Citric Acid Cycle

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CITRIC ACID CYCLE = TCA CYCLE = KREBS CYCLE

TCA = tricarboxylic acid cycle

Definition:

-- Acetate in the form of *acetyl-CoA*, is derived from pyruvate and other metabolites, and is ***oxidized*** to CO_2 in the *citric acid cycle*.

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① One high energy compound is produced for each cycle.

② The electrons from the TCA cycle are made available to an ***electron transport chain*** in the form of three NADH and one $FADH_2$ and ultimately energy is provided for ***oxidative phosphorylation.***

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- The citric acid cycle is *central to all respiratory oxidation*, oxidizing acetyl-CoA from glucose, lipid and protein catabolism in aerobic respiration to maximize energy gain.
- The cycle also supplies some *precursors* for biosynthesis.
- All enzymes are in the mitochondrial matrix or inner mitochondrial membrane

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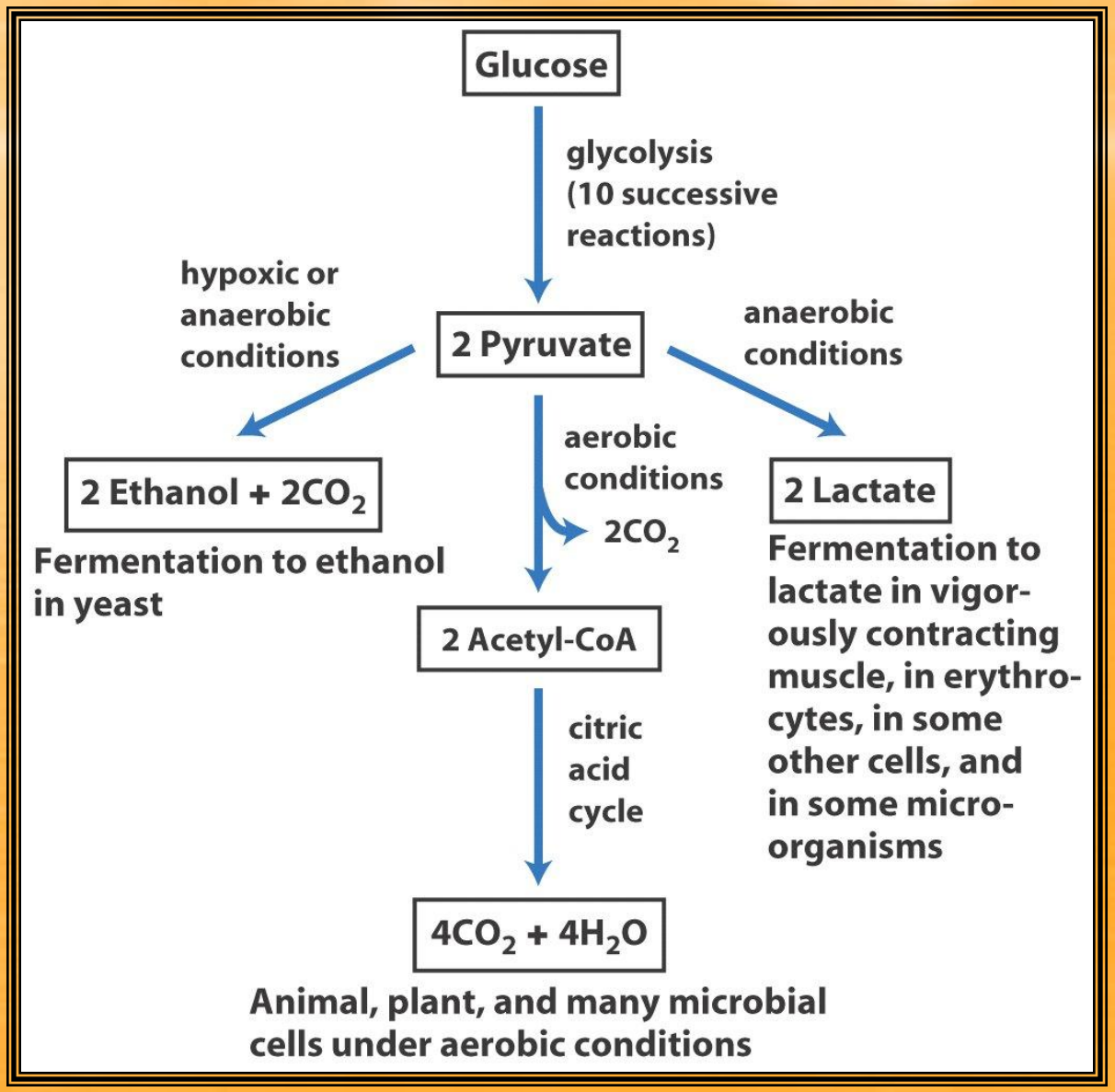
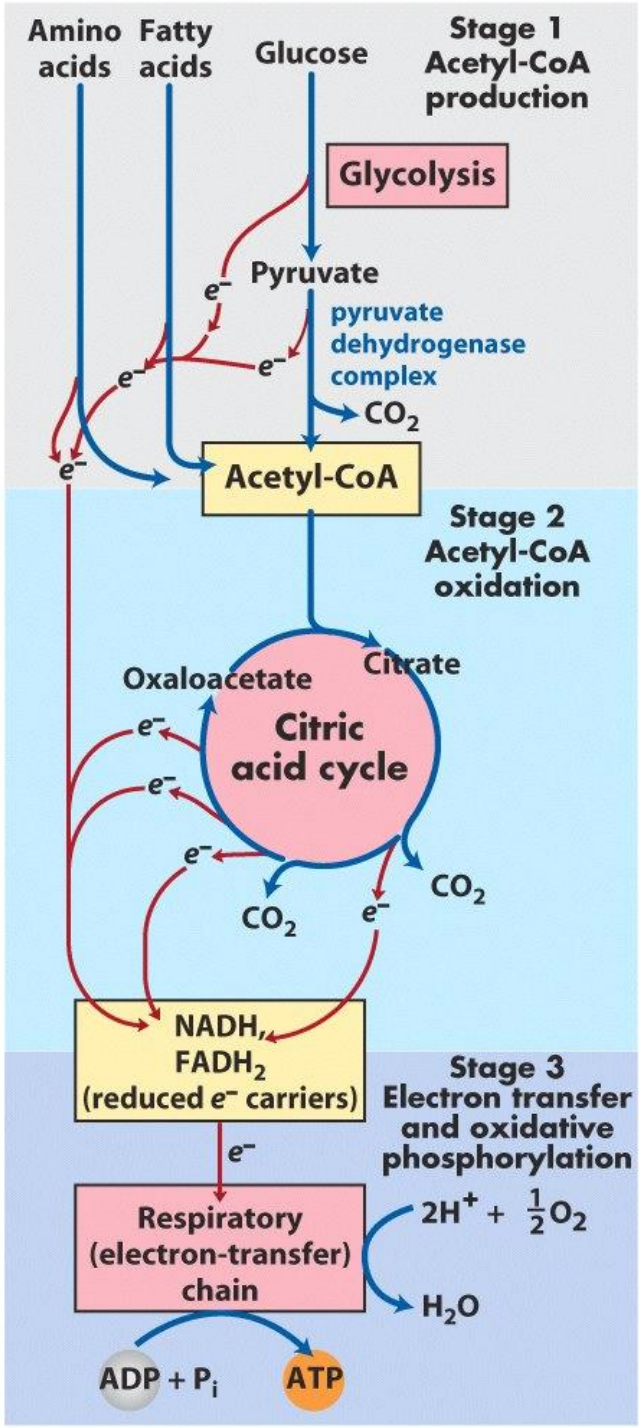
Pyruvate has Several Fates



- The citric acid cycle is a central part of the aerobic phase of metabolism.
- Pyruvate from glycolysis is first oxidized to acetate and linked to CoA to form acetyl-CoA.
- It is in this form that carbon enters the citric acid cycle.
- In the citric acid cycle acetyl-CoA is condensed with oxaloacetate to give citrate.
- In a turn of the cycle, two carbons are oxidized to CO2 and oxaloacetate is regenerated.

