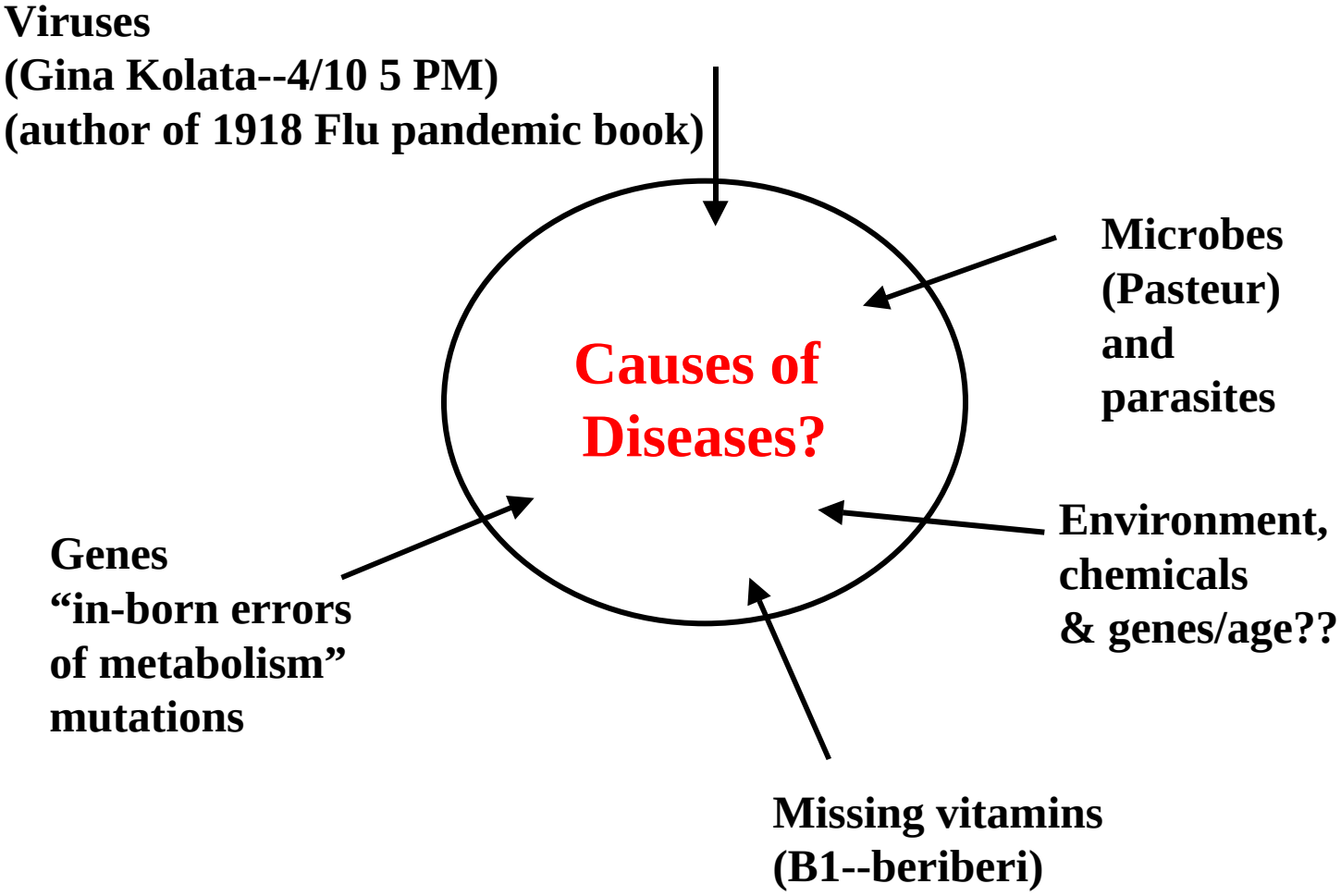
Chapter 14--Citric Acid Cycle

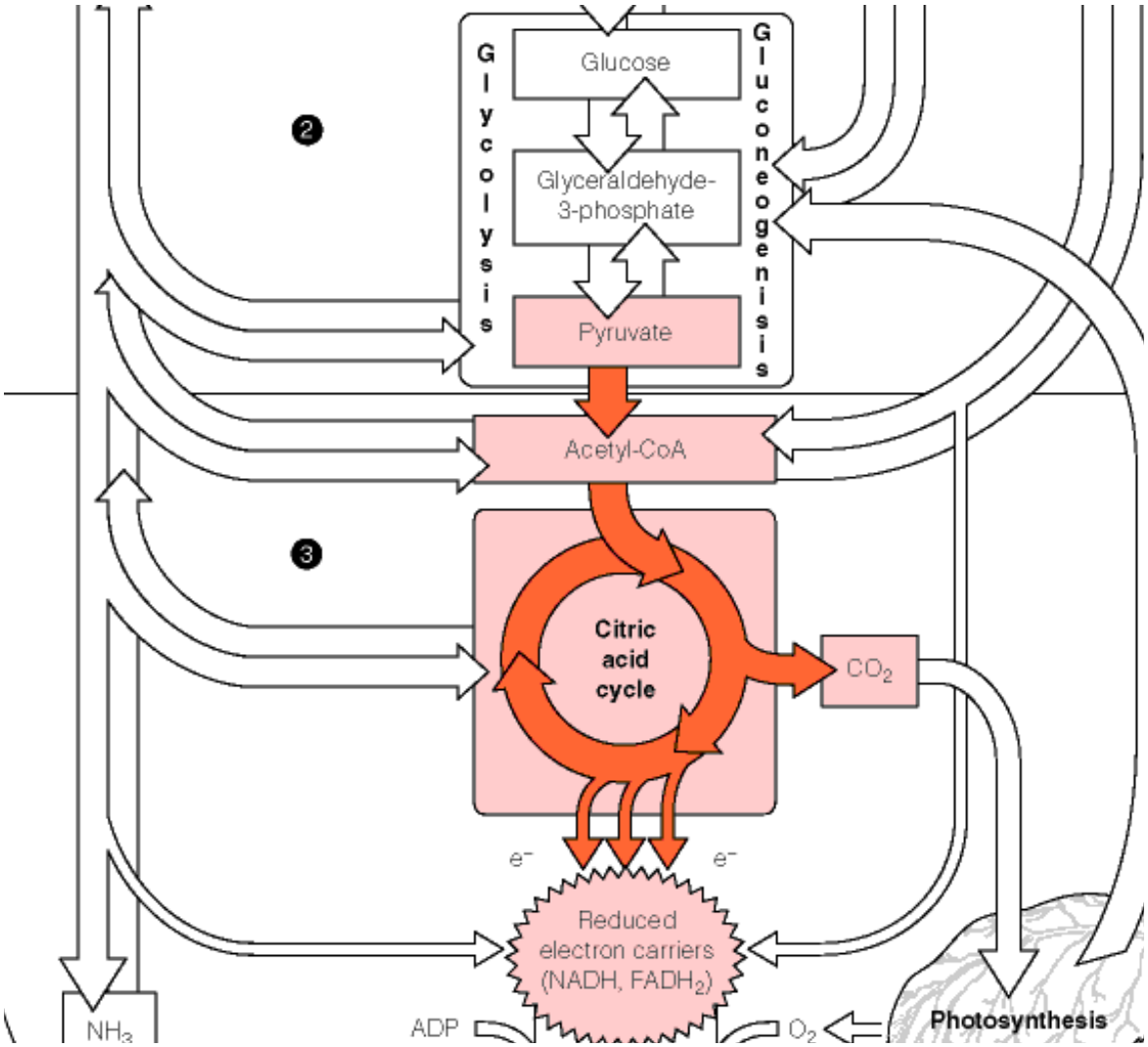
Pyruvate to Acetyl Co-A--Pyruvate dehydrogenase

The cycle--phase I and phase II

Co-factor structures and functions

Recognizing oxidations


Viruses
(Gina Kolata--4/10 5 PM)
(author of 1918 Flu pandemic book)
Causes of Diseases?
Microbes (Pasteur) and parasites
Environment, chemicals & genes/age??
Genes
“in-born errors of metabolism” mutations
Missing vitamins
(B1--beriberi)

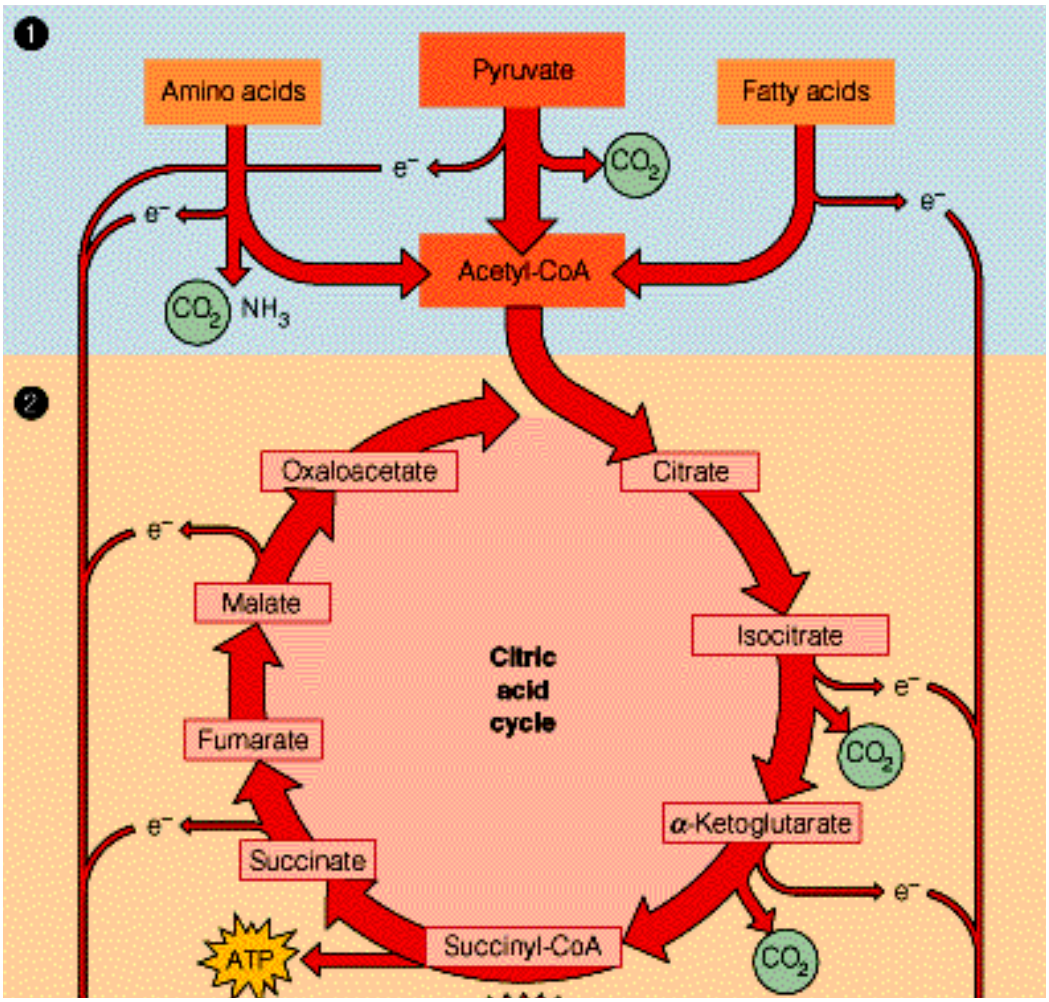

Glycolysis
Gluconeogenesis
Glucose
Glyceraldehyde-3-phosphate
Pyruvate
Acetyl-CoA
Citric acid cycle
CO_2
e^-
e^-
Reduced electron carriers ($NADH$, $FADH_2$)
NH_3
ADP
O_2
Photosynthesis

