Oxidative Pyruvate Decarboxylation and the Citric Acid Cycle

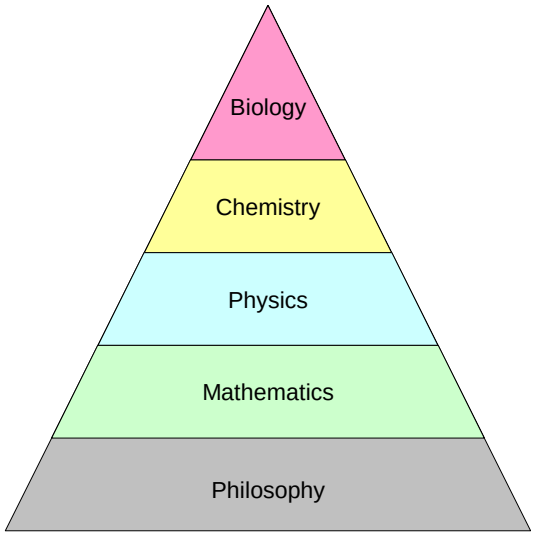
Fates of pyruvate

- Aerobic: oxidize to CO_2
- Anaerobic: fermentation

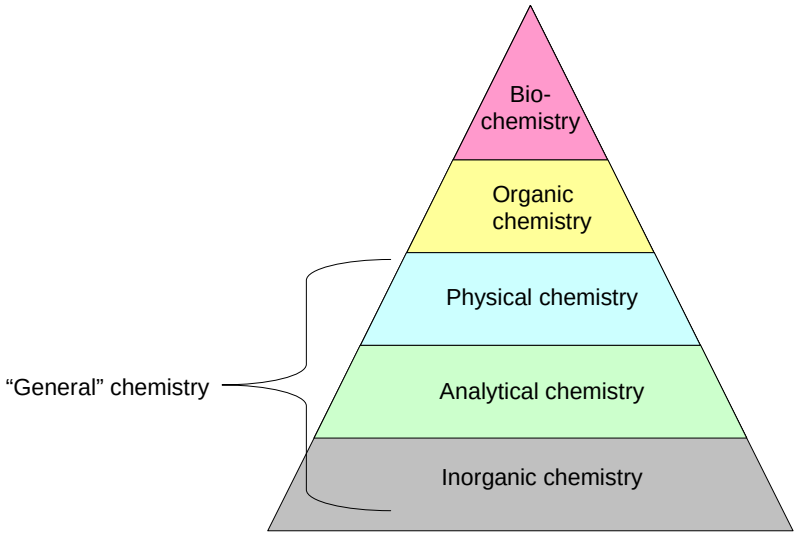
Core concept mastery check…..

If you want to really understand oxidative metabolism, you have to be able to keep track of electrons. To keep track of electrons, you have to know how to determine oxidation numbers!!

The hierarchy of scientific learning….



The hierarchy of biochemical learning..

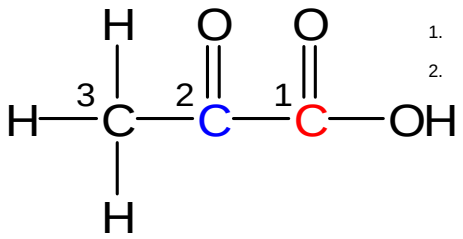


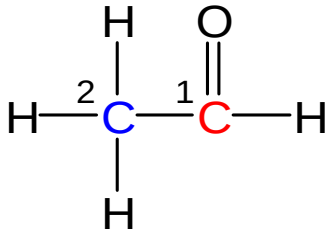
Rules for assigning oxidation numbers

1. For an atom in it’s elemental form, oxidation # = 0
2. For a monatomic ion, oxidation # = ion charge
3. For binary compounds of nonmetals: Assign the element with greater electronegativity the “ion charge” it would have if it was in an ionic compound
4. The sum of oxidation #s is 0 in a neutral compound, while the sum is the overall charge for polyatomic ions

Oxidation numbers for bioorganic compounds

1. Select the carbon atom you wish to assign an oxidation number
2. Assign oxidation numbers to the atoms bonded to the carbon atom of interest based on their electronegativities relative to the C you are focusing on:
    1. H = +1
    2. O = -2 (except for peroxides, where O = -1)
    3. N = -3
    4. S = -2
    5. C = 0
    6. Phosphorus-carbon bonds are rare in biology; in phosphate compounds, P = +5
3. If the atom bonded to the C of interest is in turn bonded to additional groups, this must be accounted for in assigning an oxidation number for the entire “group” relative to the C. For example, the “OH group” has oxidation number -1 since O is -2 and H is +1 (same for SH). The “NH2 group” also has oxidation number -1 since N is -3 and each H is +1. For an ether O or S, the -2 charge on the central O/S must be split between the two carbons to which it is bonded, so O/S contributes -1 to each C (see examples following pages).
4. When focusing on another C atom in the same compound, remember that any C atoms bonded to it are assigned oxidation numbers of “0” for the purpose of calculating oxidation numbers, even if they have nonzero oxidation numbers relative to the overall molecule
5. The sum of oxidation #s for all atoms is 0 in a neutral compound, while the sum of oxidation #s is the overall charge for an ionic species. For organic compounds that have ionizable groups, I recommend drawing the structure of the compound in the nonionized form so you don’t have to keep track of the net charge.
6. When assigned properly, oxidation numbers will allow you to keep track of which specific C atom(s) gain or lose electrons during an oxidation/reduction reaction





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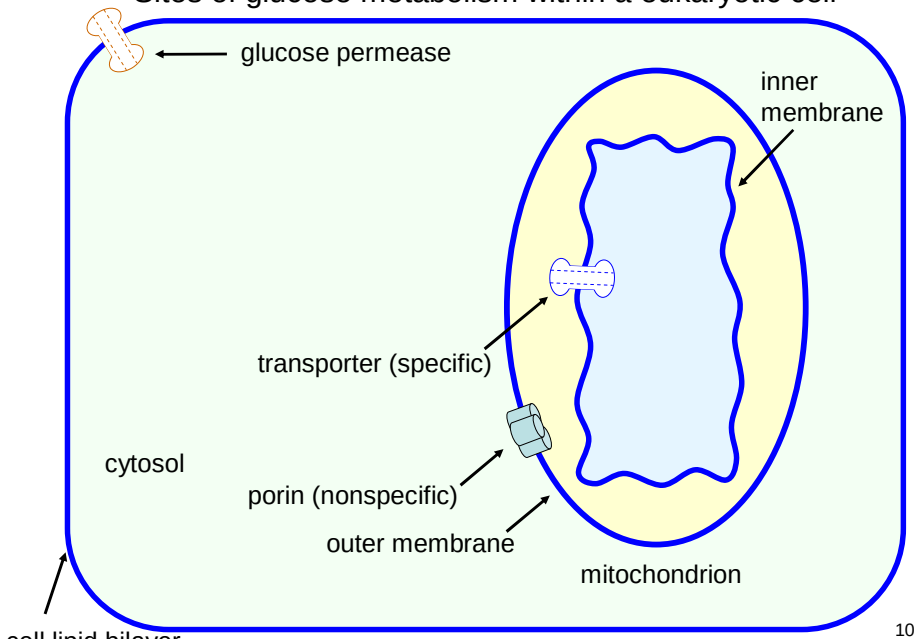
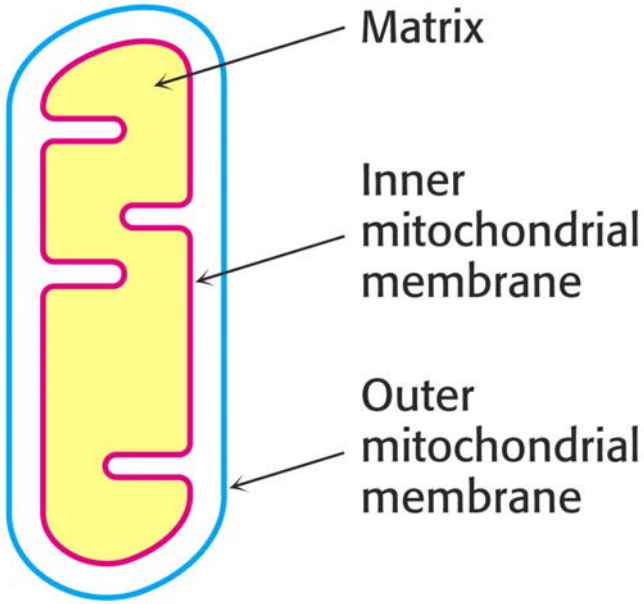
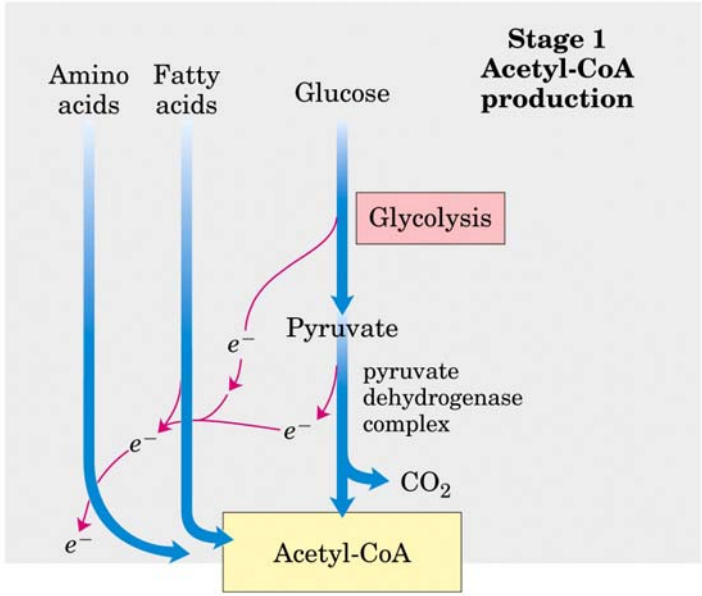
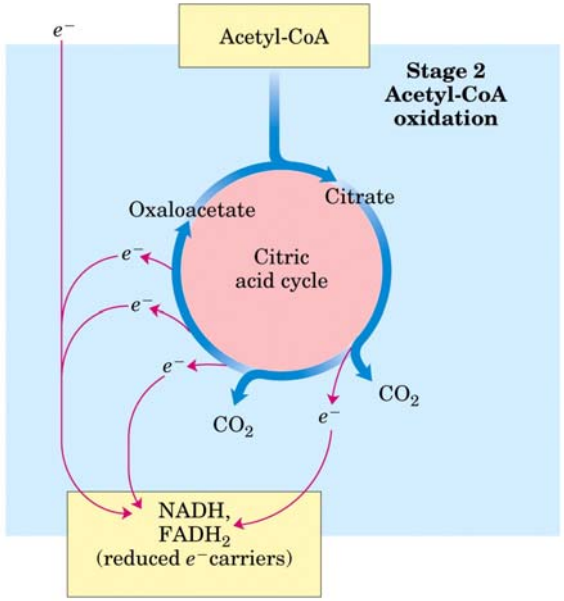
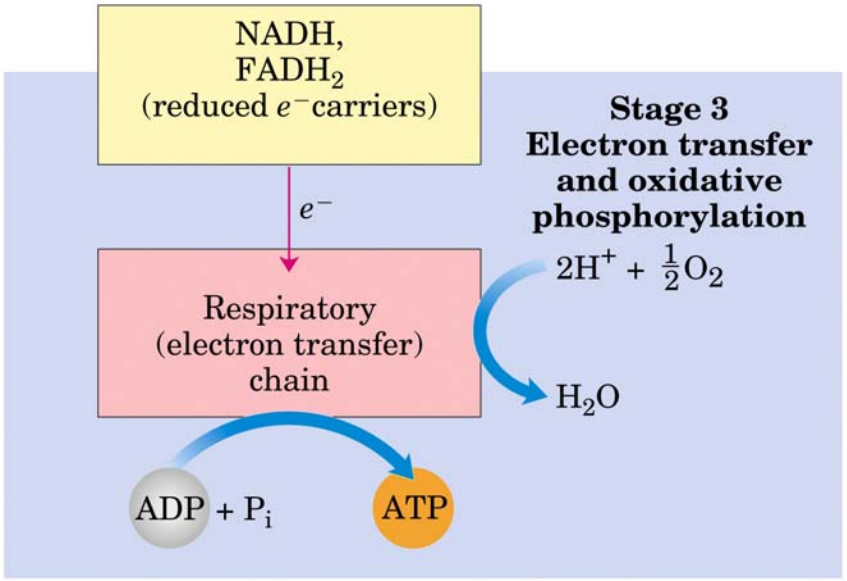
Sites of glucose metabolism within a eukaryotic cell