Steps of Aerobic Metabolism



Glycolysis

Citric Acid Cycle

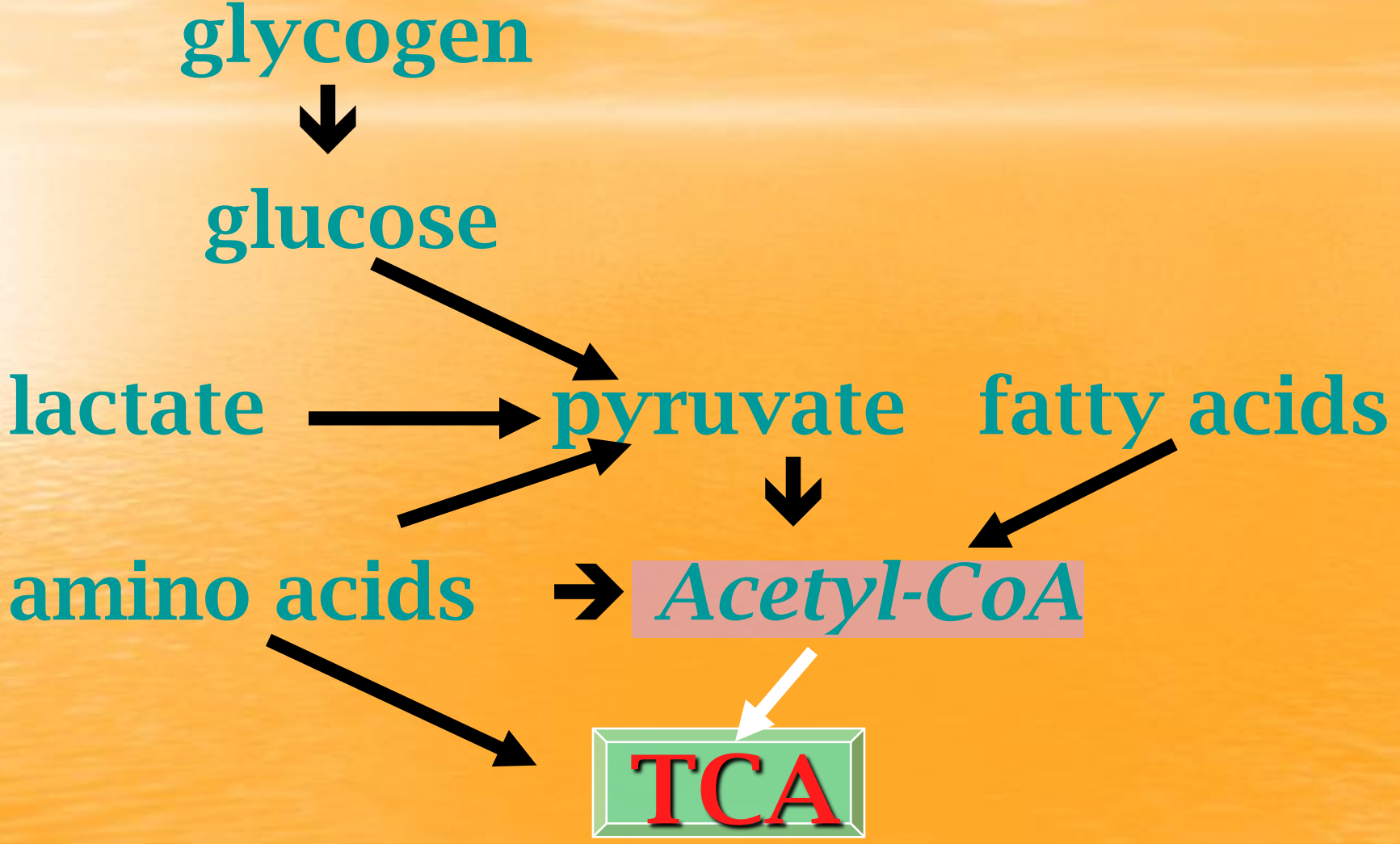
Electron transfer/ Oxidative phosphorylation

1. Introduction to the citric acid cycle and oxidative metabolism

- For most eukaryotic cells and many bacteria, glycolysis is only the first step in the catabolism of glucose.
- In fermentation there is no net oxidation: glucose is oxidized to give pyruvate, but then pyruvate is reduced to ethanol or lactate.
- In contrast, in oxidative metabolism glucose is completely oxidized to CO2. This oxidative metabolism is called **respiration**.