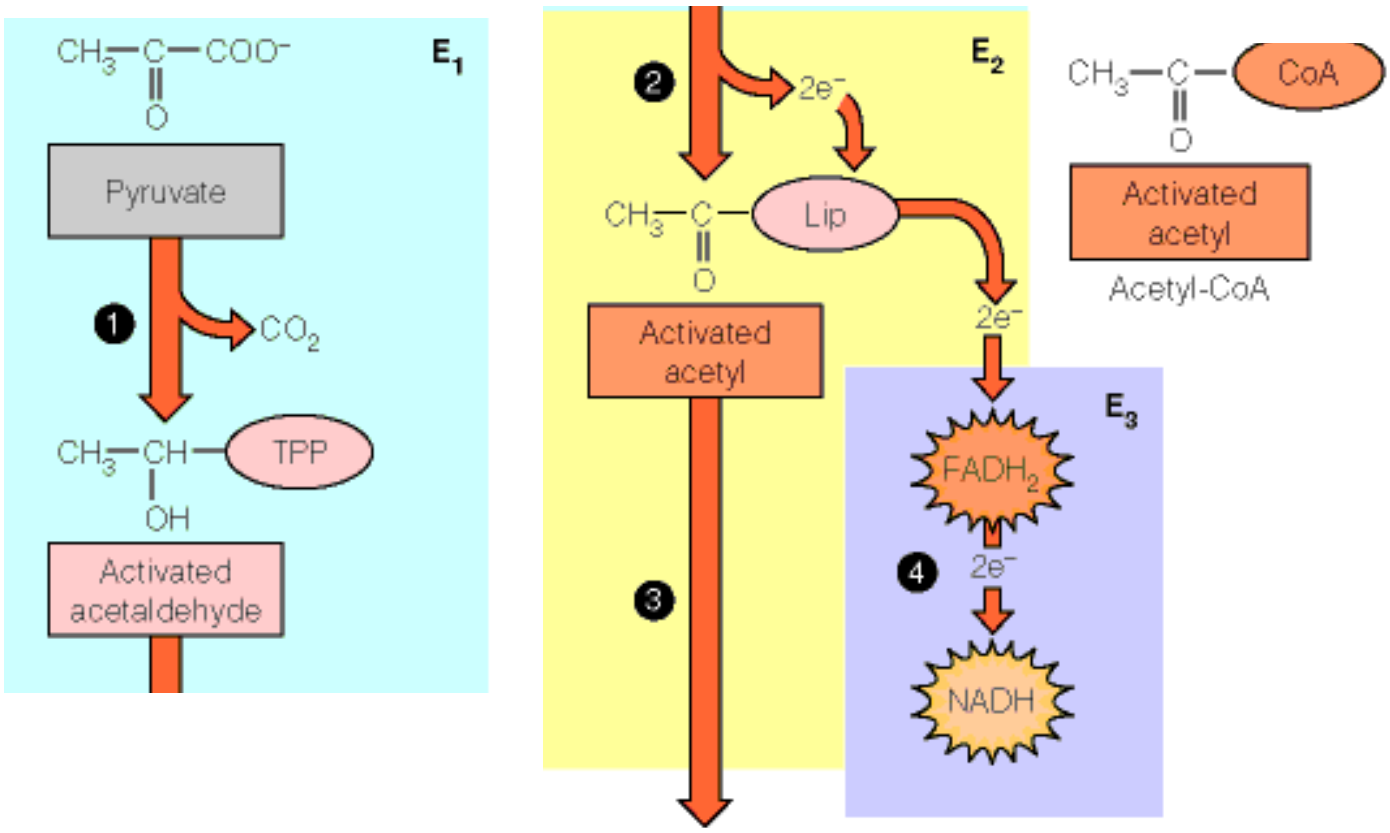
Historical Discovery--Krebs 1937
System--Respiring minced pigeon muscles.
Cyclic vs. linear pathway

Observations
****tricarboxylic acids stimulated**
More oxygen consumption and pyruvate
Oxidation than predicted by stoichimetry.
Cycle or catalyst???

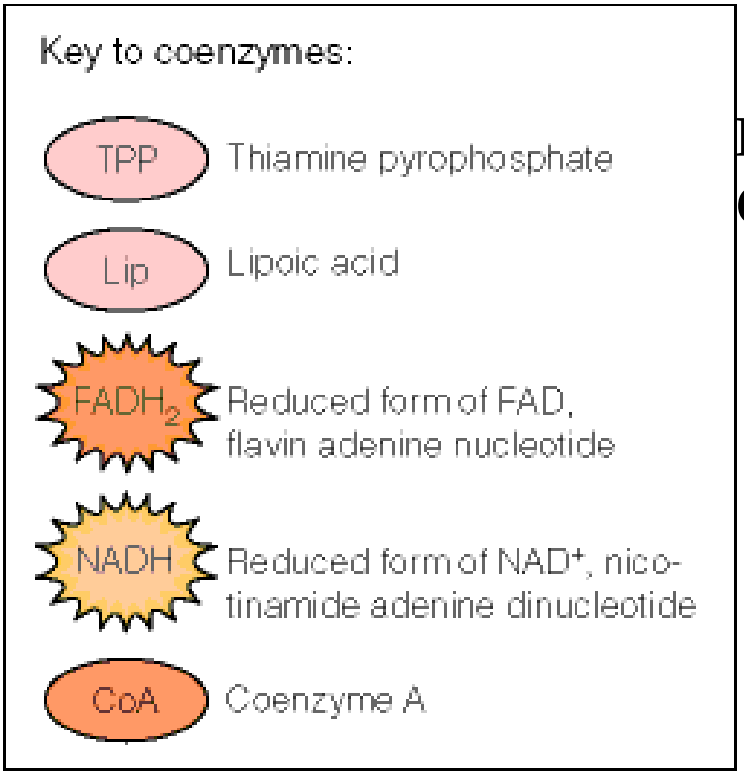
****Malonate inhibits succinate dehydrogenase and**
Leads to pyruvate, citrate, succinate, and alpha ketoglutarate build-up.

****addition of pyruvate and oxaloacetate produce**
Citrate.

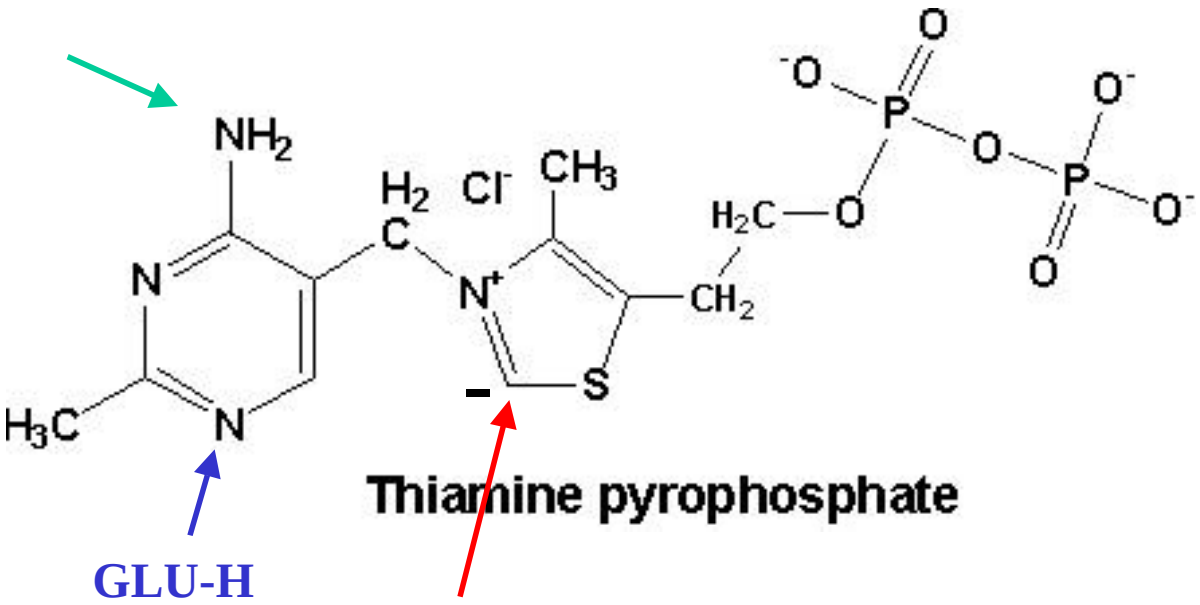

1
Amino acids
Pyruvate
Fatty acids
e⁻
CO2
e⁻
e⁻
Acetyl-CoA
CO2
NH3
2
Oxaloacetate
Citrate
e⁻
Malate
Isocitrate
Citric
acid
cycle
e⁻
CO2
Fumarate
α-Ketoglutarate
e⁻
Succinate
e⁻
ATP
Succinyl-CoA
CO2



Pyruvate Dehydrogenase Complex in E. coli has
24 E1, 24 E2, 12 E3 and MW 4.6 million g/mol!
3 enzymes and 5 co-factors are arranged in a cube