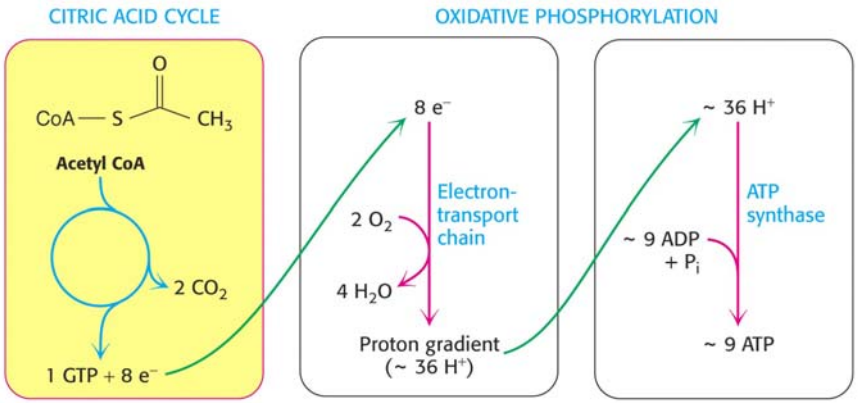




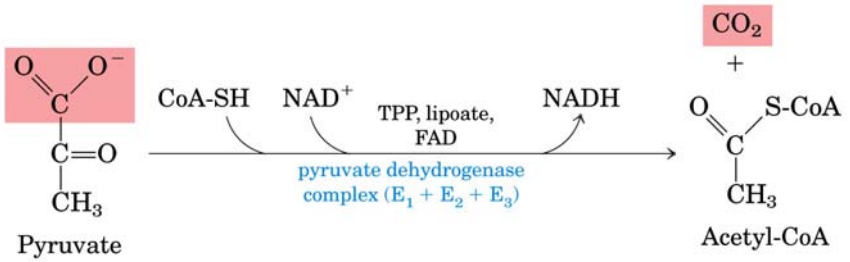








Oxidative pyruvate decarboxylation



$\Delta G'^{\circ} = -33.4$ kJ/mol

Pyruvate dehydrogenase

- A multienzyme complex consisting of three enzymes that work in unison
- E_1: pyruvate dehydrogenase
- E_2: dihydrolipoyl transacetylase
- E_3: dihydrolipoyl dehydrogenase

Pyruvate dehydrogenase cofactors

Cofactor	Vitamin precursor	Function	Bound or dissociable?
Thiamine pyrophosphate	Thiamin	Decarboxyl-ation	Bound, E_1
Lipoic acid (lipoamide)		e^- and acyl carrier	Bound, E_2
FAD	Riboflavin	e^- carrier	Bound, E_3
NAD^+	Niacin	e^- transfer	Diss. E_3
Coenzyme A	pantothenate	Acyl carrier	Diss. E_2

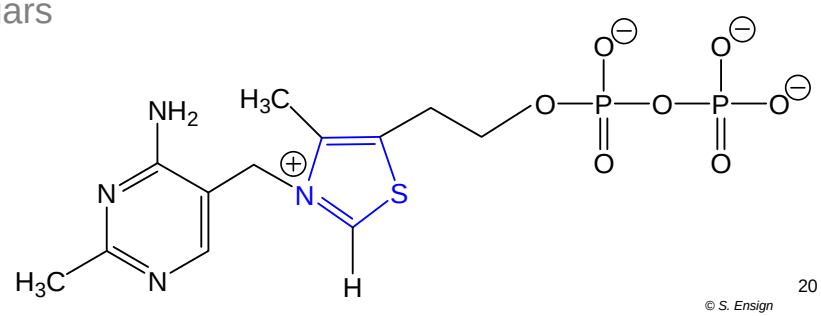
Introduction to the cofactors of the pyruvate dehydrogenase complex

- Three you have seen already
- Two which are new
- Tightly bound (catalytic):
  - TPP (noncovalent)
  - Lipoamide (covalent)
  - FAD (covalent in some enzymes, noncovalent in others
- Substrates in the reaction (get transformed):
  - NAD^+
  - Coenzyme A

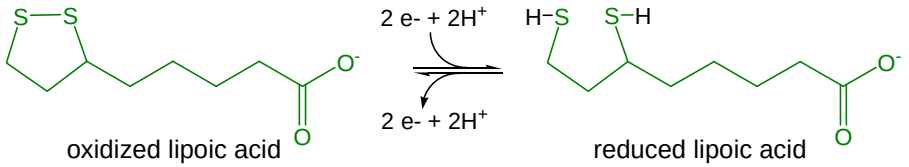
Review..

Thiamine pyrophosphate

- Pyruvate decarboxylase: “nonoxidative” decarboxylation- production of acetaldehyde
- Pyruvate dehydrogenase: “oxidative” decarboxylation
- Transketolase: intermolecular group transfer between sugars



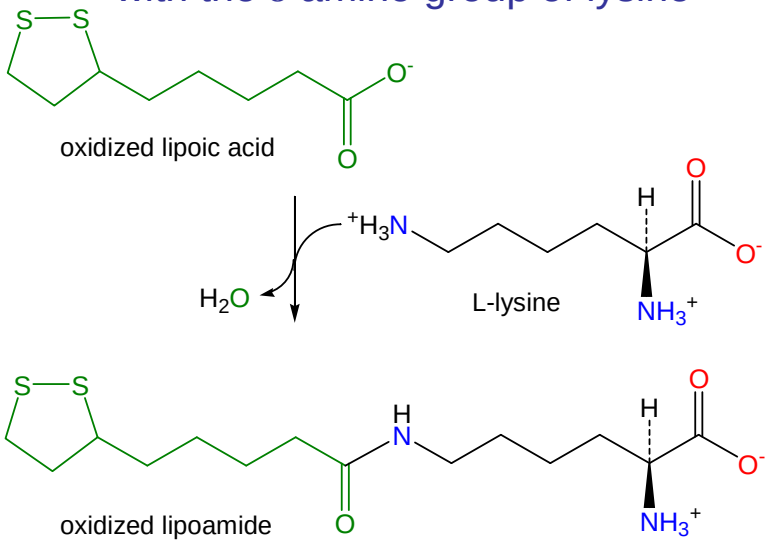
Lipoic acid



oxidized lipoic acid

reduced lipoic acid

Lipoamide formation by reaction with the ε-amino group of lysine

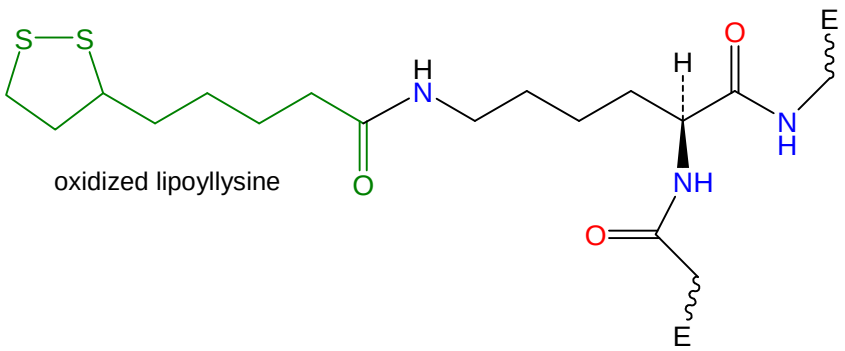


oxidized lipoic acid

L-lysine

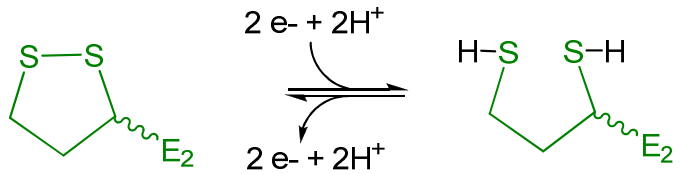
oxidized lipoamide

Lipoamide in a polypeptide



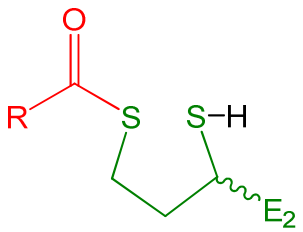
oxidized lipoyllysine

Biologically important forms of lipoamide



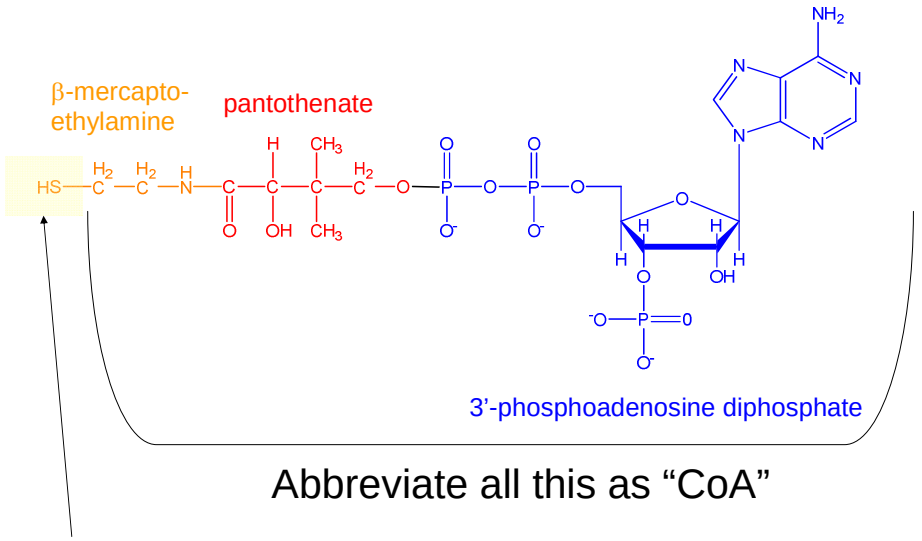
oxidized lipoamide

reduced lipoamide



Acyl lipoamide

Coenzyme A, an acyl group carrier



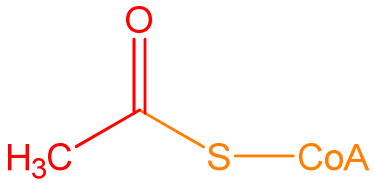
Abbreviate all this as “CoA”

