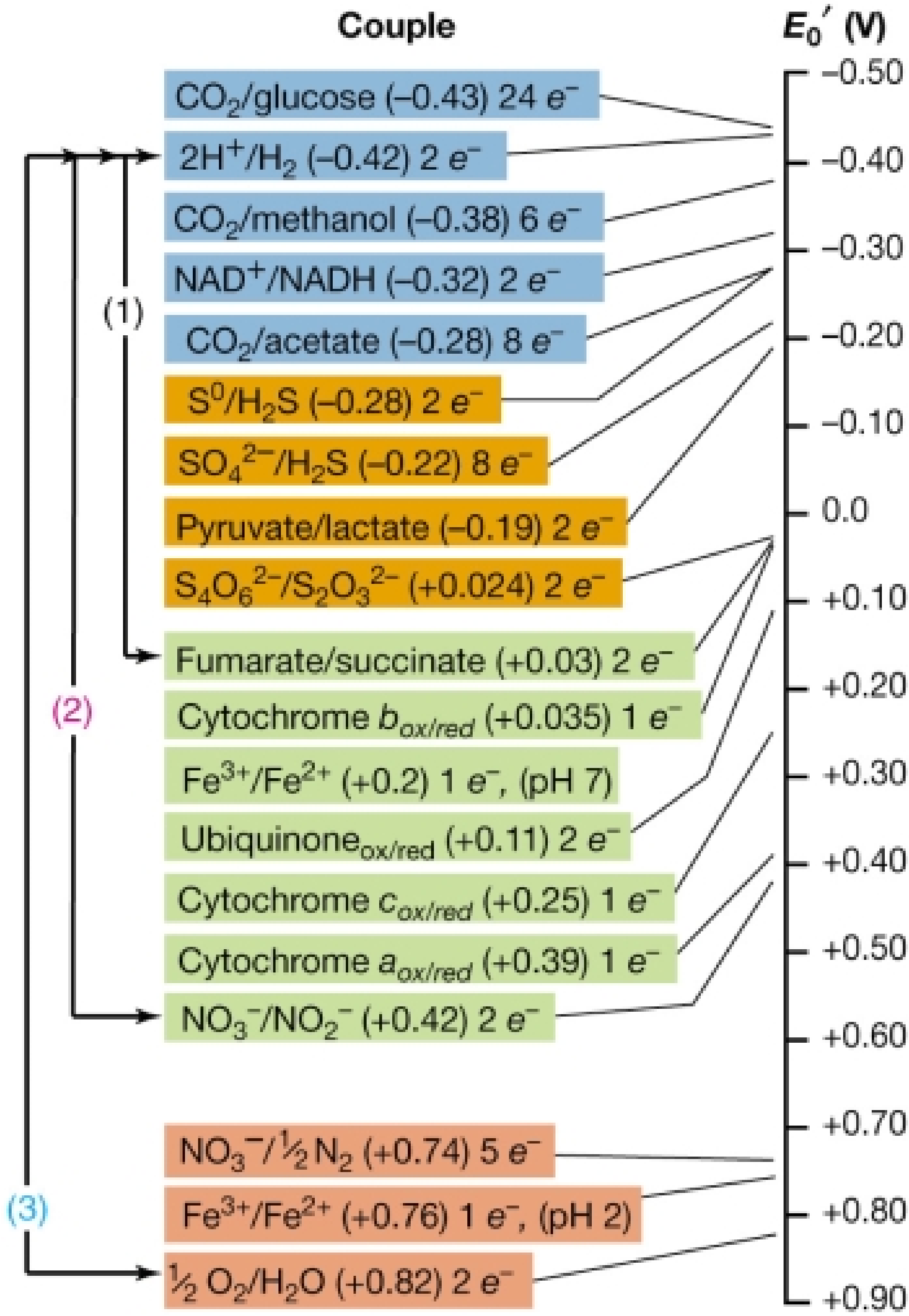
Using hydrogen gas as the electron donor:

Fumarate (.03)-(-.43) = 0.46 V

Nitrate (0.42)-(-.43) = 0.85 V

Oxygen (.82)-(-.43) = 1.25 V

Electrons flow down!



How do these values compare to $\Delta G^{o'}$?

$$\Delta G^{o'} = -nF\Delta E_o{}'$$

F = 96.48 kJ/mol

n = number of electrons

So we can compare the three reactions!

	$\Delta E_o{}'$	$\Delta G^{o'}$
H_2 + fumarate → succinate	0.46 V	~-85 kJ/mol
$H_2 + NO_3^- \rightarrow NO_2^- + H_2O$	0.85 V	~-160 kJ/mol
$H_2 + ½\ O_2 \rightarrow H_2O$	1.25 V	~-240 kJ/mol

Can estimate the ATP available from such processes on the basis of –32 kJ/mol = ~0.16 V (for a 2 electron process)

Things to think about regarding oxidation

- What is needed for a cell to carry out aerobic respiration of glucose?
- What components would change if the cell is carrying out nitrate respiration, a type of anaerobic respiration?
- How might a cell regulate which system to synthesize?
- How might pH affect the bioenergetics of respiration?
- How could two microbes work together to allow “unfavorable” respirations to occur? (syntrophy)