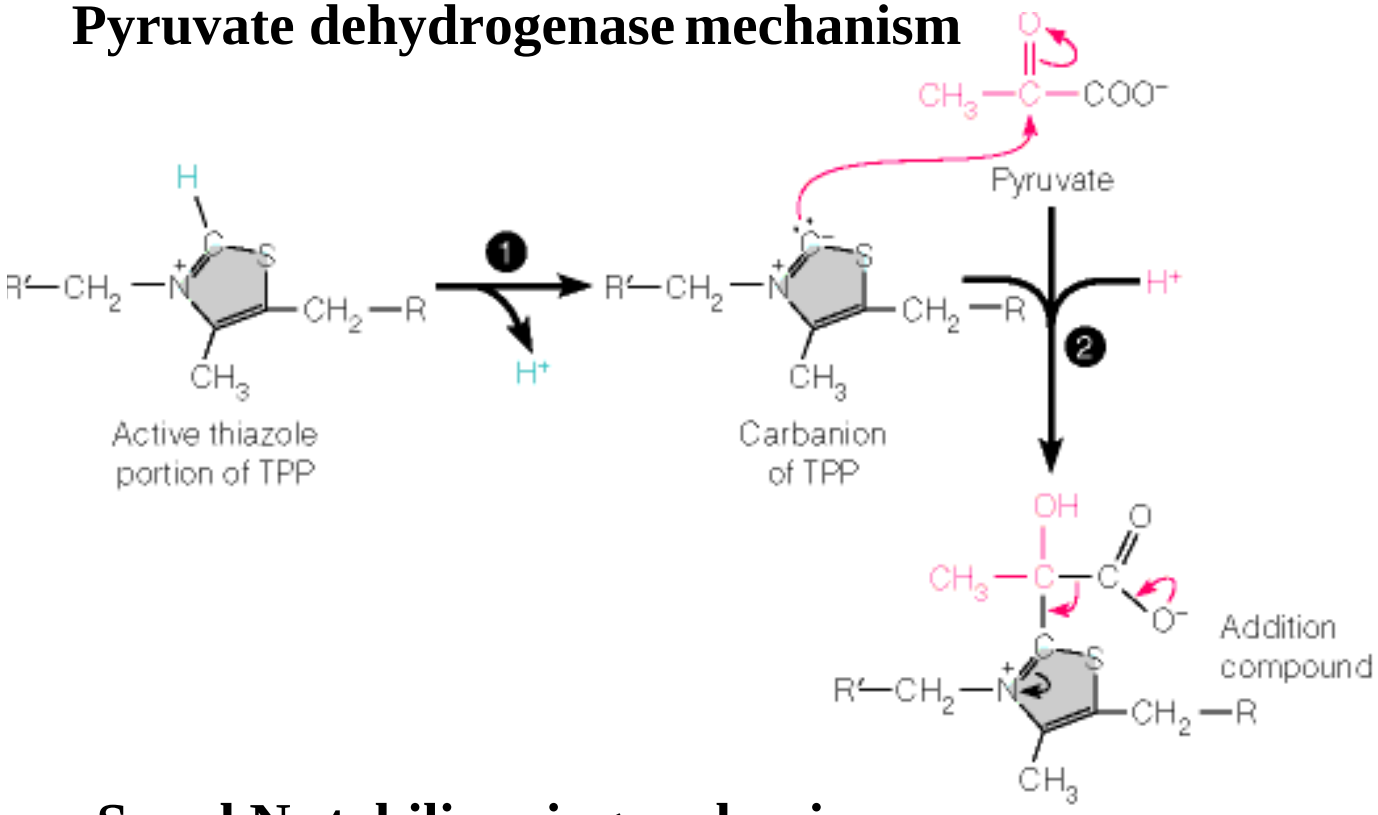
Pyruvate dehydrogenase Co-factors



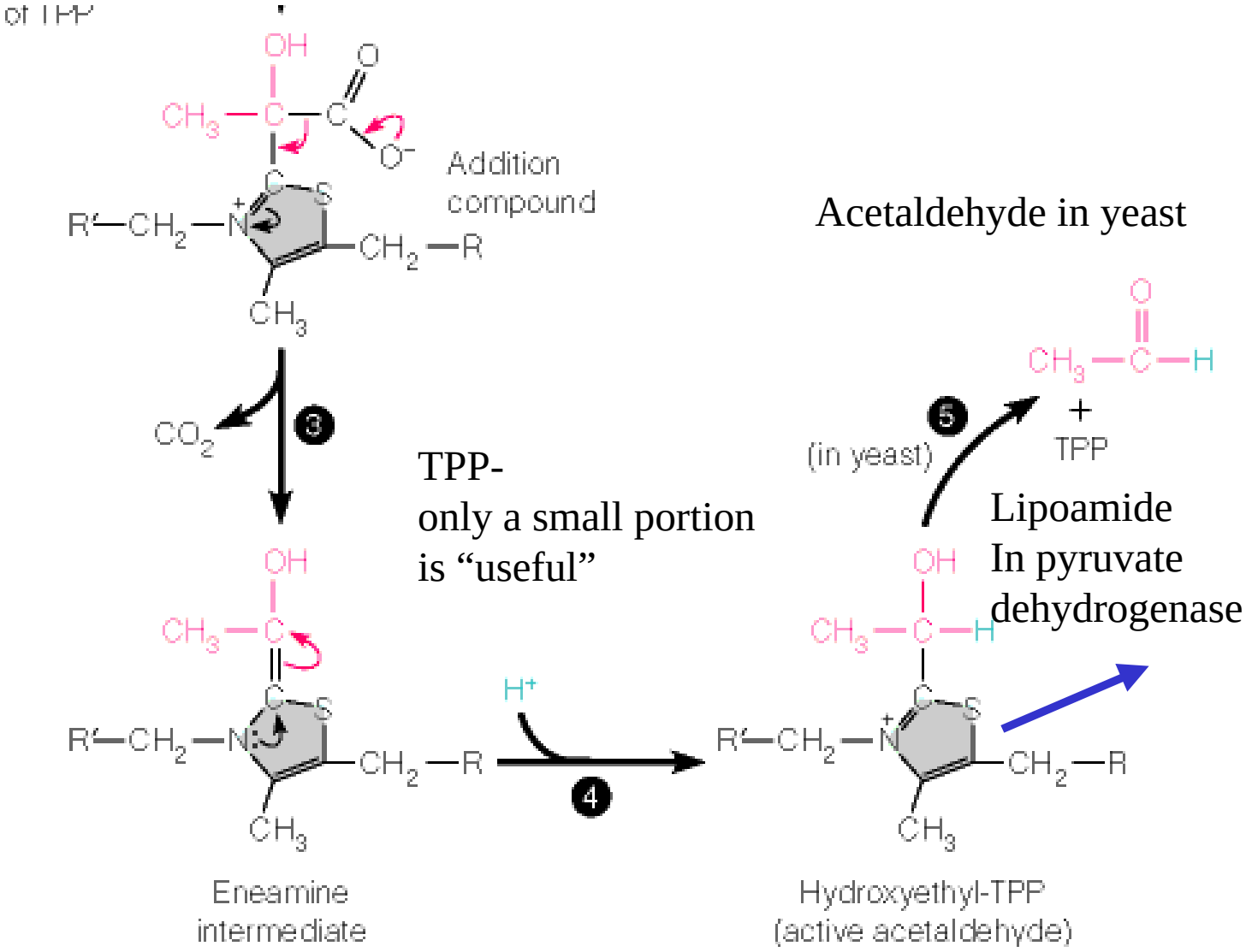
Acidic proton abstracted to produce NH2 (enamine) following Glu abstraction of N-H

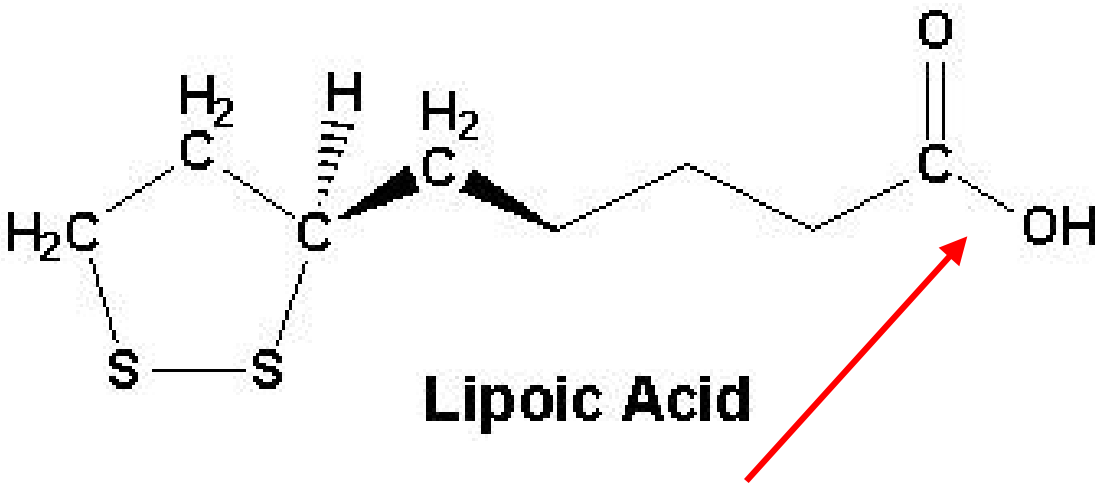
See page 491 for more correct form

Pyruvate dehydrogenase mechanism



S and N stabilize ring carbanion

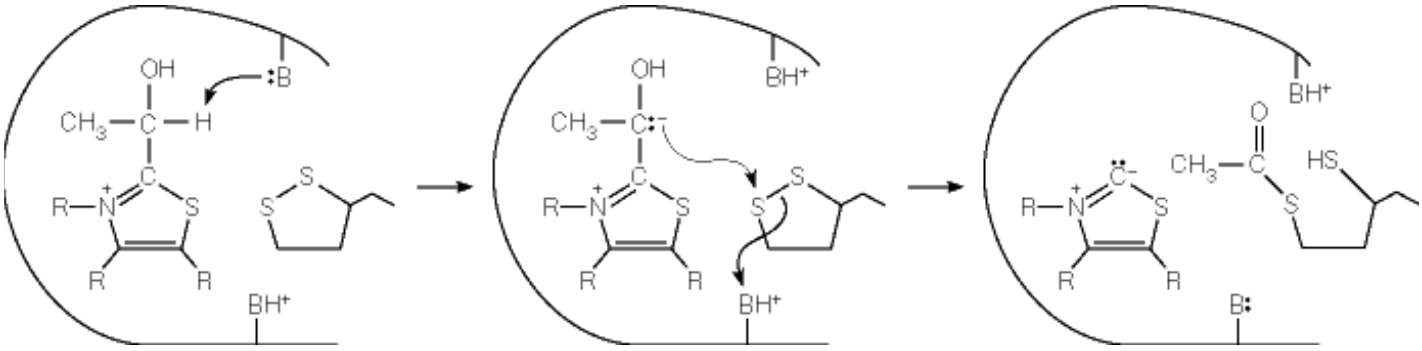

Addition compound
CO2
3
TPP-
only a small portion
is “useful”
H+
4
Eneamine intermediate
Hydroxyethyl-TPP (active acetaldehyde)
Acetaldehyde in yeast
(in yeast)
5
TPP
Lipoamide
In pyruvate
dehydrogenase



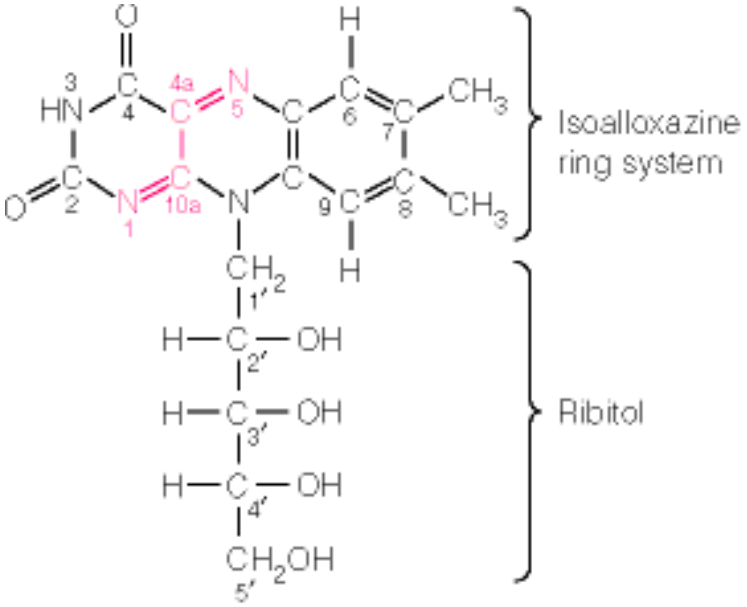
Lipoic Acid

Amide to lysine

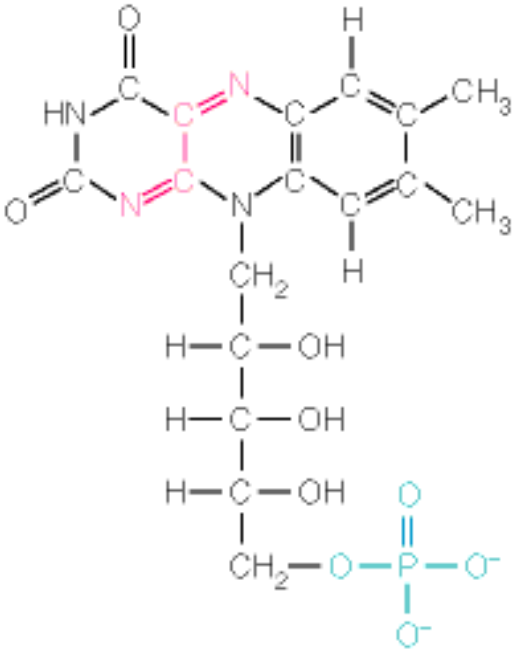
14 Å arm--Length & flexibility are important



The TPP hand off to lipoamide.