Reactive thiol group

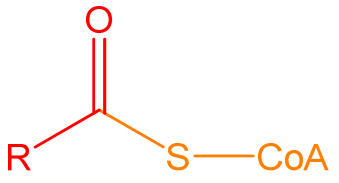
Coenzyme A, an acyl group carrier

Coenzyme A

Coenzyme A thiolate

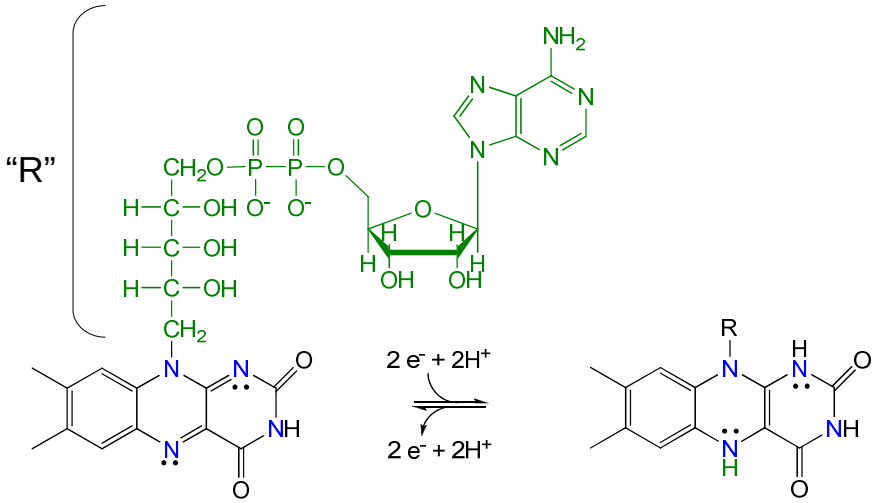


Acetyl-coenzyme A



Acyl-coenzyme A

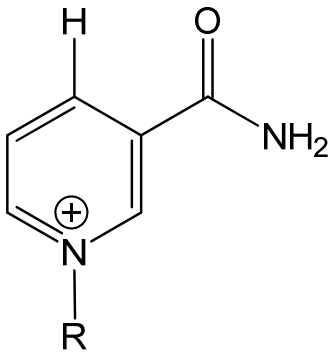
Flavin adenine dinucleotide



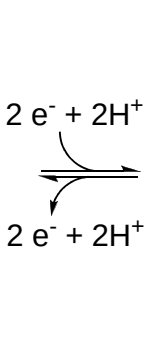
Oxidized FAD (FAD)

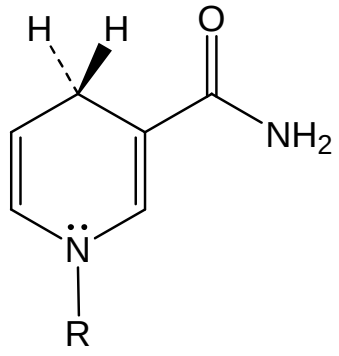
reduced FAD ($FADH_2$)

Nicotinamide adenine dinucleotide



NAD^+





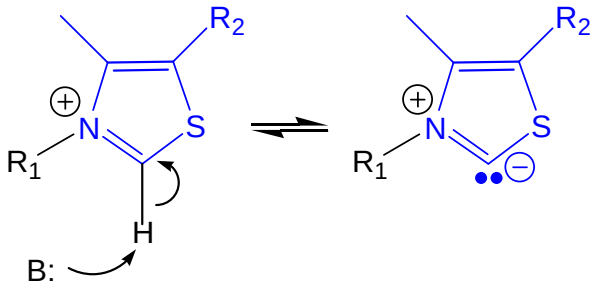
NADH

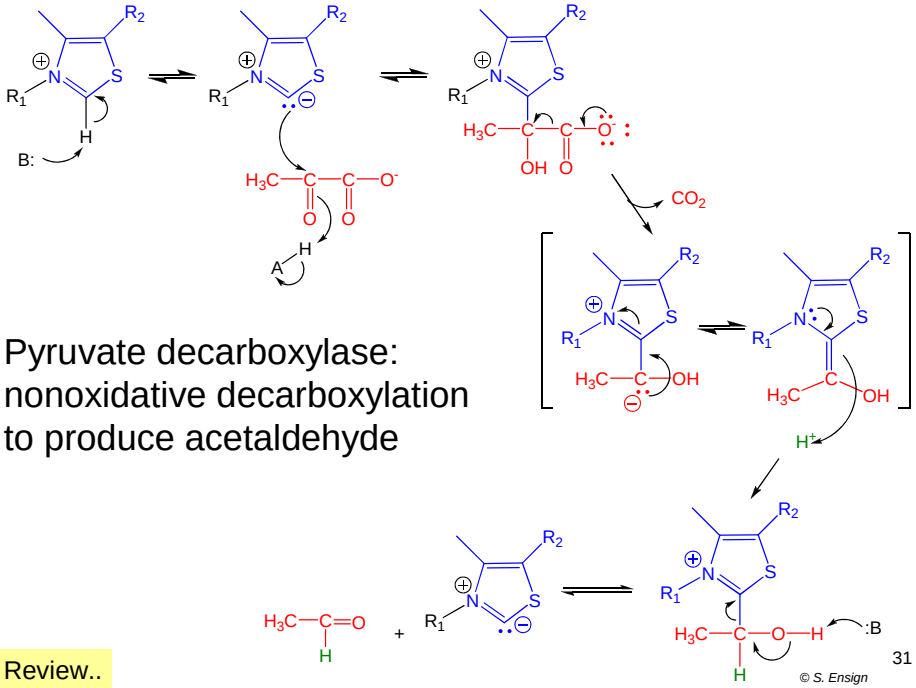
Pyruvate dehydrogenase mechanism

- The three enzyme components work in unison
- E_1 (pyruvate dehydrogenase) decarboxylates pyruvate as seen for pyruvate decarboxylase
- E_2 (dihydrolipoyl transacetylase) accepts the acetyl derived from pyruvate, and then transfers it to Coenzyme A. In the process lipoamide is reduced by two electrons
- E_3 (dihydrolipoyl dehydrogenase) reoxidizes lipoamide using FAD as the initial e^- acceptor and NAD^+ as the terminal e- acceptor
- The lipoate group "pivots" on E_2, translocating between E_1 and E_3 during catalysis

Review..

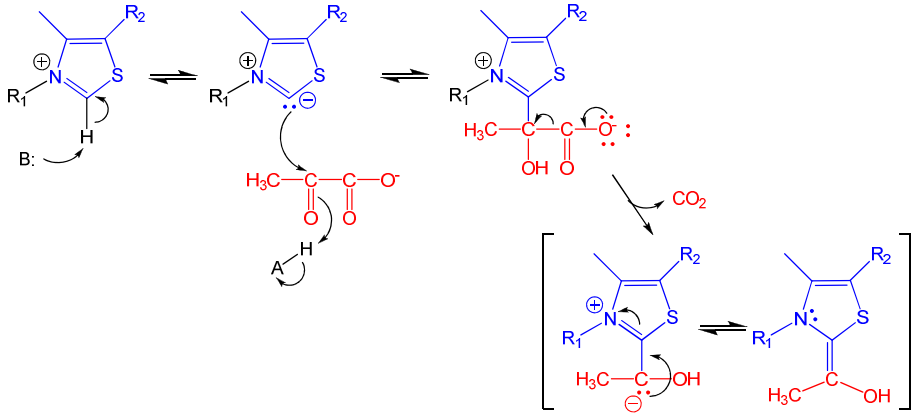
Recall mechanism of pyruvate decarboxylase





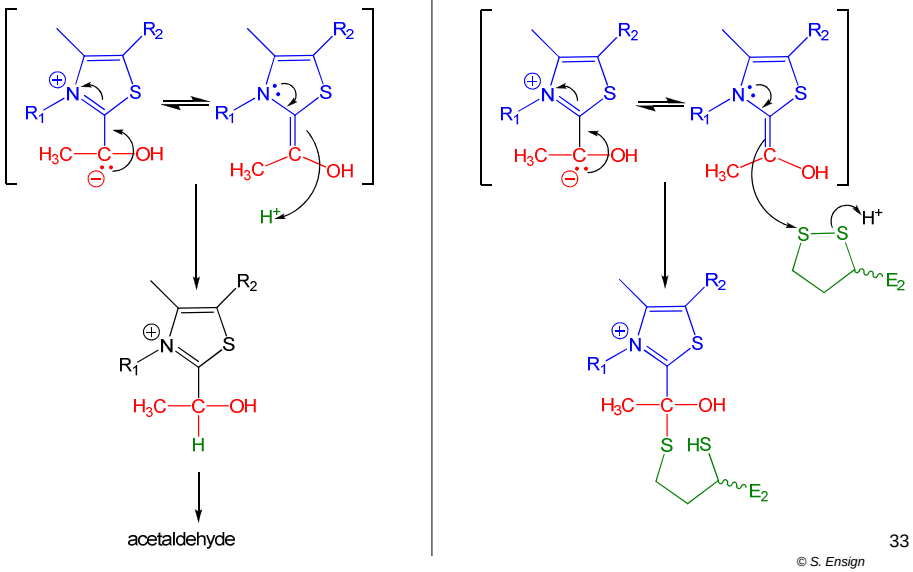
Pyruvate decarboxylase: nonoxidative decarboxylation to produce acetaldehyde

Review..



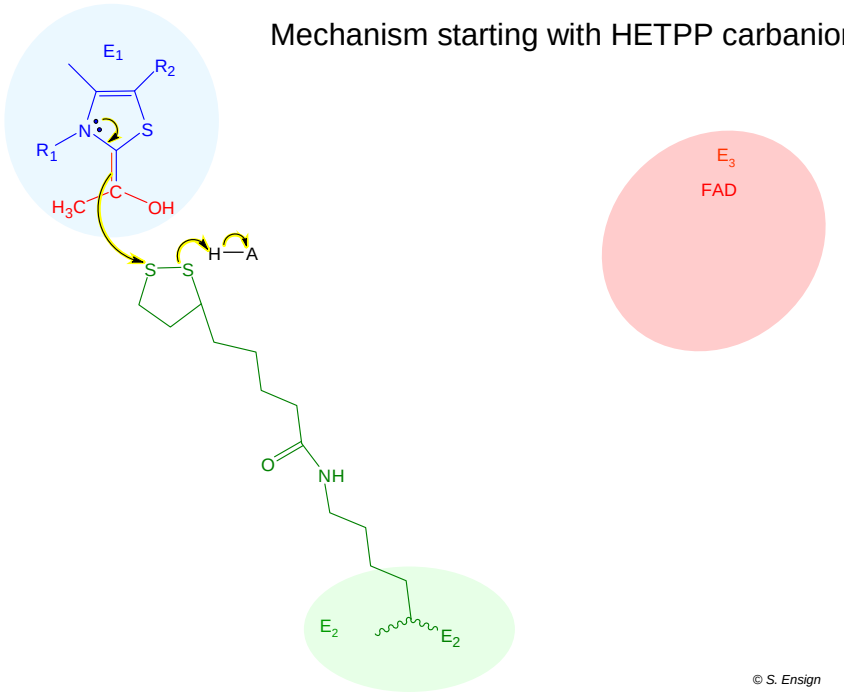
The mechanisms of pyruvate carboxylase and pyruvate dehydrogenase diverge at the level of the hydroxyethyl-TPP carbanion

In oxidative pyruvate decarboxylation, the hydroxyethyl-TPP anion is transferred to lipoamide rather than H+