- Three stages of cellular respiration .
- First, glucose, fatty acids, and some amino acids are oxidized to acetate (as part of acetyl CoA).
- Second, these acetyl groups enter the citric acid cycle, which catalyzes their oxidation to CO_2.
- The electrons are transferred to the carriers NADH and $FADH_2$.
- In the third stage the reduced coenzymes are themselves oxidized, transferring the electrons to O_2 through a series of electron-carrying molecules known as the respiratory chain.
- This electron transfer process releases a large amount of energy, most of which is conserved by the synthesis of ATP in **oxidative phosphorylation.**

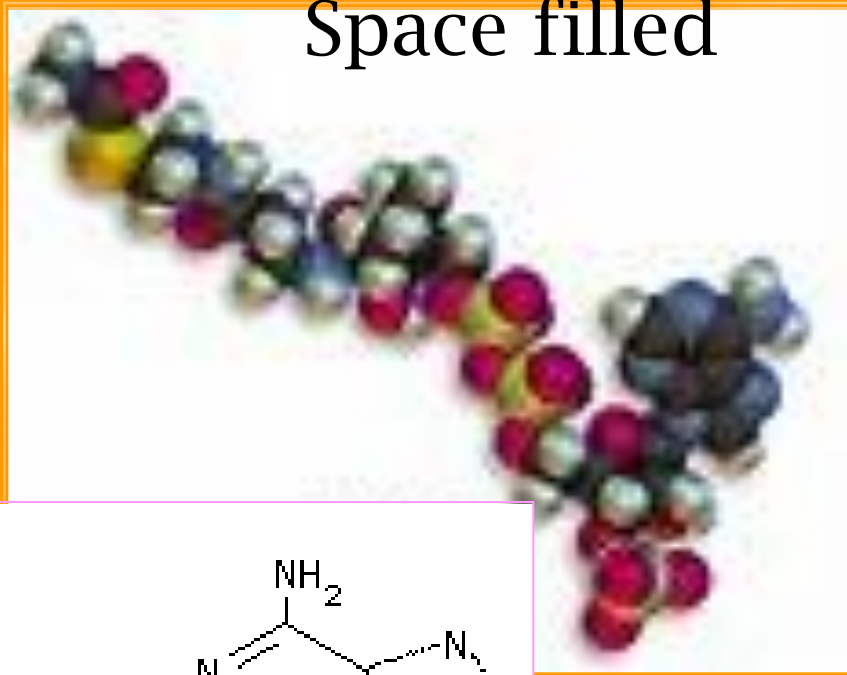
Many catabolic pathways yield acetyl CoA for the TCA cycle