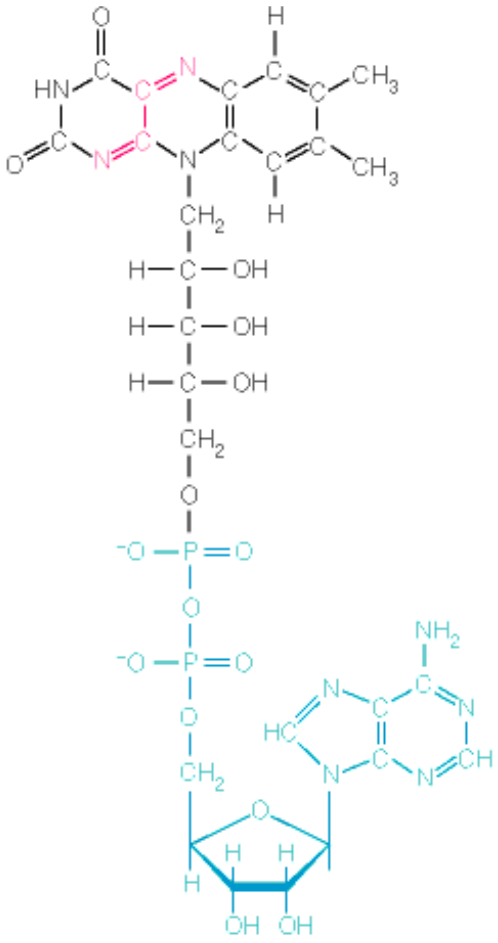


Riboflavin



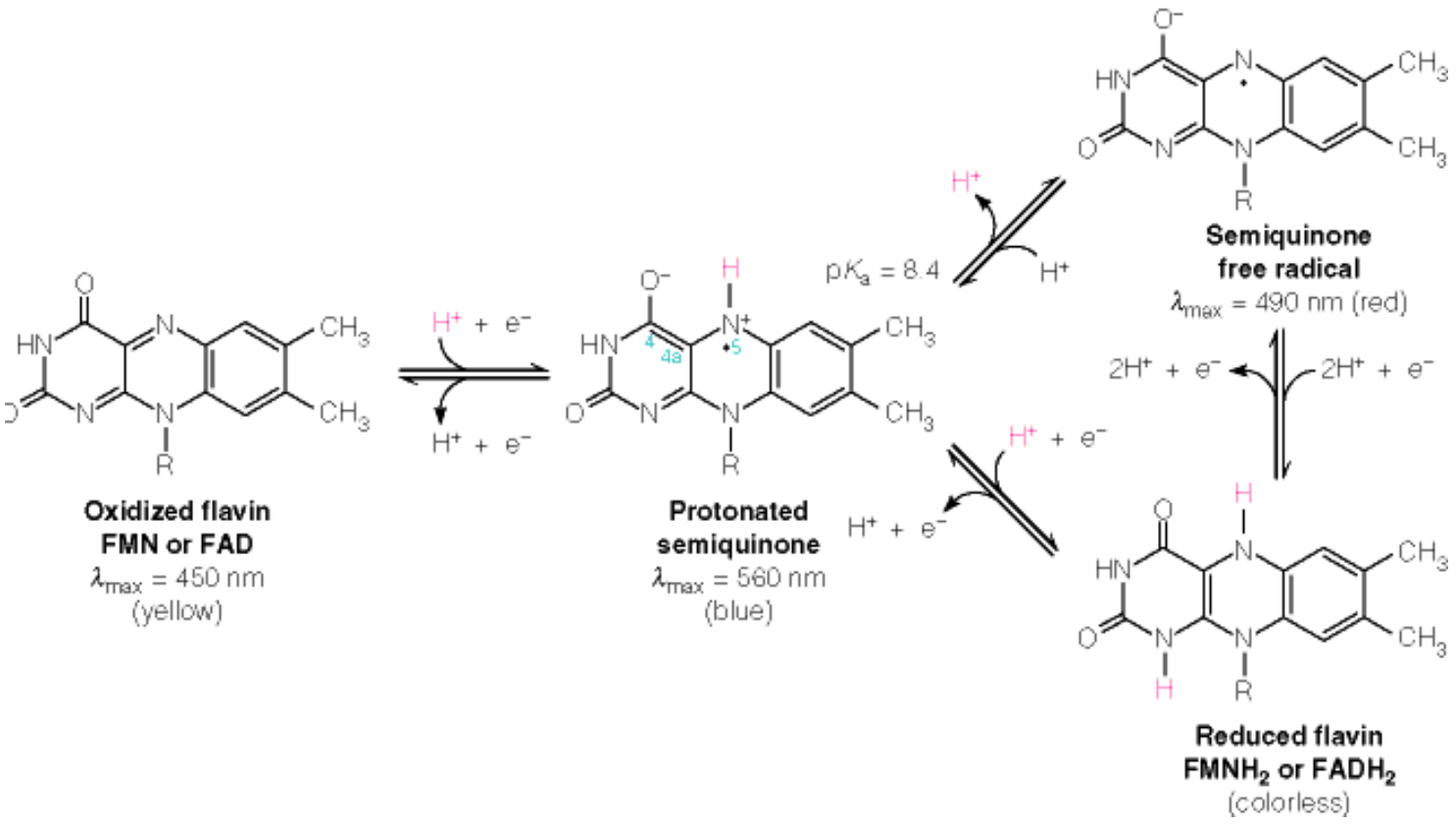
Flavin mononucleotide (FMN)
(also called riboflavin phosphate)

FLAVINS

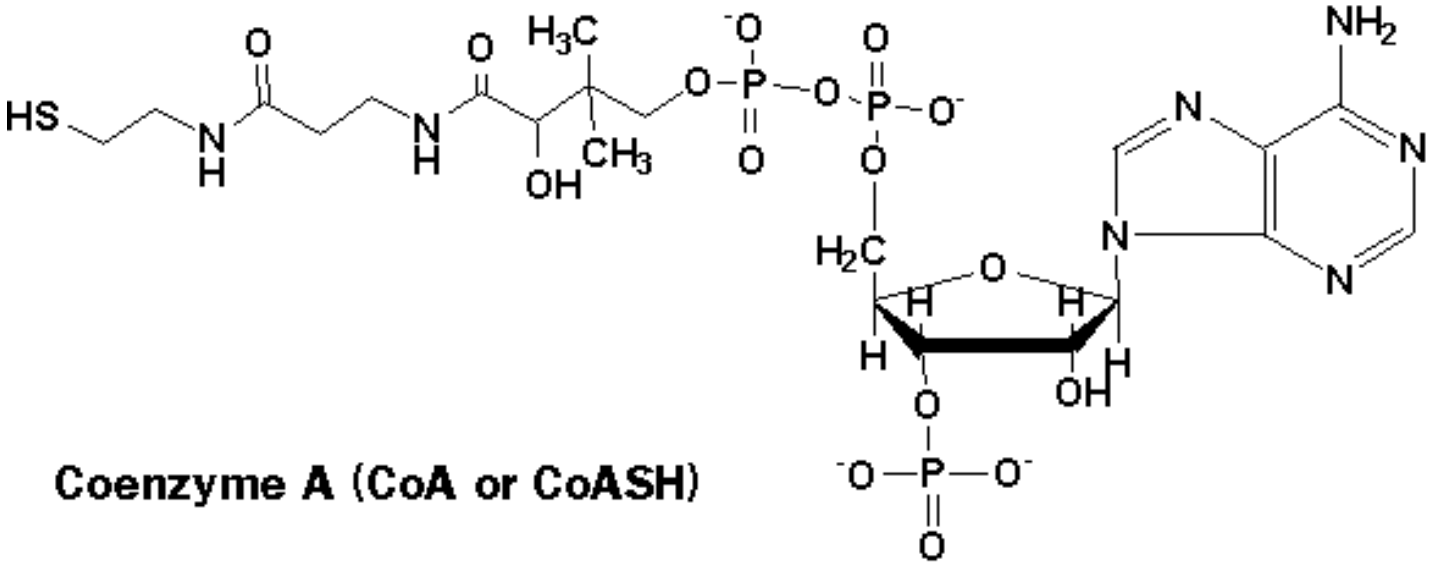


Flavin adenine dinucleotide (FAD)

FAD



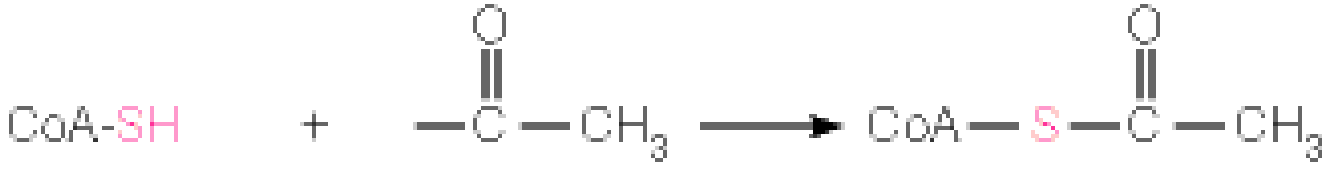
Flavins can accept one electron at a time.



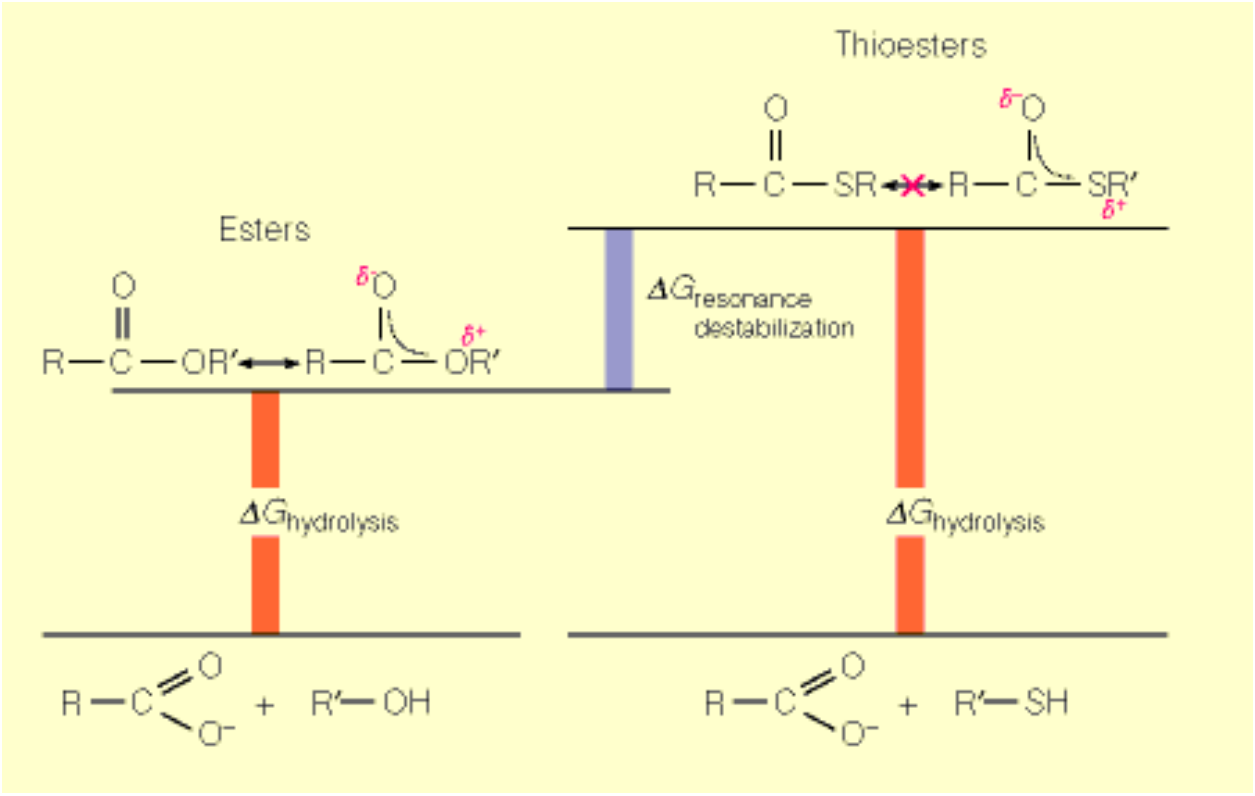
Coenzyme A (CoA or CoASH)