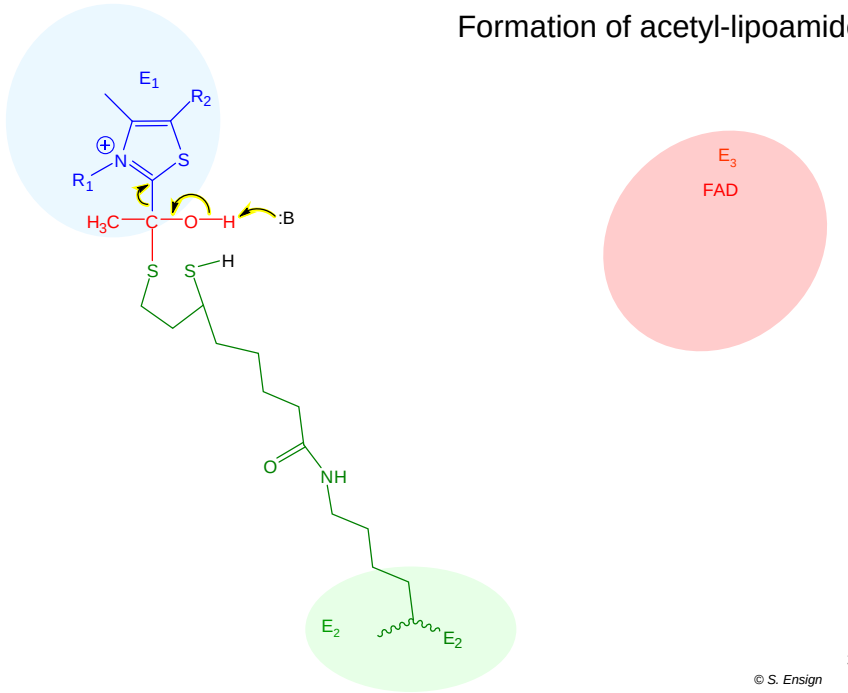


Watch how the interplay of the activities of the three subunits is mediated by the movement of the flexible linker arm connecting the “business end” of lipoamide

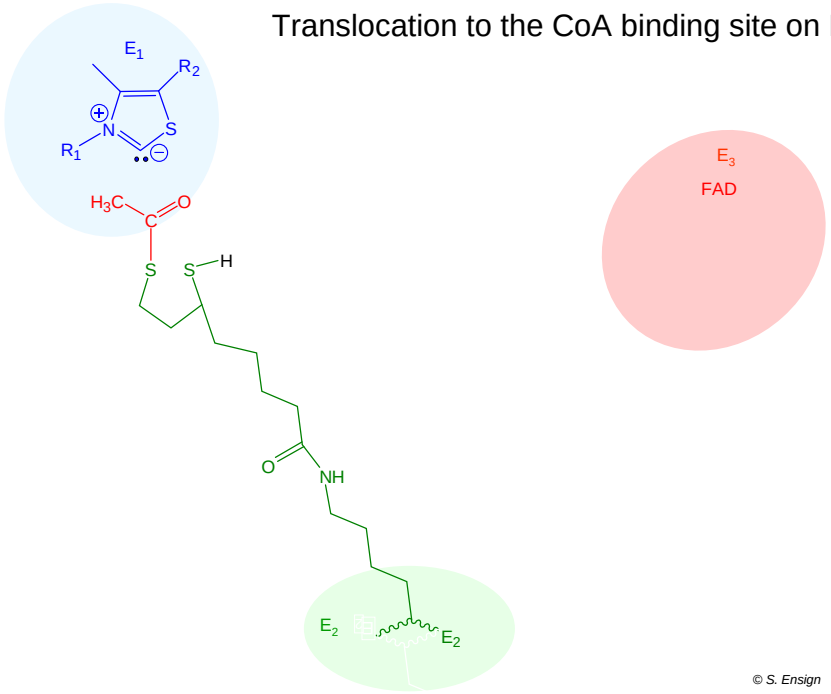
Mechanism starting with HETPP carbanion



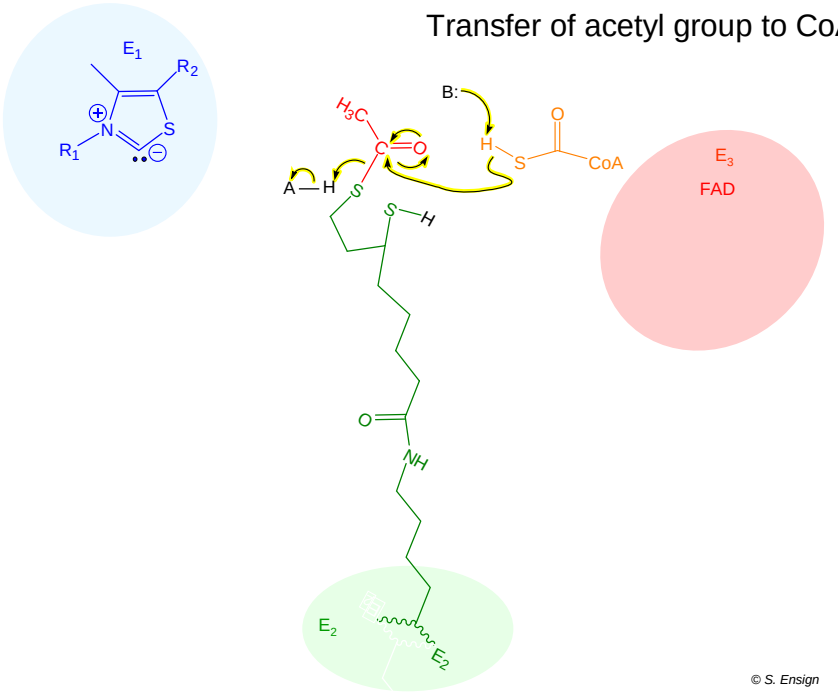
Formation of acetyl-lipoamide



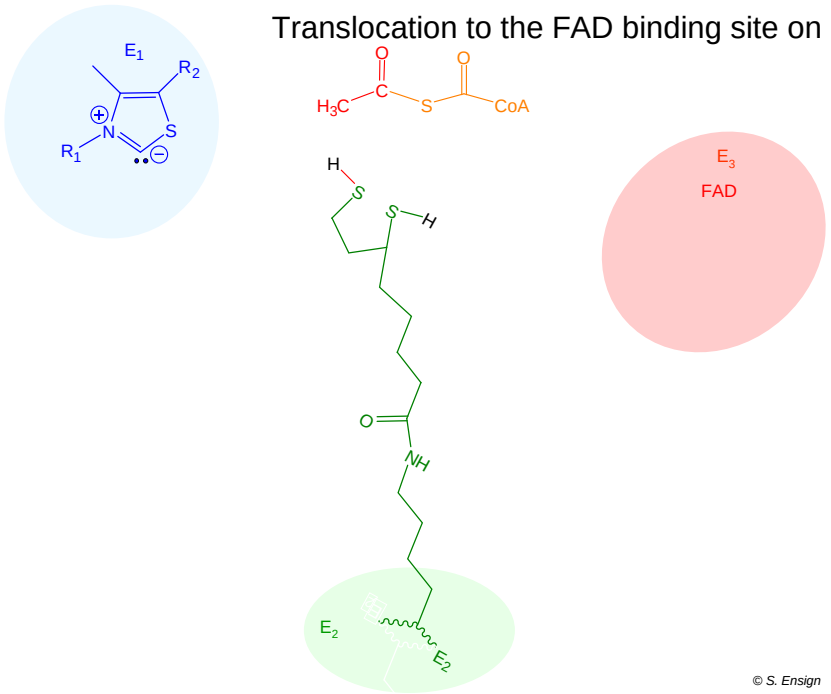
Translocation to the CoA binding site on E_2



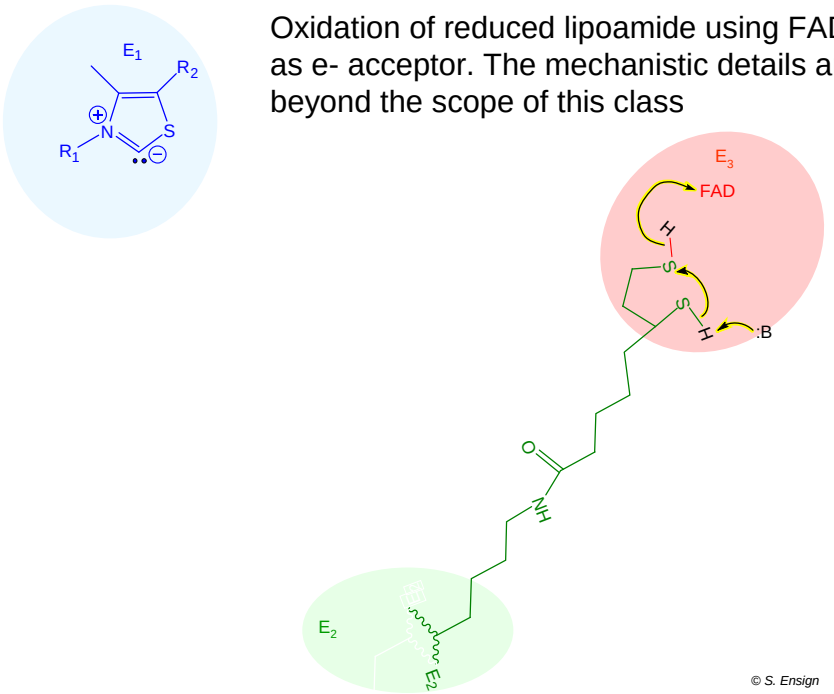
Transfer of acetyl group to CoA

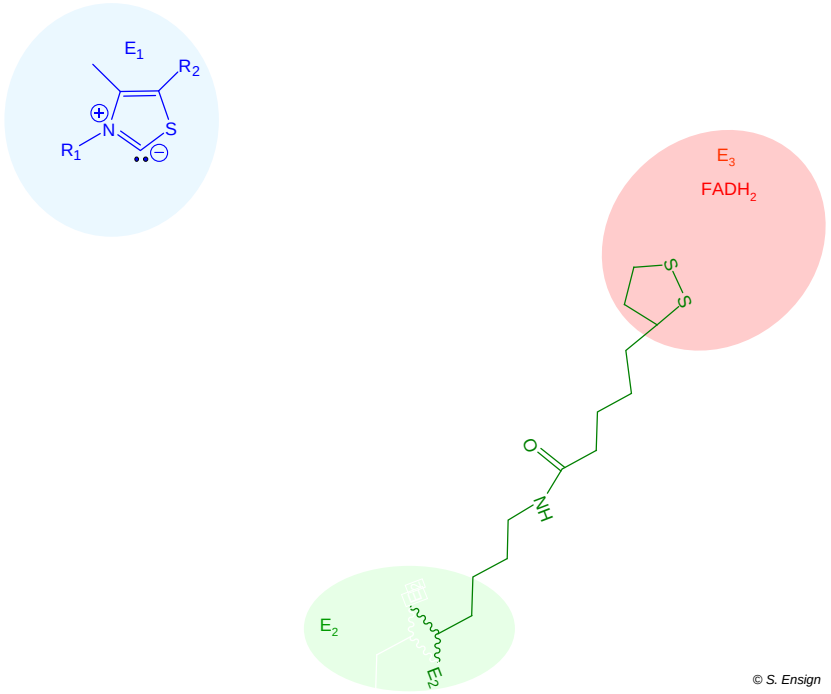


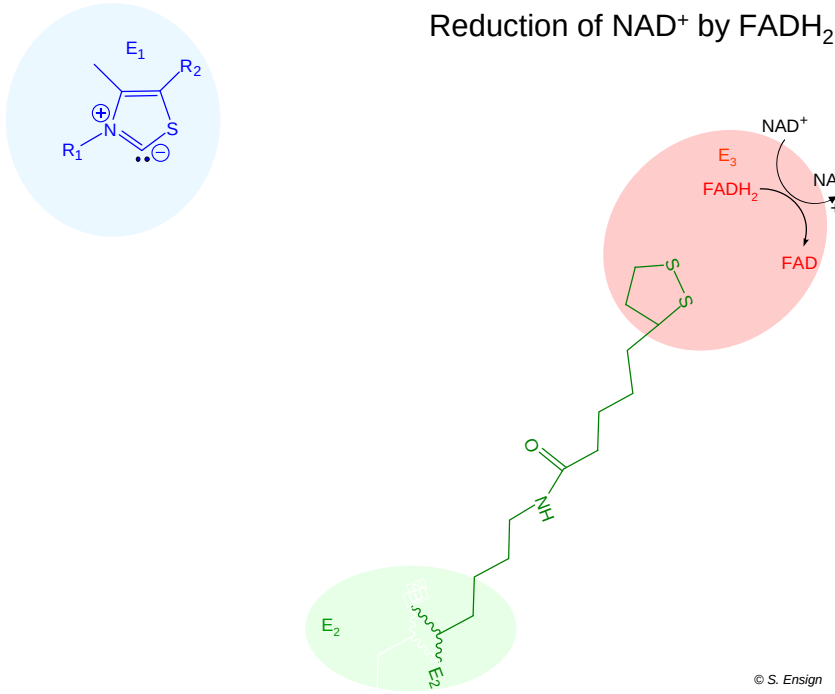
Translocation to the FAD binding site on E_3