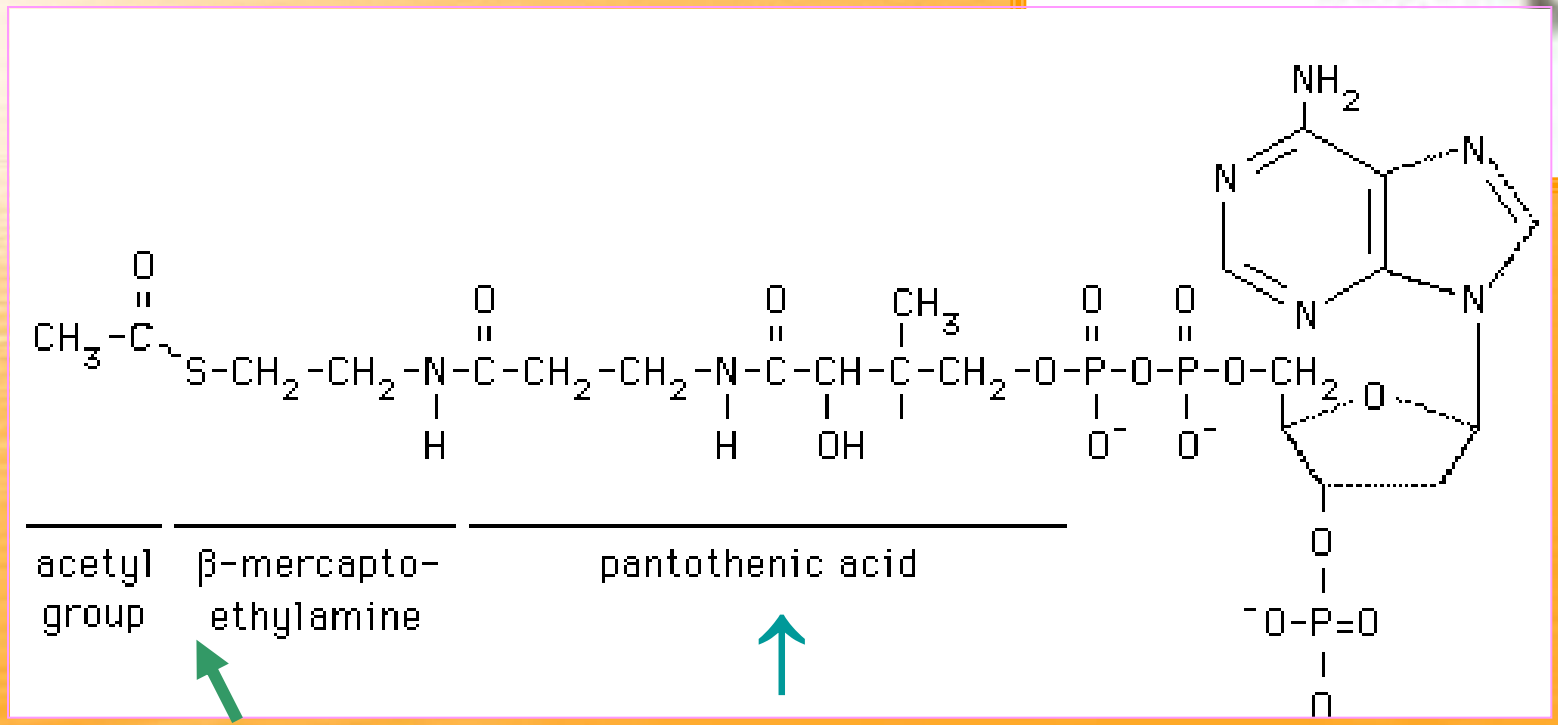


(Note: aa more than one entry point)