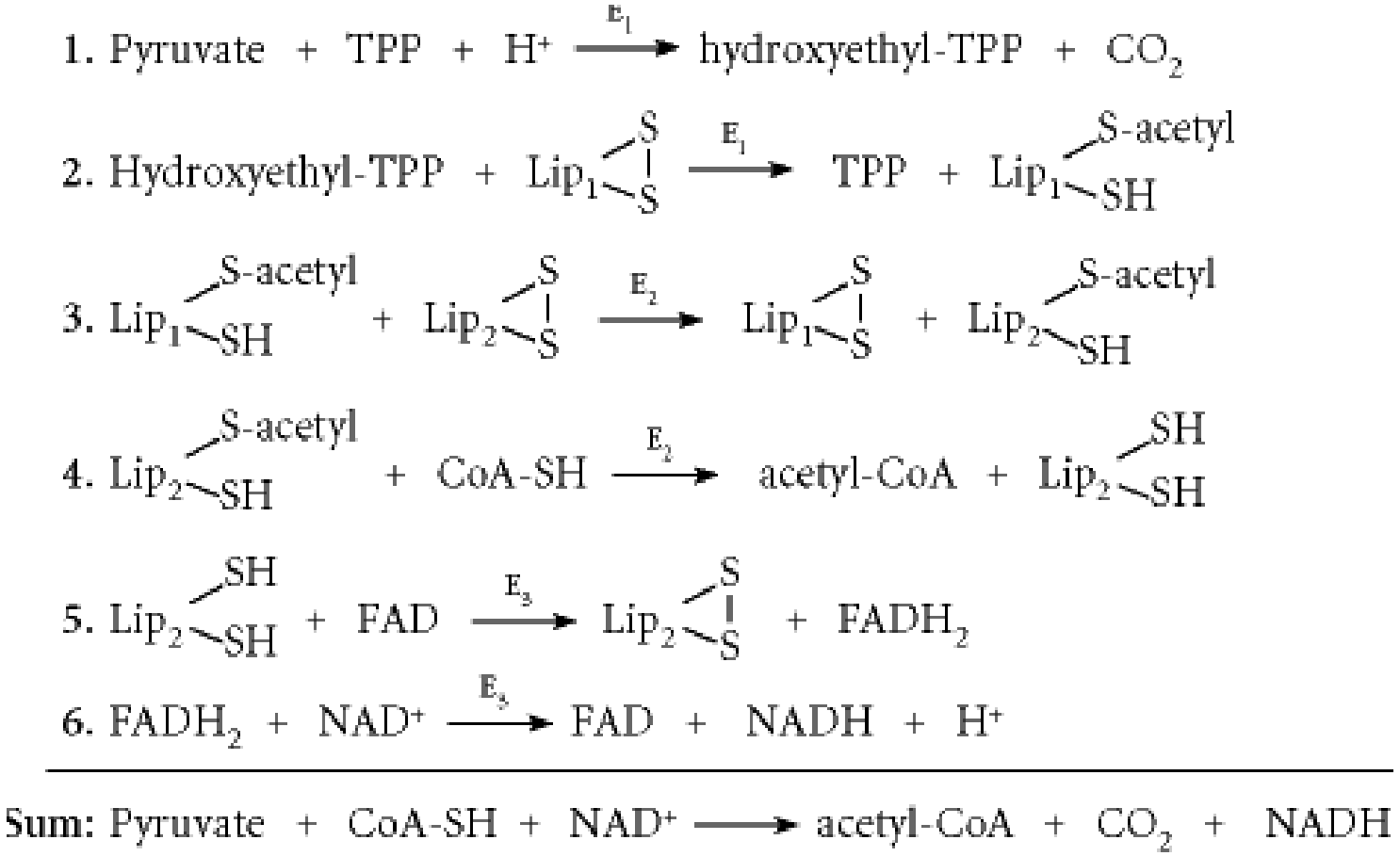
Co-enzyme A

Adenine-ribose-diphosphate-pantothenic acid-β mercaptoethylamine



Coenzyme A **Acetyl group** **Acetyl-CoA**



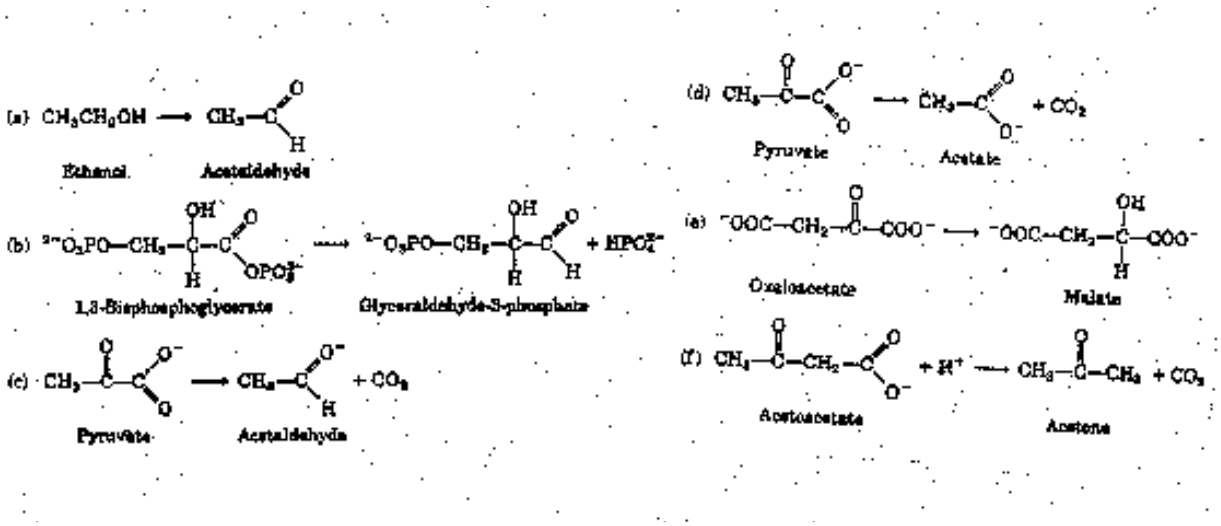

1. Pyruvate + TPP + H+ E1 hydroxyethyl-TPP + CO2
2. Hydroxyethyl-TPP + Lip1 S S E1 TPP + Lip1 S-acetyl SH
3. Lip1 S-acetyl SH + Lip2 S S E2 Lip1 S S + Lip2 S-acetyl SH
4. Lip2 S-acetyl SH + CoA-SH E2 acetyl-CoA + Lip2 SH SH
5. Lip2 SH SH + FAD E3 Lip2 S S + FADH2
6. FADH2 + NAD+ E3 FAD + NADH + H+
Sum: Pyruvate + CoA-SH + NAD+ acetyl-CoA + CO2 + NADH

Oxidation and Reduction

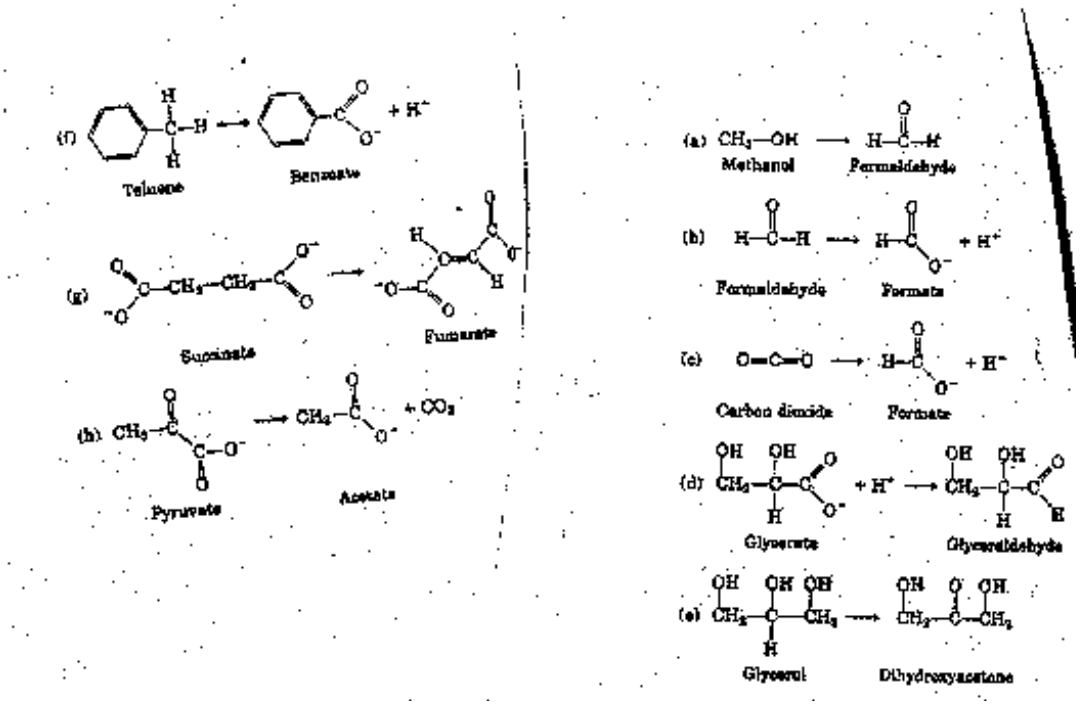
Oxidation--dehydrogenases
C combines with O
C loses H