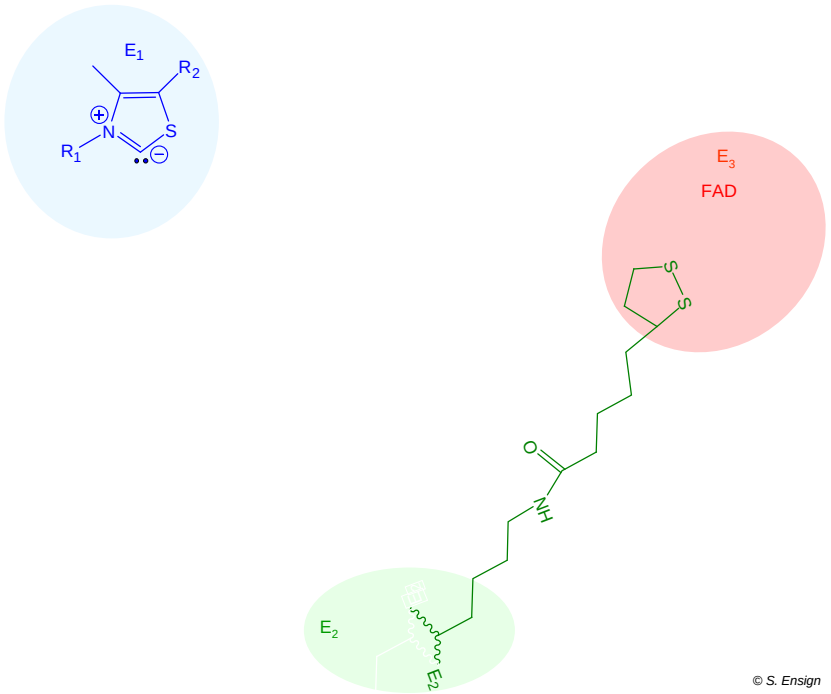
Oxidation of reduced lipoamide using FAD as e- acceptor. The mechanistic details are beyond the scope of this class

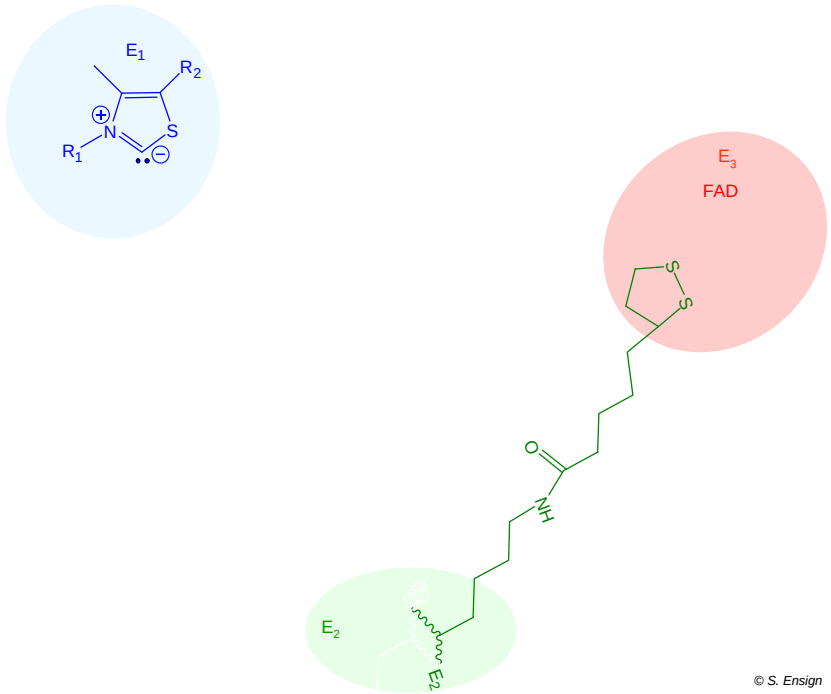


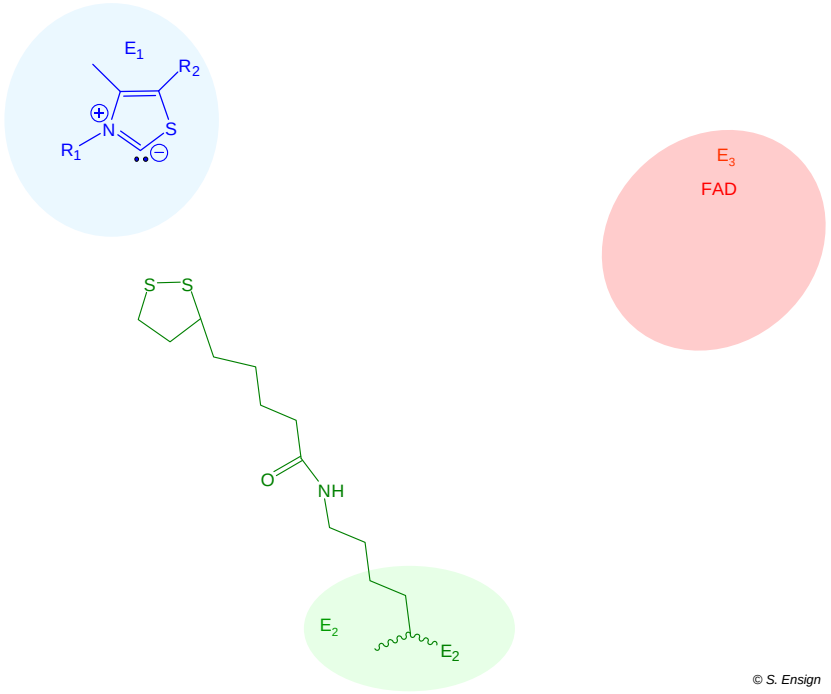


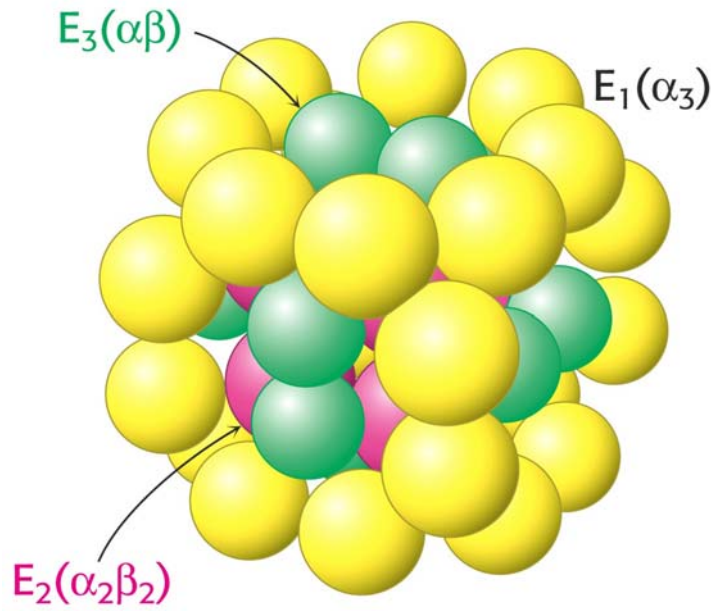


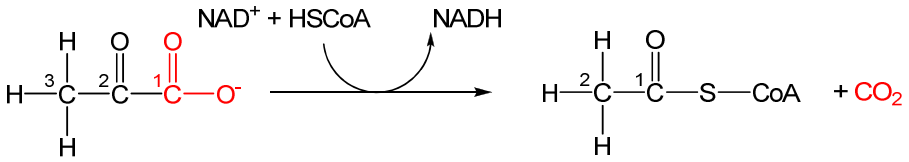
Reduction of NAD^+ by $FADH_2$





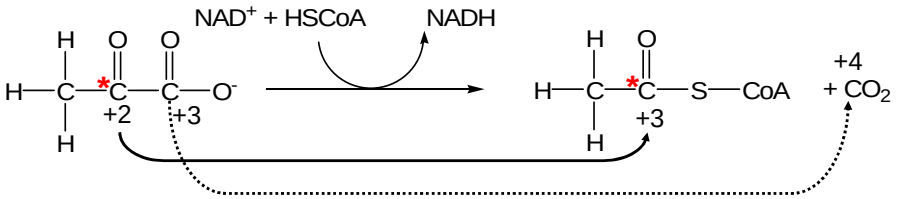


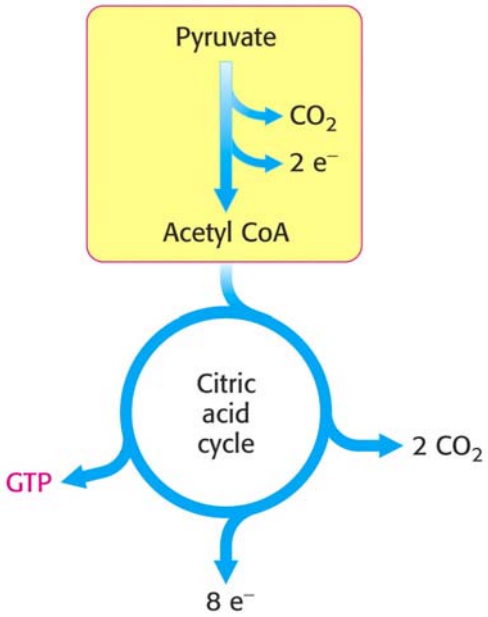




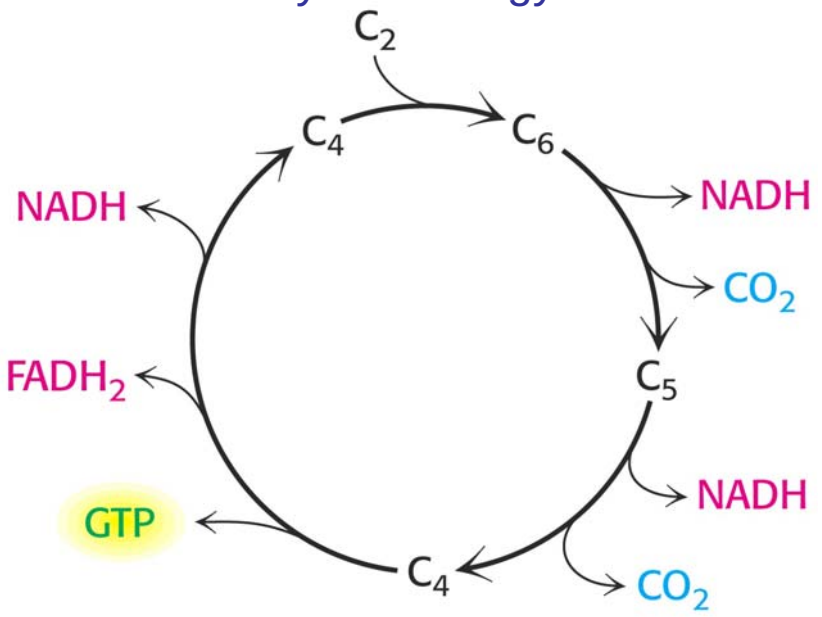
"Oxidative decarboxylation" because pyruvate lost two electrons (gained by NAD^+) in the course of the reaction