Acetyl CoA

Space filled

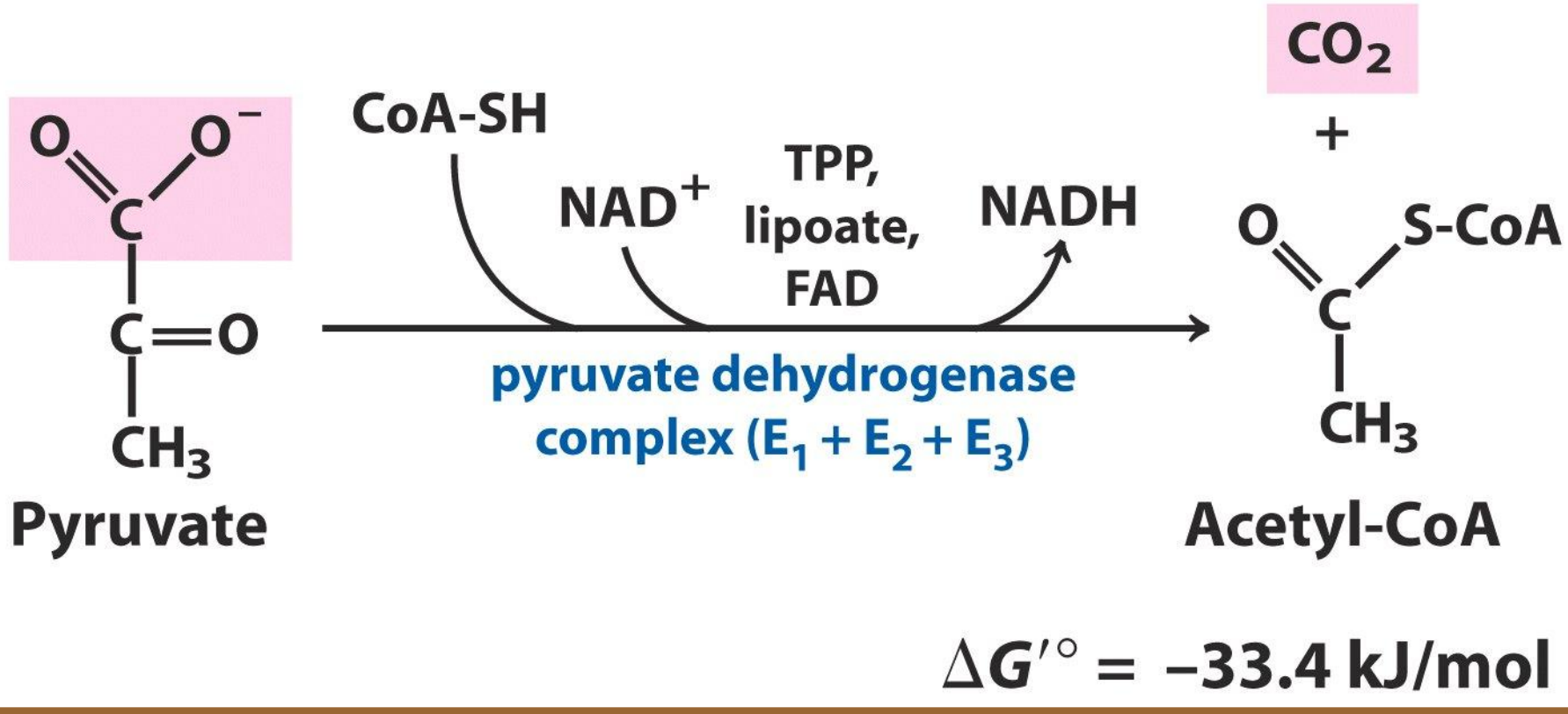




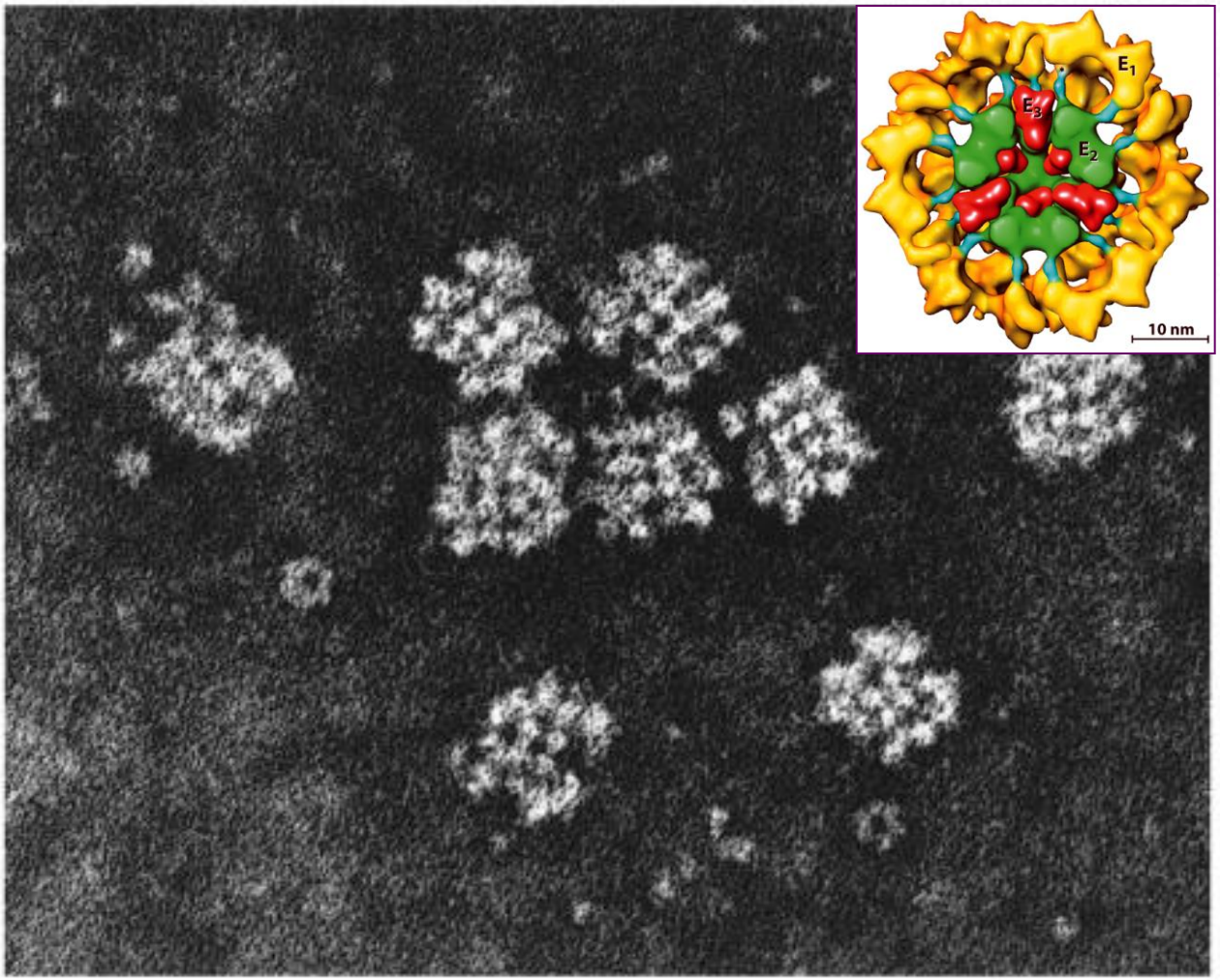
Acetyl

HS-CoA

Before the Citric Acid Cycle: Conversion of Pyruvate to Acetyl CoA