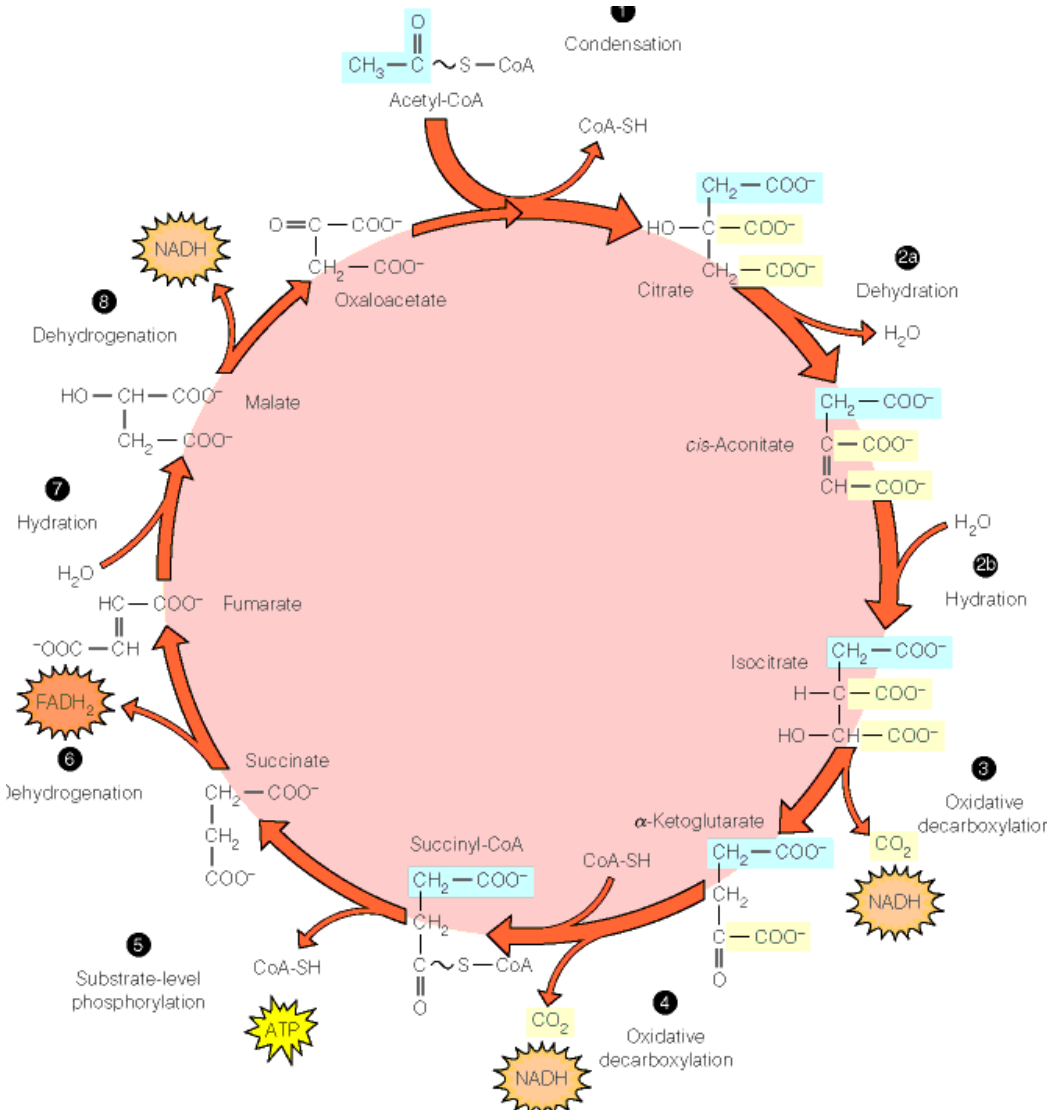
Reduction
C loses O
C combines with H

Redox Worksheet Part I --Circle the more reduced species

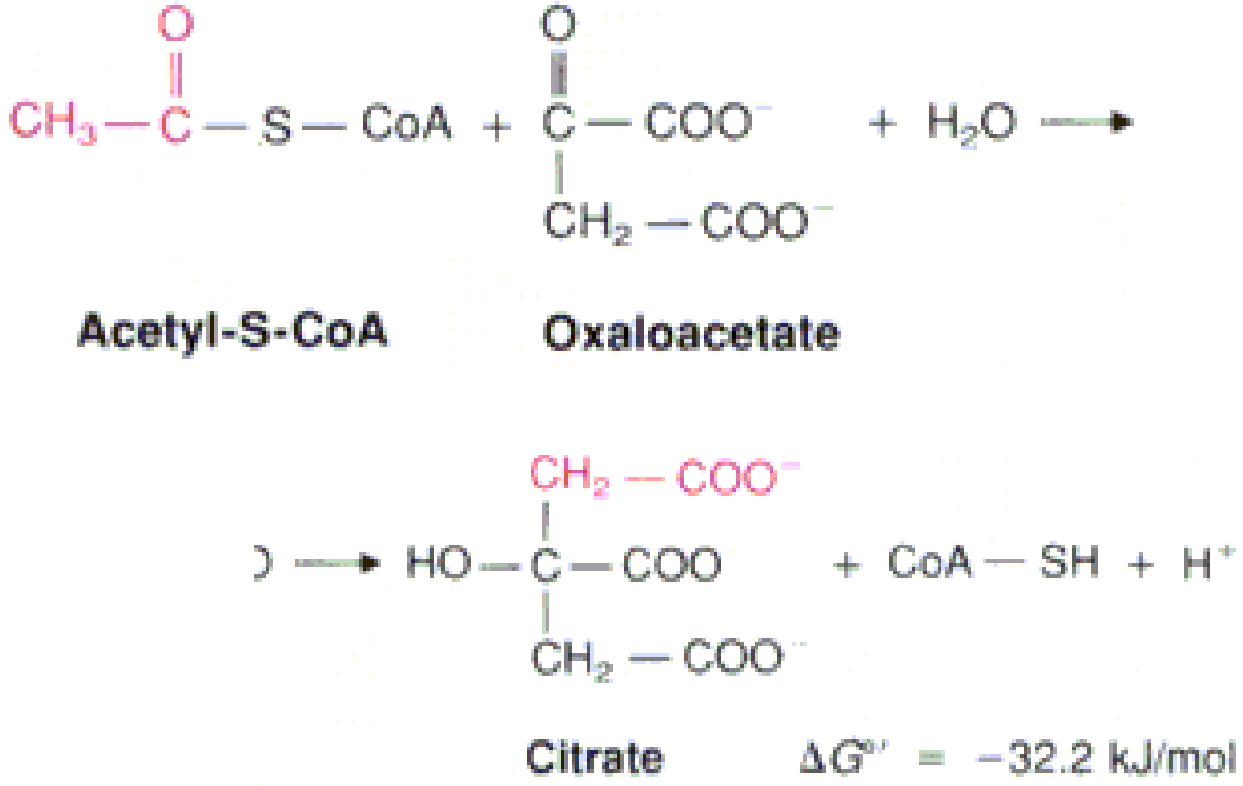


Redox Worksheet Part II --Circle the more reduced species



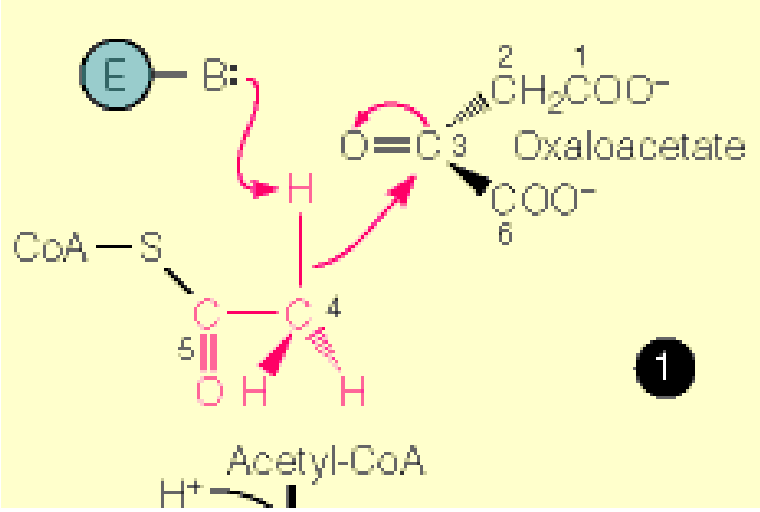

1
Condensation
Acetyl-CoA
CoA-SH
Citrate
2a
Dehydration
H_2O
cis-Aconitate
H_2O
2b
Hydration
Isocitrate
3
Oxidative decarboxylation
CO_2
NADH
α-Ketoglutarate
CoA-SH
4
Oxidative decarboxylation
CO_2
NADH
Succinyl-CoA
CoA-SH
5
Substrate-level phosphorylation
ATP
Succinate
6
Dehydrogenation
$FADH_2$
Fumarate
7
Hydration
H_2O
Malate
8
Dehydrogenation
NADH
Oxaloacetate

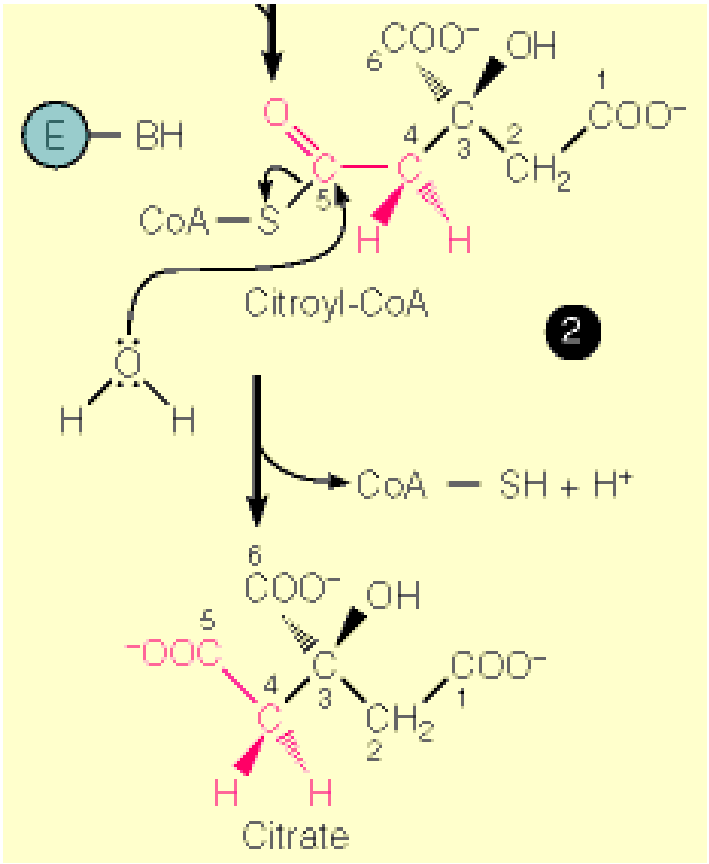
TCA RXN 1



Acetyl-S-CoA **Oxaloacetate**

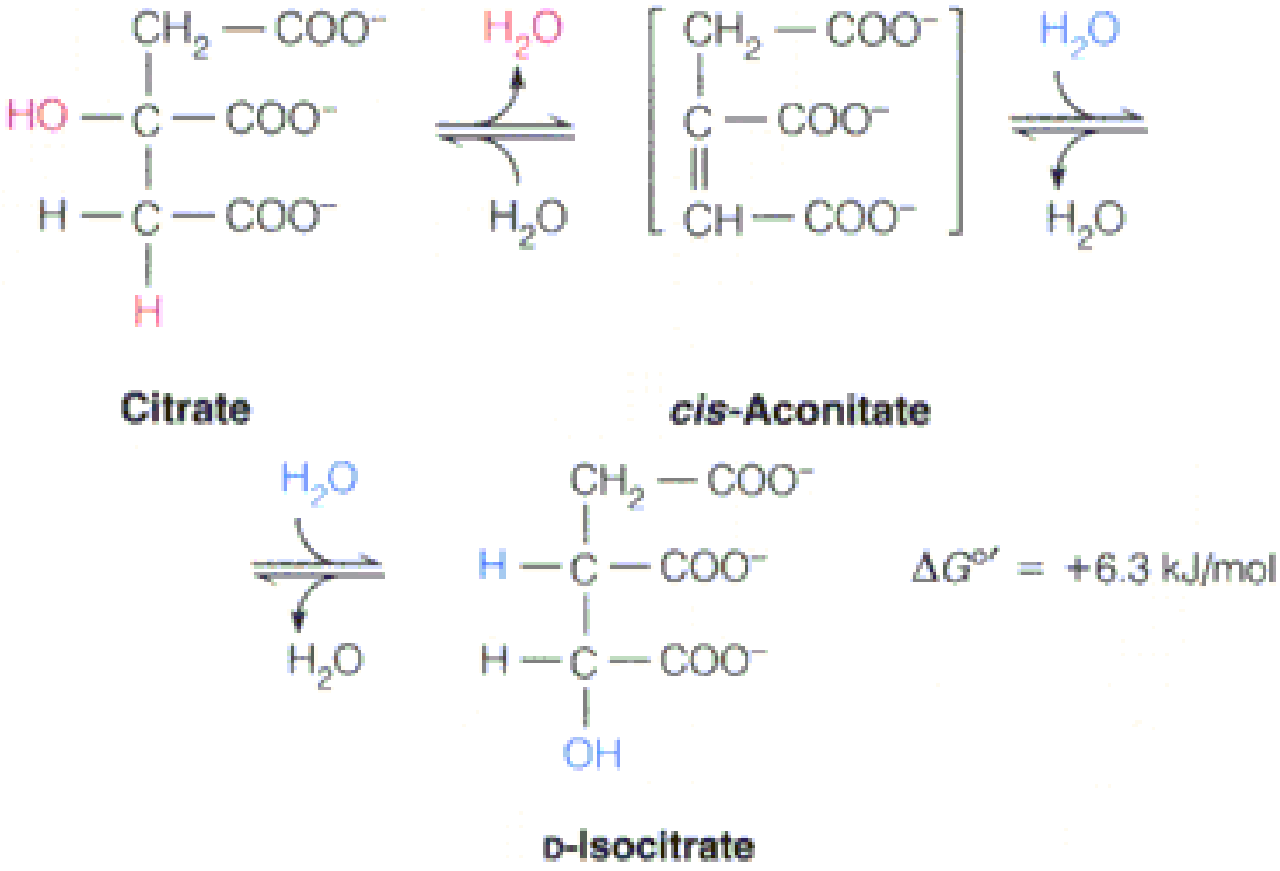
Citrate $\Delta G^{\circ\prime} = -32.2$ kJ/mol





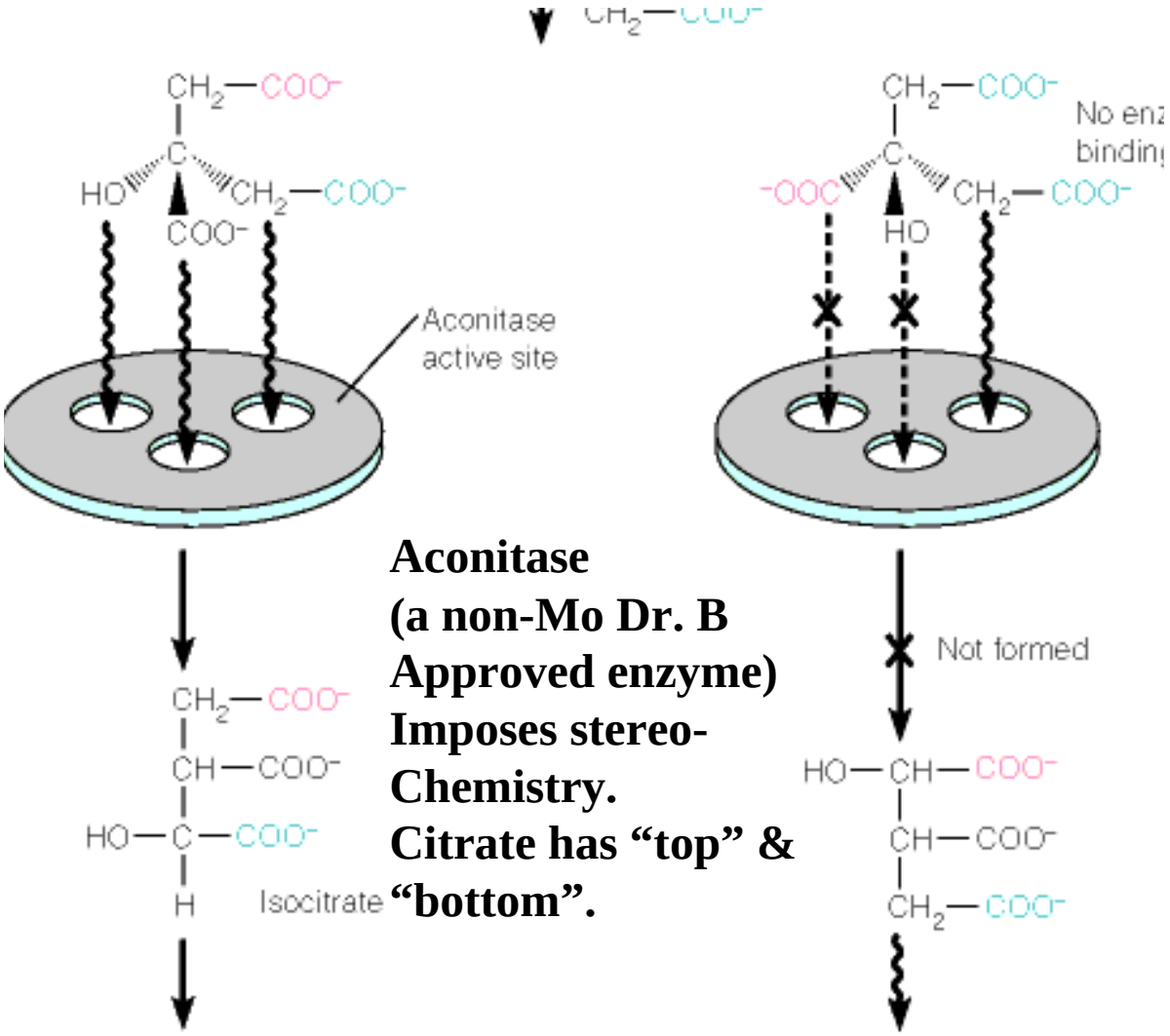
Mechanism--TCA Rxn 1

Formation of C-C bond
1) Proton Abstracted C4
2)C-C bond formation
3)Hydrolysis of S-C bond