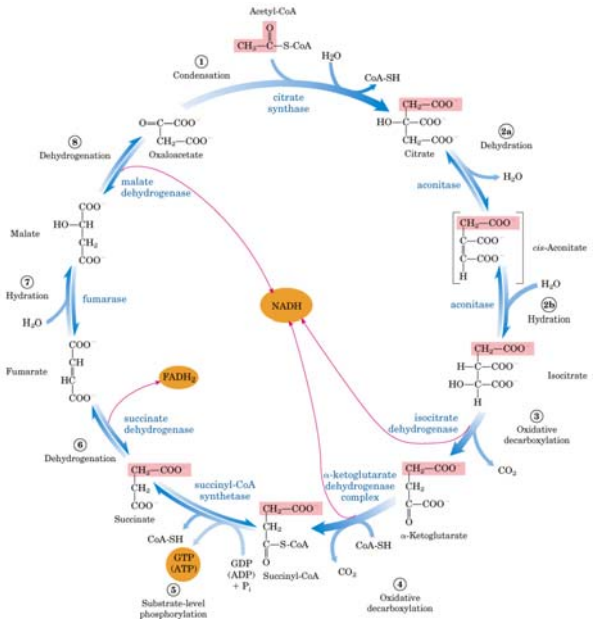
Where did the other electron for NADH come from??:



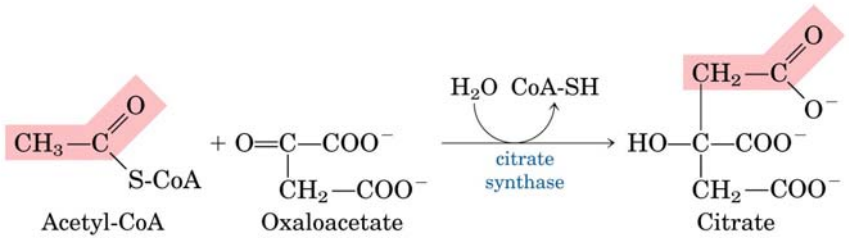


Citric acid cycle strategy

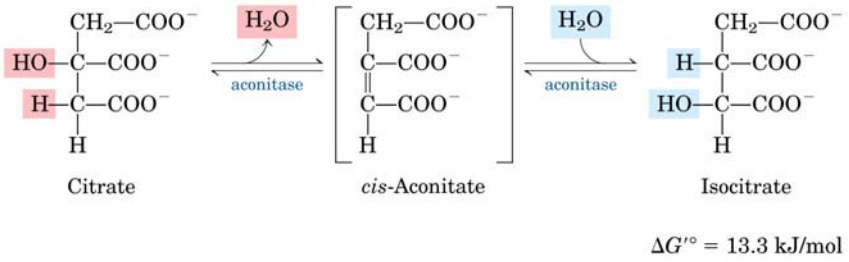




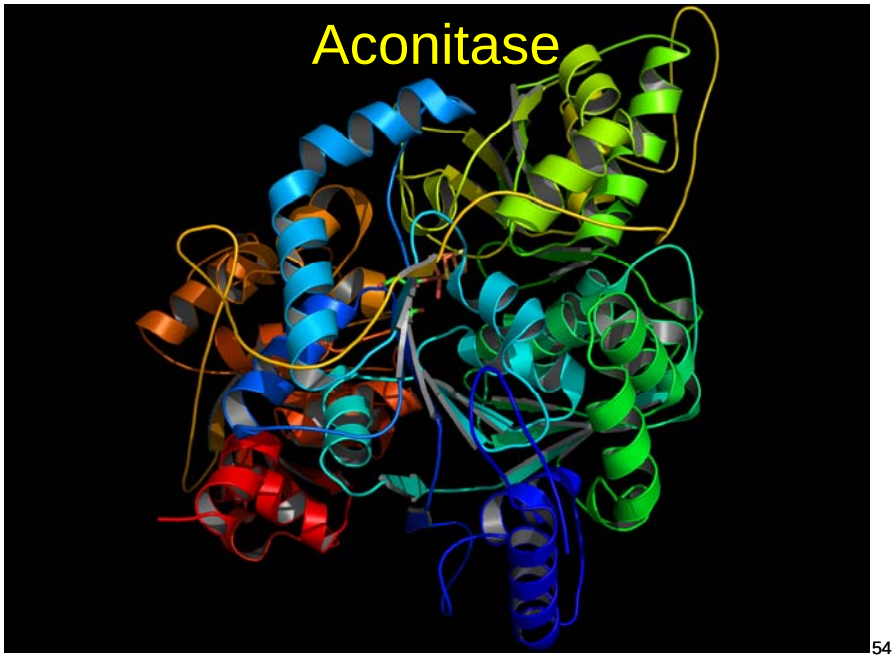
(1) Claisen condensation of Acetyl-CoA and OAA

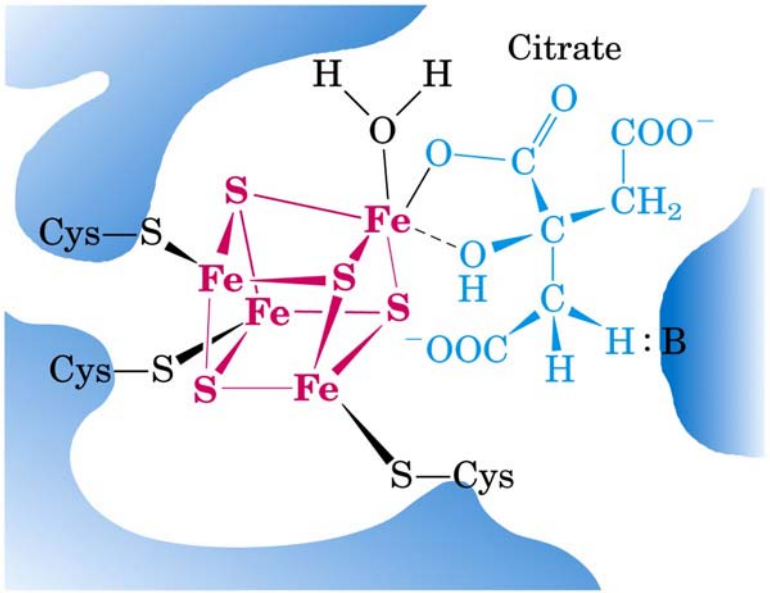


(2) Isomerization of citrate

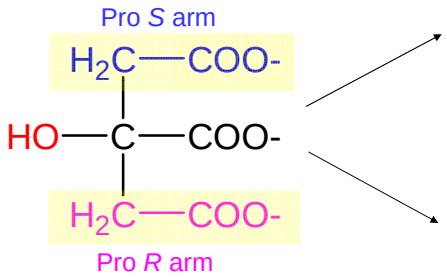


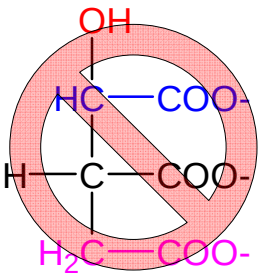
$\Delta G'^{\circ} = 13.3$ kJ/mol



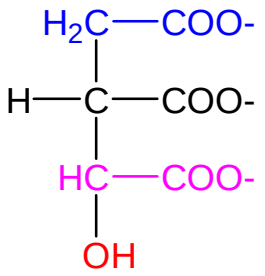


Discrimination between the two arms of citrate by aconitase

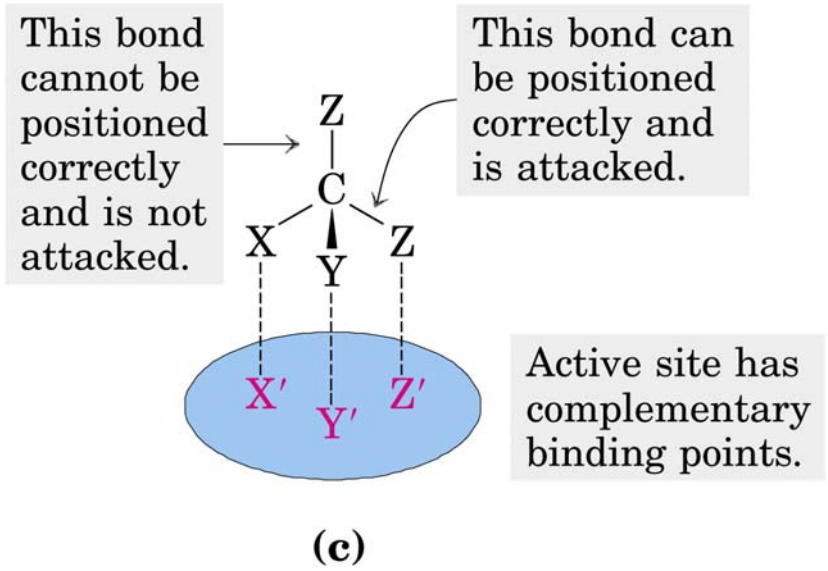


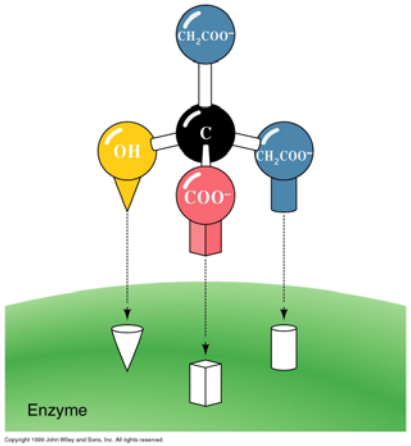


NO

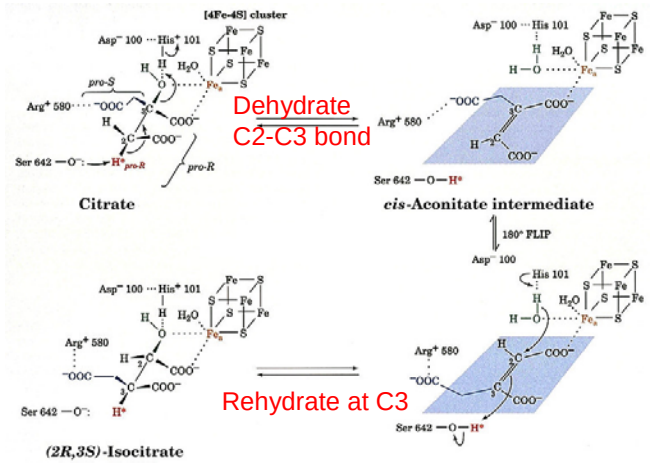


YES



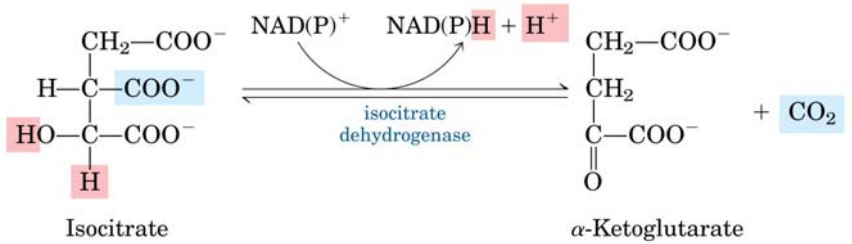


Aconitase mechanism



(3) Oxidative decarboxylation of isocitrate

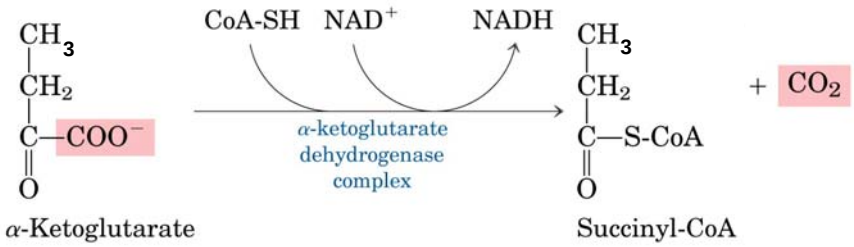
TPP is not used for this decarboxylation



$\Delta G'^{\circ} = -20.9$ kJ/mol

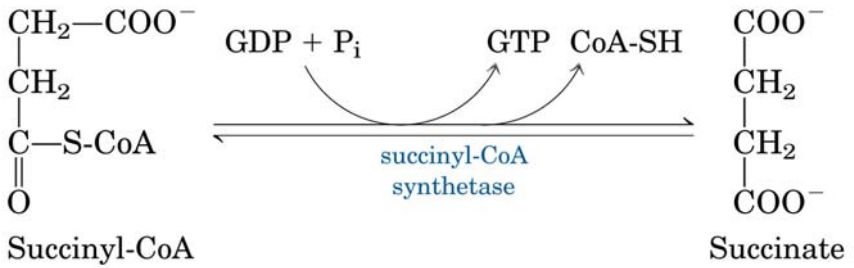
(4) Oxidative decarboxylation of α-KG

The α-KGDH complex is analogous to the PDH complex



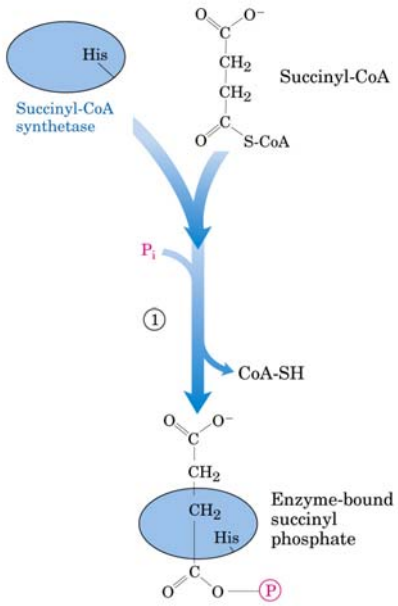
$\Delta G'^{\circ} = -33.5$ kJ/mol

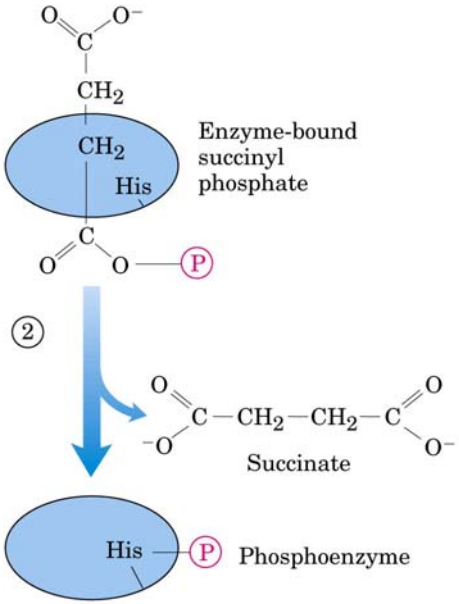
(5) Substrate level phosphorylation

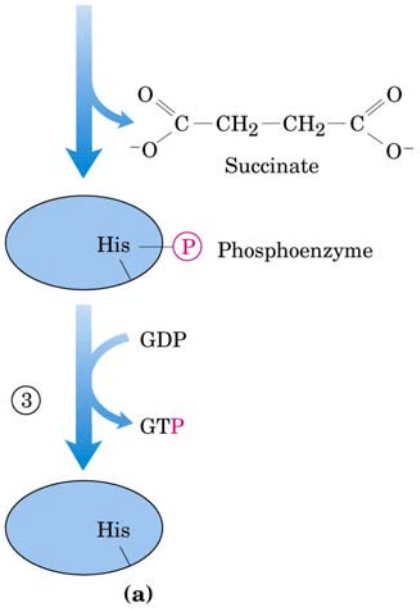


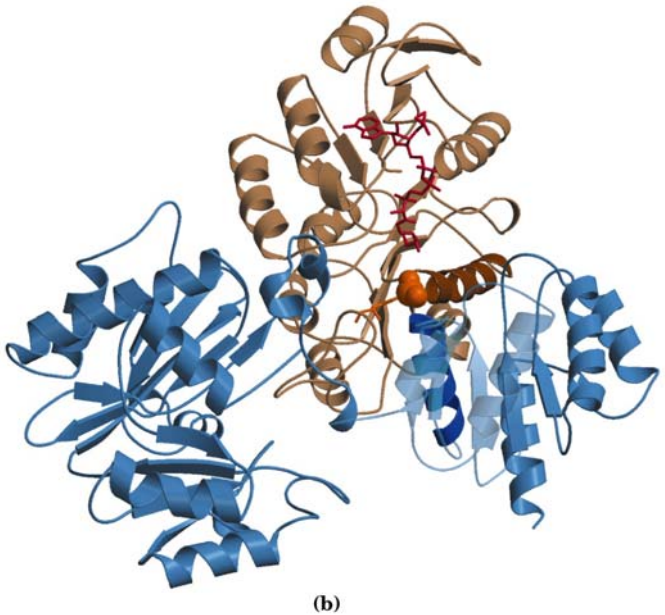
$\Delta G'^{\circ} = -2.9$ kJ/mol

A multstep reaction proceeding as follows…

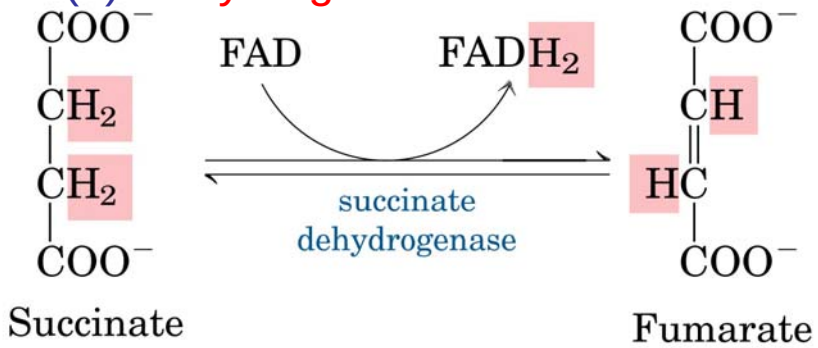








(6) Dehydrogenation of succinate



$$\Delta G'^{\circ} = 0 \text{ kJ/mol}$$

- For a redox reaction to be spontaneous, E°' for the overall reaction must have a positive value
- When writing a half reaction in the oxidative direction, the sign of E°' is switched
- The oxidized form of a given redox pair will react spontaneously with the reduced form of any redox pair that is below it in a table of standard reduction potentials (under standard conditions)

Table 14–7

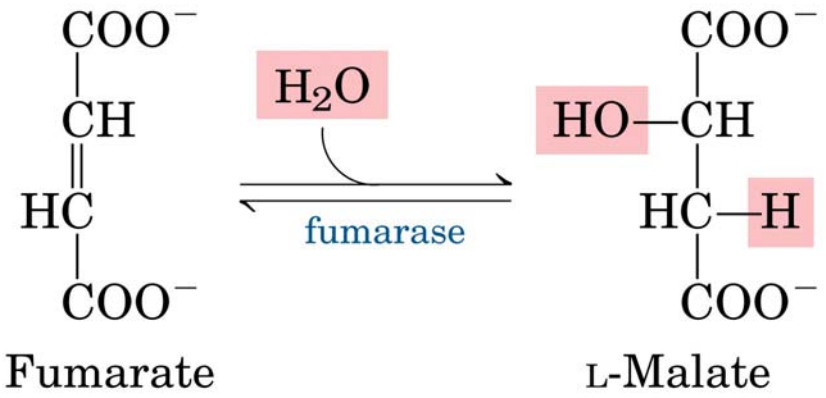
Standard Reduction Potentials of Some Biologically Important Half-Reactions, at 25 °C and pH 7

Half-reaction	E'° (V)
$\frac{1}{2}O_2 + 2H^+ + 2e^- \longrightarrow H_2O$	0.816
$Fe^{3+} + e^- \longrightarrow Fe^{2+}$	0.771
$NO_3^- + 2H^+ + 2e^- \longrightarrow NO_2^- + H_2O$	0.421
Cytochrome f $(Fe^{3+}) + e^- \longrightarrow$ cytochrome f (Fe^{2+})	0.365
$Fe(CN)_6^{3-}$ (ferricyanide) $+ e^- \longrightarrow Fe(CN)_6^{4-}$	0.36
Cytochrome a_3 $(Fe^{3+}) + e^- \longrightarrow$ cytochrome $a_3(Fe^{2+})$	0.35
$O_2 + 2H^+ + 2e^- \longrightarrow H_2O_2$	0.295
Cytochrome a $(Fe^{3+}) + e^- \longrightarrow$ cytochrome a (Fe^{2+})	0.29
Cytochrome c $(Fe^{3+}) + e^- \longrightarrow$ cytochrome c (Fe^{2+})	0.254
Cytochrome c_1 $(Fe^{3+}) + e^- \longrightarrow$ cytochrome c_1 (Fe^{2+})	0.22
Cytochrome b $(Fe^{3+}) + e^- \longrightarrow$ cytochrome b (Fe^{2+})	0.077
Ubiquinone $+ 2H^+ + 2e^- \longrightarrow$ ubiquinol $+ H_2$	0.045
Fumarate^{2-} $+ 2H^+ + 2e^- \longrightarrow$ succinate^{2-}	0.031
$2H^+ + 2e^- \longrightarrow H_2$ (at standard conditions, pH 0)	0.000
Crotonyl-CoA $+ 2H^+ + 2e^- \longrightarrow$ butyryl-CoA	−0.015
Oxaloacetate^{2-} $+ 2H^+ + 2e^- \longrightarrow$ malate^{2-}	−0.166
Pyruvate$^-$ $+ 2H^+ + 2e^- \longrightarrow$ lactate$^-$	−0.185
Acetaldehyde $+ 2H^+ + 2e^- \longrightarrow$ ethanol	−0.197
FAD $+ 2H^+ + 2e^- \longrightarrow FADH_2$	−0.219*
Glutathione $+ 2H^+ + 2e^- \longrightarrow$ 2 reduced glutathione	−0.23
$S + 2H^+ + 2e^- \longrightarrow H_2S$	−0.243
Lipoic acid $+ 2H^+ + 2e^- \longrightarrow$ dihydrolipoic acid	−0.29
$NAD^+ + H^+ + 2e^- \longrightarrow NADH$	−0.320
$NADP^+ + H^+ + 2e^- \longrightarrow NADPH$	−0.324
Acetoacetate $+ 2H^+ + 2e^- \longrightarrow$ β-hydroxybutyrate	−0.346
α-Ketoglutarate $+ CO_2 + 2H^+ + 2e^- \longrightarrow$ isocitrate	−0.38
$2H^+ + 2e^- \longrightarrow H_2$ (at pH 7)	−0.414
Ferredoxin $(Fe^{3+}) + e^- \longrightarrow$ ferredoxin (Fe^{2+})	−0.432

Data mostly from Loach, P.A. (1976) In *Handbook of Biochemistry and Molecular Biology*, 3rd edn (Fasman, G.D., ed.), *Physical and Chemical Data*, Vol. I, pp. 122–130, CRC Press, Boca Raton, FL.