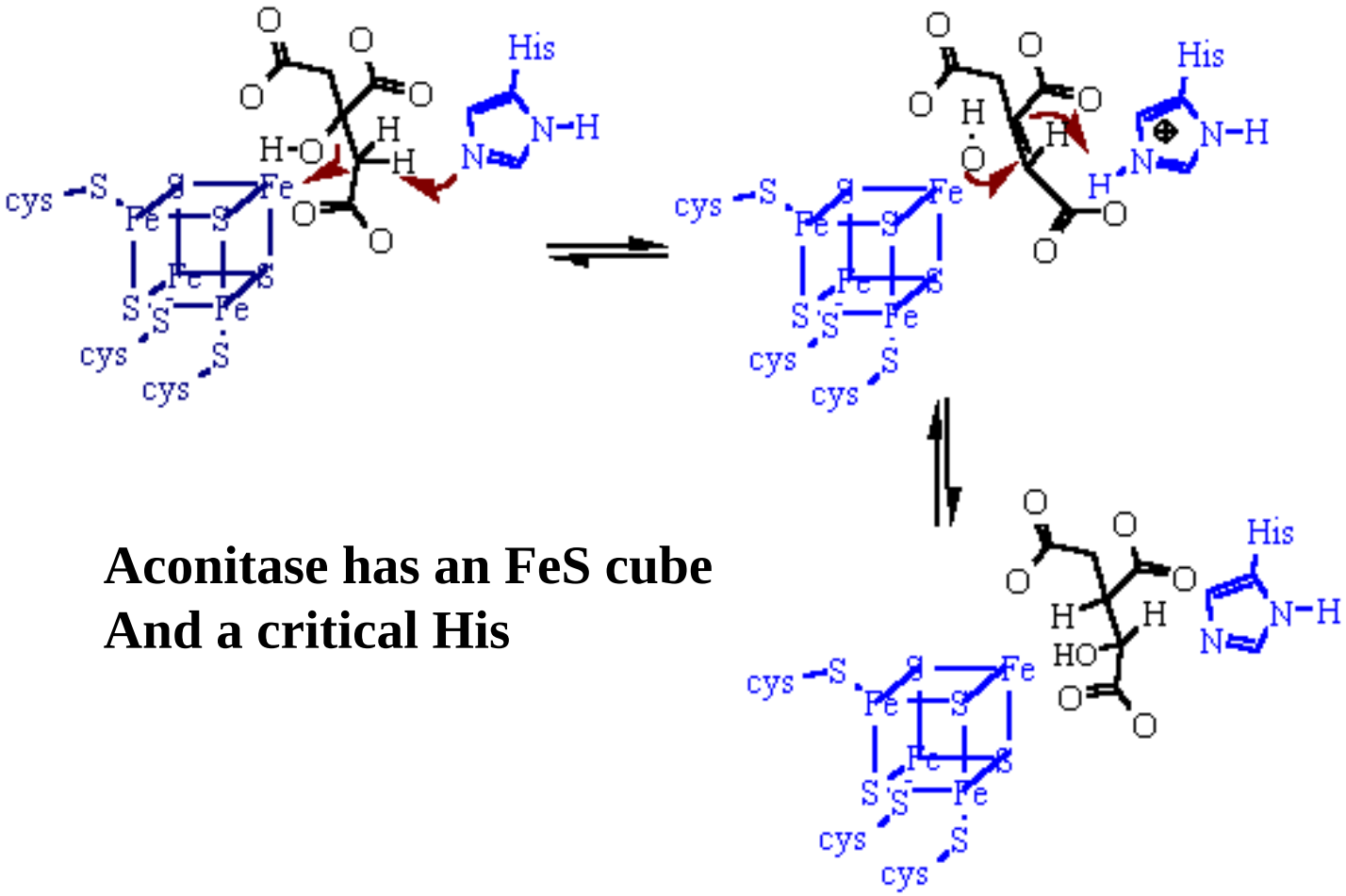


RXN 2 TCA--

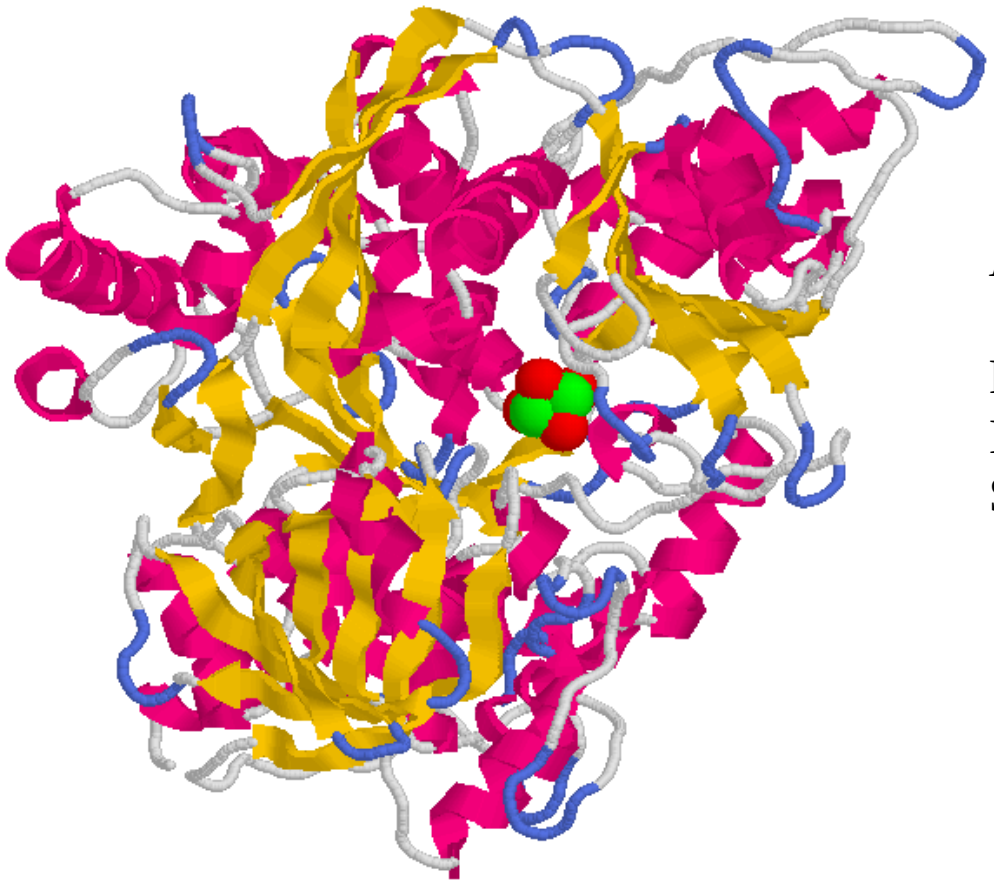
Permutation of OH via dehydrogenation/rehydrogenation



Aconitase
(a non-Mo Dr. B Approved enzyme)
Imposes stereo-Chemistry.
Citrate has “top” & “bottom”.

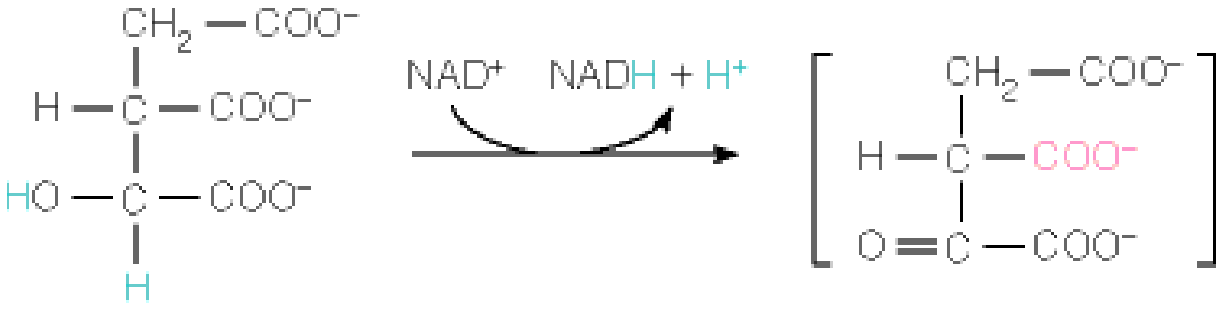


Aconitase has an FeS cube
And a critical His



Aconitase

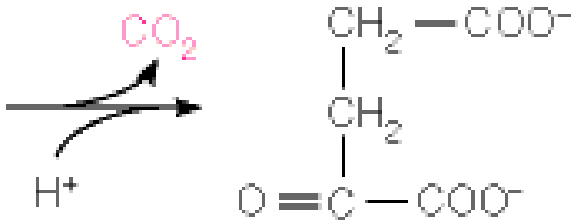
FeS cube
In red/green
Space filling



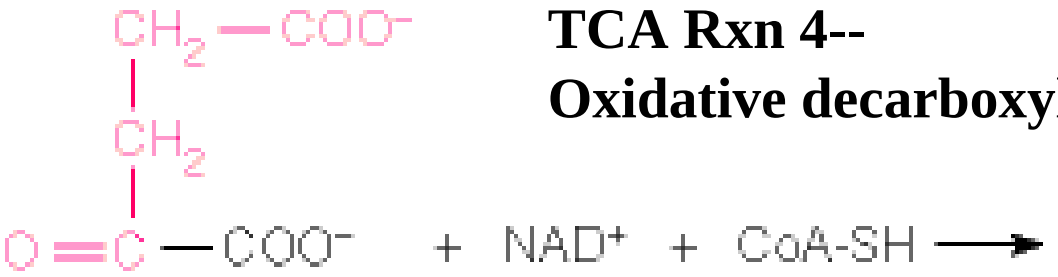
Isocitrate

Oxalosuccinate

TCA--Rxn 3--
isocitrate dehydrogenase
1) Oxidation of "bottom" OH
2) Decarboxylation of "middle" C



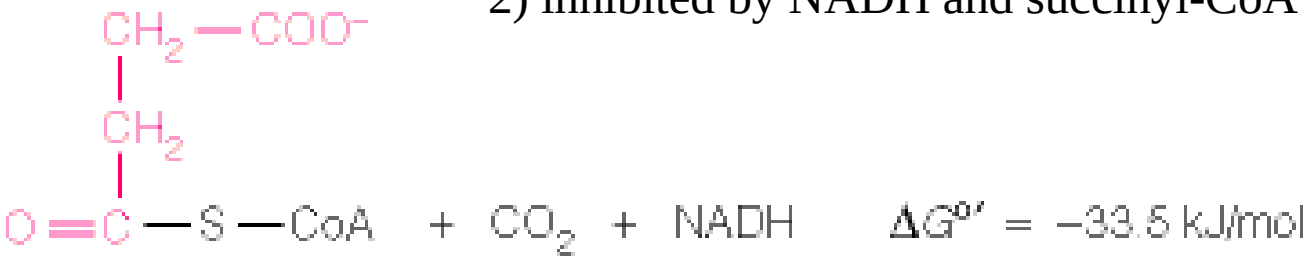
α-Ketoglutarate



α-Ketoglutarate + NAD+ + CoA-SH →

TCA Rxn 4--
Oxidative decarboxylation

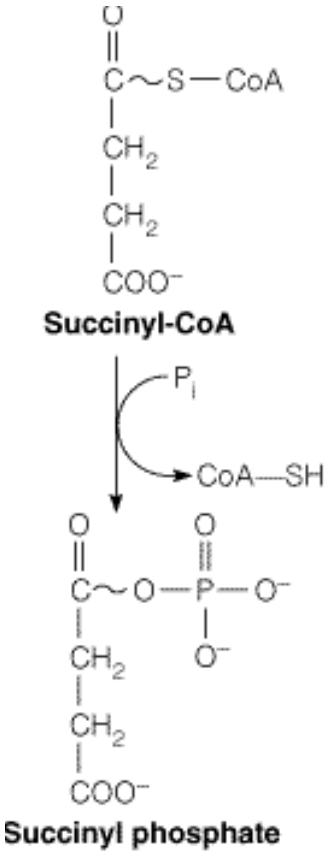
Alpha ketoglutarate dehydrogenase
1) large enzyme complex--
TPP, lipoic, FAD^+, NADH, CoASH
2) inhibited by NADH and succinyl-CoA

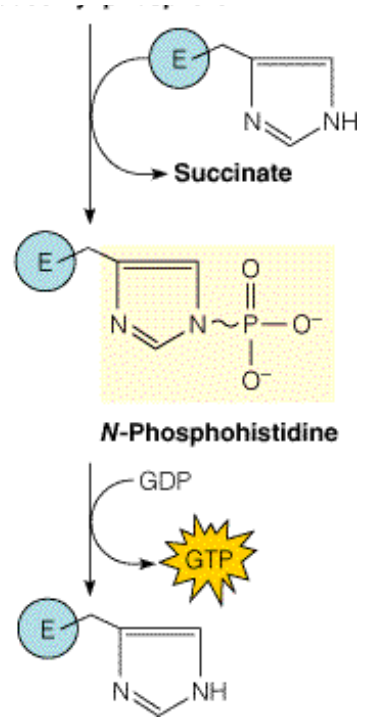


Succinyl-CoA + CO_2 + NADH $\Delta G^{o\prime} = -33.5$ kJ/mol

3)Mech. TPP decarboxylation >>>lipoic >>>attachment of CoA>>>re-oxidation of Lipoic arms>>>re-oxidation of FADH2 to Produce NADH