*This is the value for free FAD; FAD bound to a specific flavoprotein (for example succinate dehydrogenase) has a different E'°.

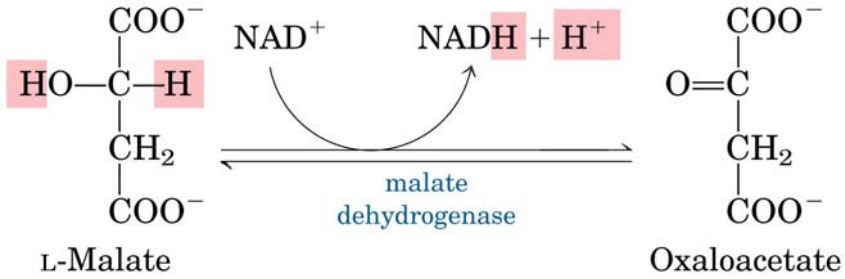
(7) Hydration of fumarate



Fumarate L-Malate

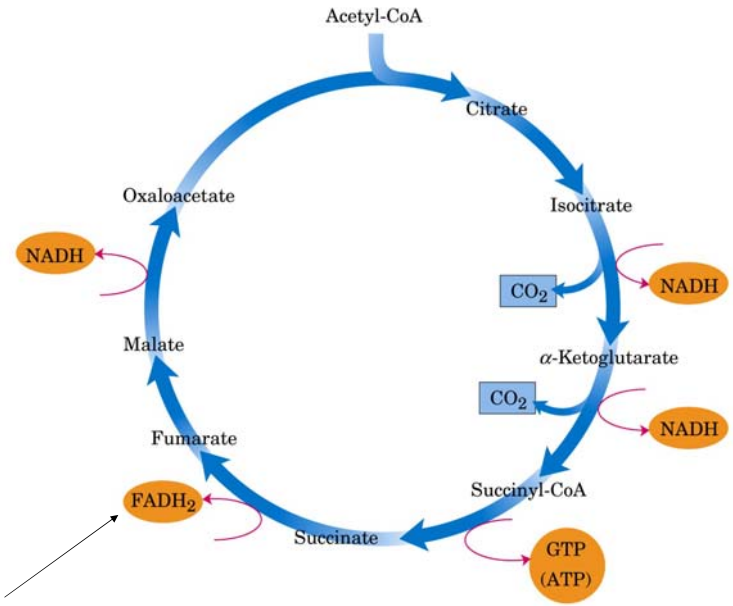
$\Delta G'^{\circ} = -3.8$ kJ/mol

(8) Dehydrogenation of L-malate



L-Malate Oxaloacetate

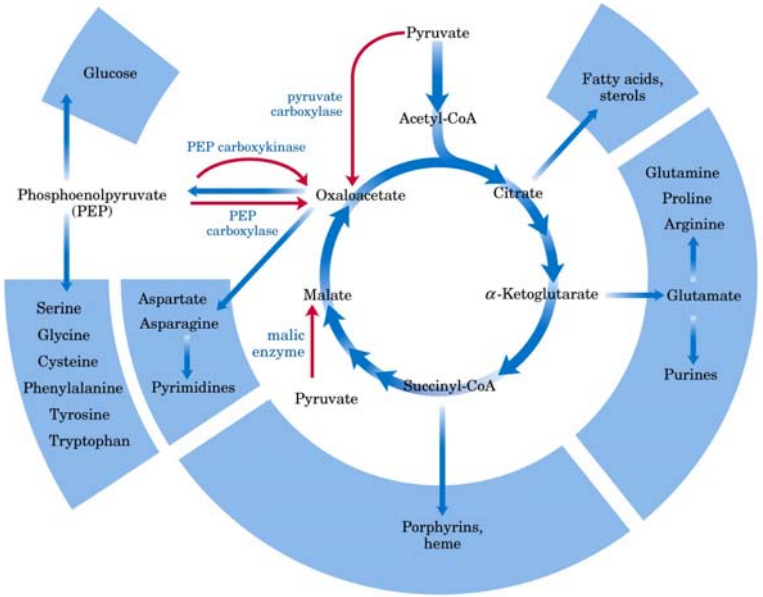
$\Delta G'^{\circ} = 29.7$ kJ/mol



Not the exogenous electron acceptor for succinate dehydrogenase!

Why a cyclic pathway?

- Feed in other than C2
- Take stuff out
- Avoid toxic C1 compounds



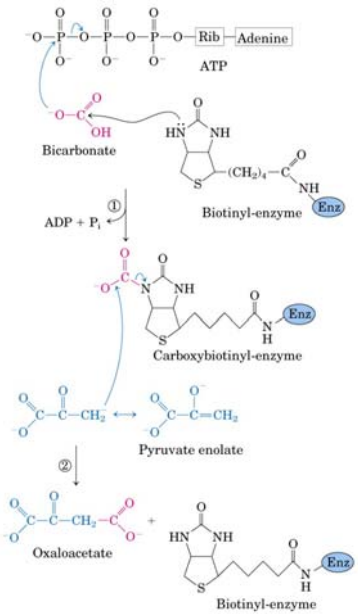
Replenishment of citric acid cycle intermediates: anaplerotic reactions

C3 + C1 → C4

table 16–2

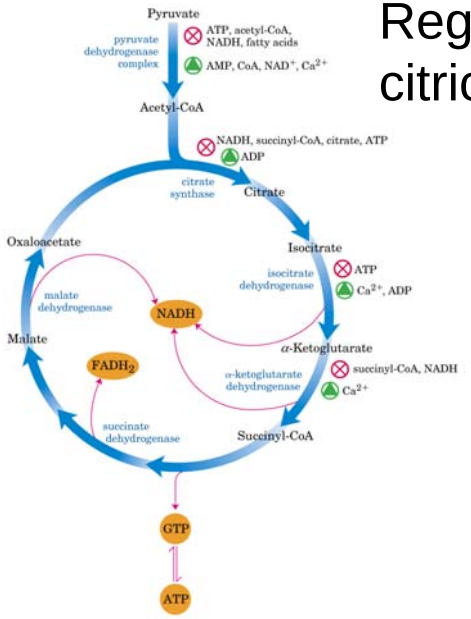
Anaplerotic Reactions

Reaction	Tissue(s)/organism(s)
$Pyruvate + HCO_3^- + ATP \xrightleftharpoons{\text{pyruvate carboxylase}} oxaloacetate + ADP + P_i$	Liver, kidney
$Phosphoenolpyruvate + CO_2 + GDP \xrightleftharpoons{\text{PEP carboxykinase}} oxaloacetate + GTP$	Heart, skeletal muscle
$Phosphoenolpyruvate + HCO_3^- \xrightleftharpoons{\text{PEP carboxylase}} oxaloacetate + P_i$	Higher plants, yeast, bacteria
$Pyruvate + HCO_3^- + NAD(P)H \xrightleftharpoons{\text{malic enzyme}} malate + NAD(P)^+$	Widely distributed in eukaryotes and prokaryotes



Biotin: the cofactor for many organic substrate carboxylation reaction

Biotin activates *abundant bicarbonate* to *electrophilic* CO_2



Regulation of the citric acid cycle

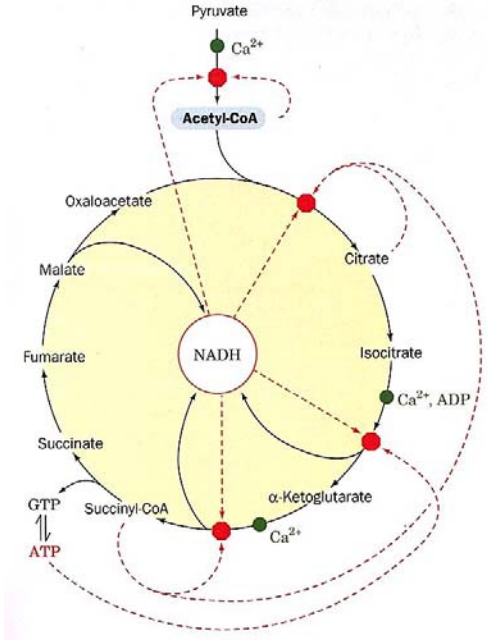
Four key sites:

(1) Pyr → Ac-CoA

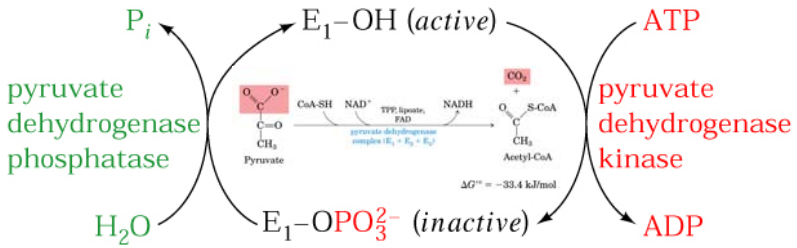
(2) Ac-CoA → citrate

(3) Isocitrate → α-KG

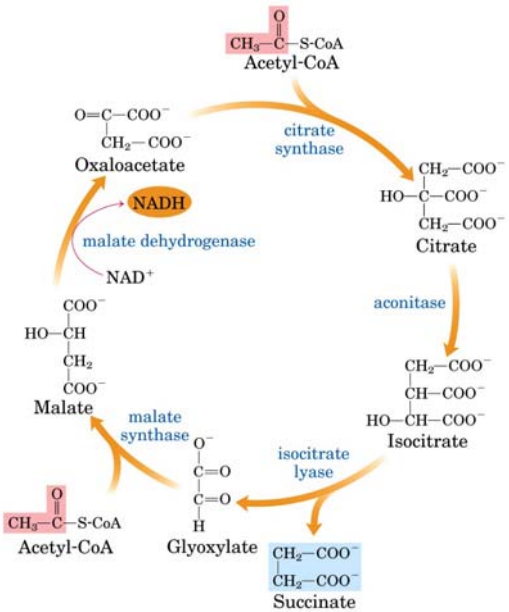
(4) α-KG → Succ-CoA



Regulation of PDH



- *Allosteric* product inhibition: Ac-CoA and NADH ⊗
- *Allosteric* inhibition by ATP ⊗
- *Allosteric* activation by AMP, CoA, NAD^+ ▲
- *On/off regulation* by PDH phosphatase and PDH kinase
  - How do you suppose these enzymes are regulated?



The Glyoxylate cycle: let's save this for next semester. Basically, it's the means by which plants and some bacteria carry out anaplerotic reactions from Ac-CoA. The main objective is to synthesize C3 and C4 units from Ac-CoA. Mammals cannot do this.