Succinyl-CoA + P_i + GDP $\rightleftharpoons$ succinate + GTP + CoA-SH
or ADP or ATP



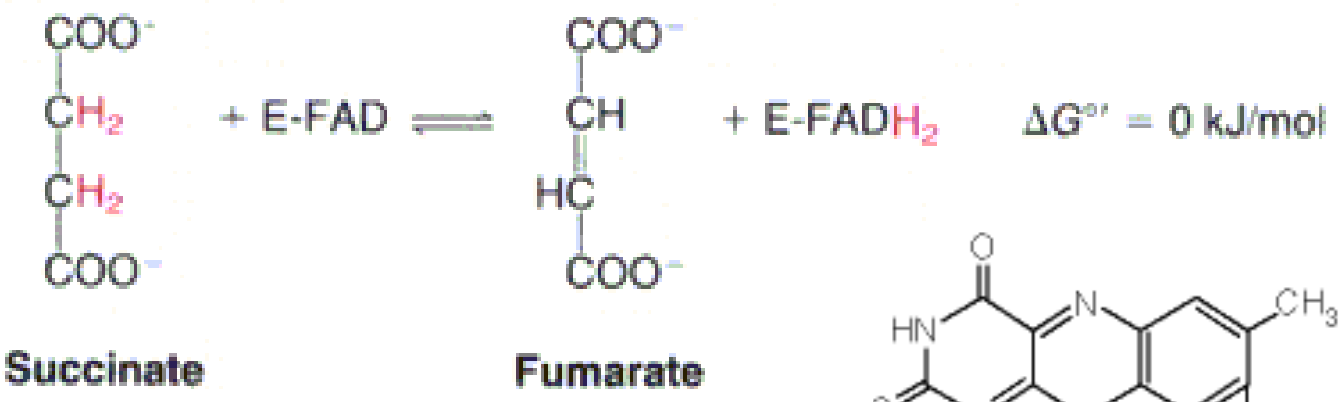


$\Delta G^{\circ\prime} = -2.9$ kJ/mol

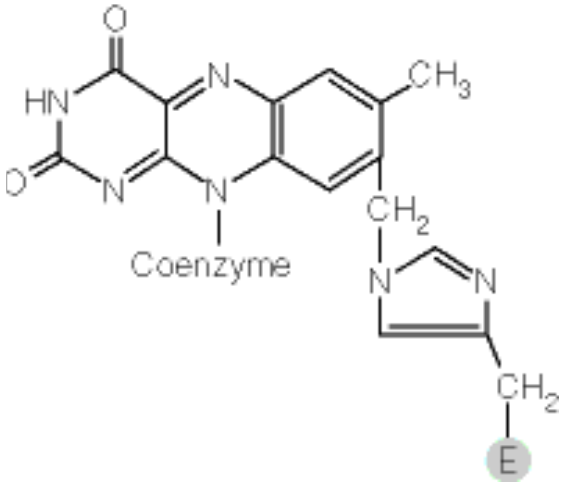
Succinyl CoA Synthetase

Phosphate transfer to Succ to enzyme to NDP

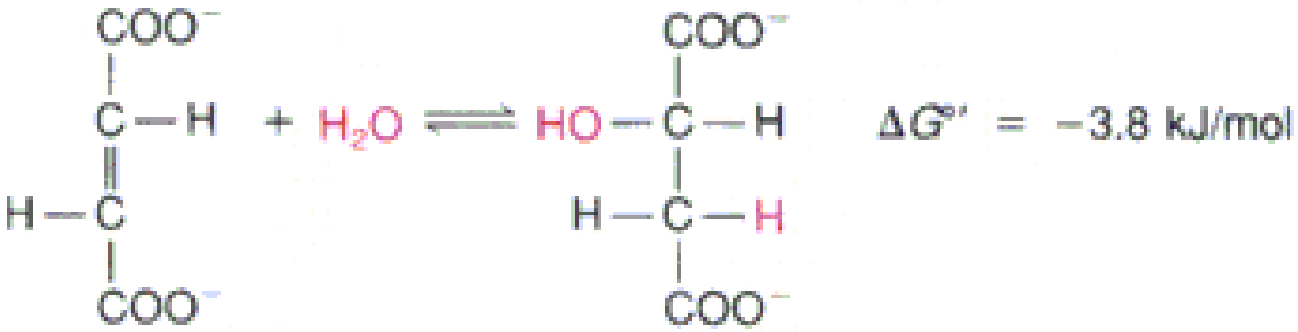
ATP(brain) or GTP (liver)
Depends on species/tissue



Always TRANS



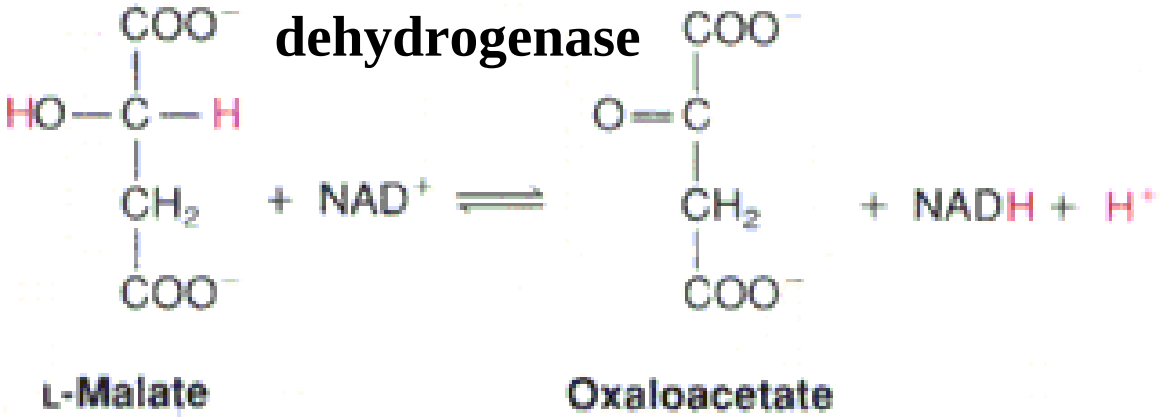
Reduced electron carriers, $FADH_2$ and NADH, are also stored forms of energy. Flavin is a stronger oxidant than NAD. Succinate dehydrogenase makes a covalent Bond to FAD and re-oxidizes it by passing electrons to Fe/S center.



Fumarase specifically hydrates the C=C (and won't work on the cis isomer.

Rxn 7 TCA

Malate dehydrogenase



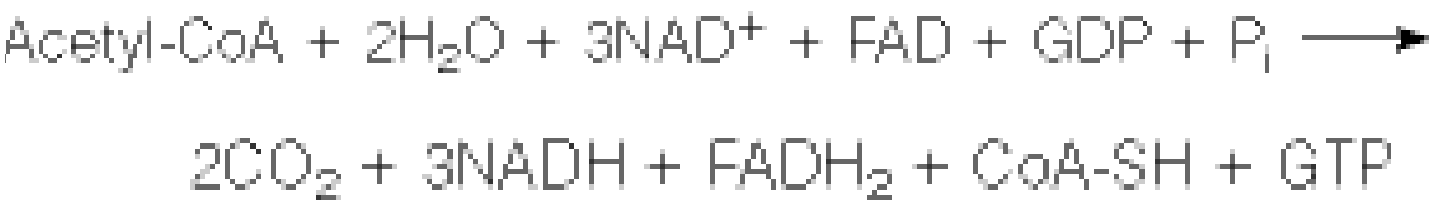
L-Malate Oxaloacetate

$\Delta G^{\circ\prime}$ = +29.7 kJ/mol

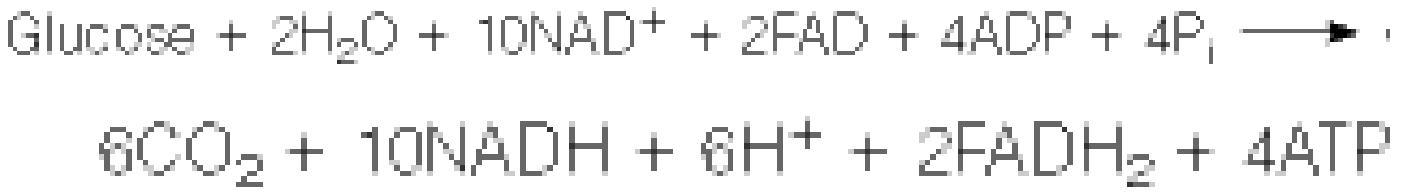
Rxn 8--oxaloacetate regenerated.
Its [ ] is very low to drive rxn to right.

Reaction	Enzyme	$\Delta G^{\circ\prime}$ (kJ/mol)
1. Acetyl-CoA + oxaloacetate + H_2O ⟶ citrate + CoA-SH + H^+	Citrate synthase	−32.2
2a. Citrate ⇌ *cis*-aconitate + H_2O	Aconitase	+6.3
2b. *cis*-Aconitate + H_2O ⇌ isocitrate	Aconitase	
3. Isocitrate + NAD^+ ⇌ α-ketoglutarate + CO_2 + NADH	Isocitrate dehydrogenase	−20.9
4. α-Ketoglutarate + NAD^+ + CoA-SH ⇌ succinyl-CoA + CO_2 + NADH	α-Ketoglutarate dehydrogenase complex	−33.5
5. Succinyl-CoA + P_i + GDP ⇌ succinate + GTP + CoA-SH	Succinyl-CoA synthetase	−2.9
6. Succinate + FAD (enzyme-bound) ⇌ fumarate + $FADH_2$ (enzyme-bound)	Succinate dehydrogenase	0
7. Fumarate + H_2O ⇌ L-malate	Fumarase	−3.8
8. L-Malate + NAD^+ ⇌ oxaloacetate + NADH + H^+	Malate dehydrogenase	+29.7
	Net	−57.3

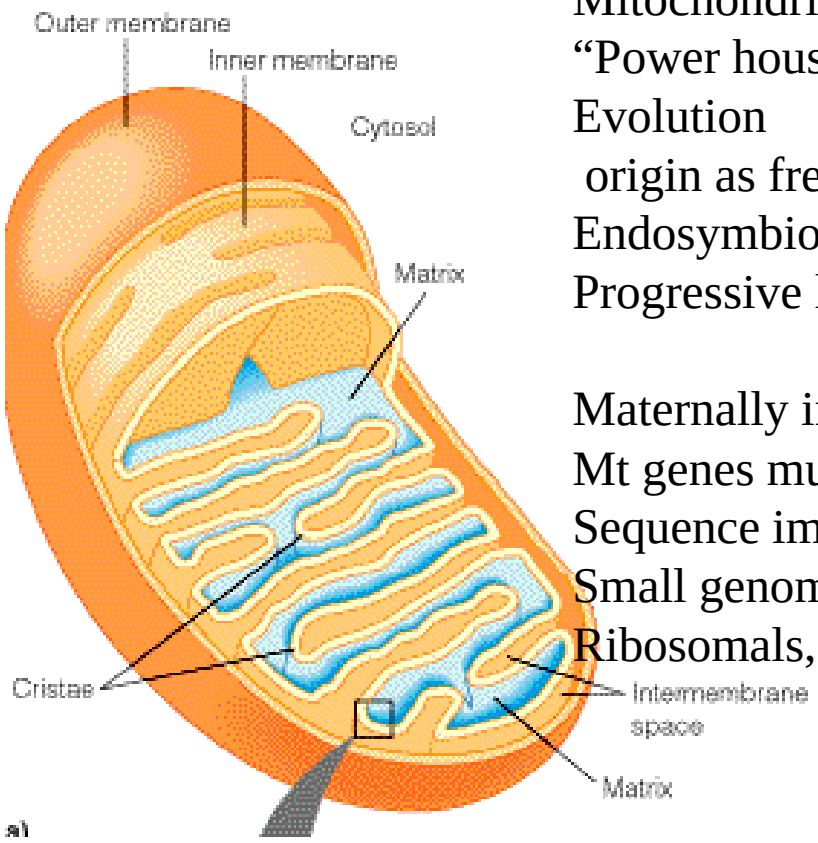
Net Citric Acid cycle reaction



Net Glycolysis 7 Citric Acid cycle reaction



Reduced electron carriers will produce even more ATP. The NADH/NAD+ ratio is important for regulation.



Mitochondria--
"Power house" of cell
Evolution
origin as free-living bacteria
Endosymbiosis/capture
Progressive loss of function/DNA

Maternally inherited--
Mt genes mutate quickly
Sequence important in human evolution
Small genomes encode ATP synthase,
Ribosomals, oxidative enzymes.

Inside Mitochondrium