Pyruvate Dehydrogenase: A Huge Complex



- ***Irreversible***

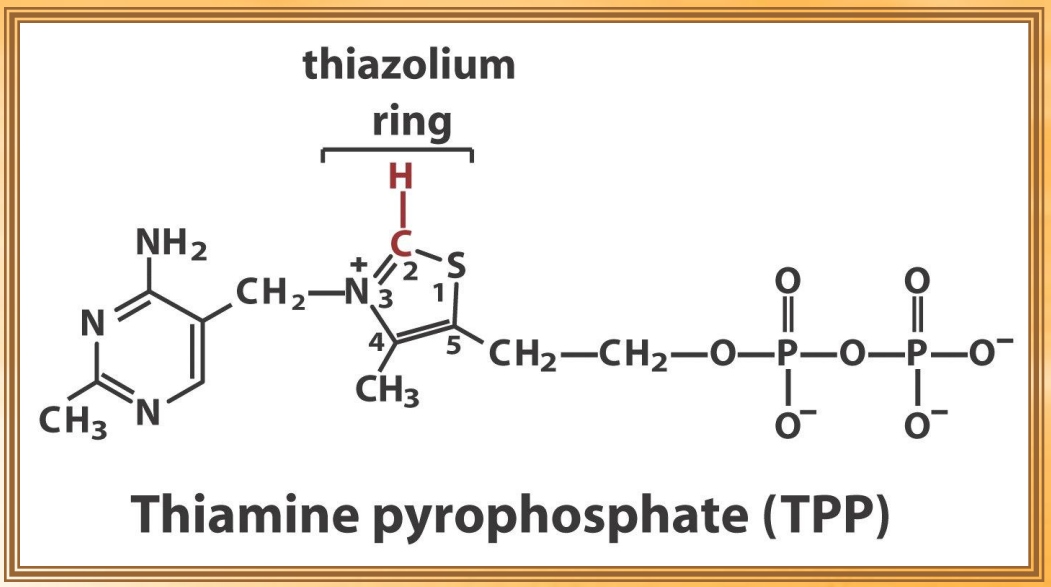
-- irreversible means acetyl-CoA cannot be converted backward to pyruvate;

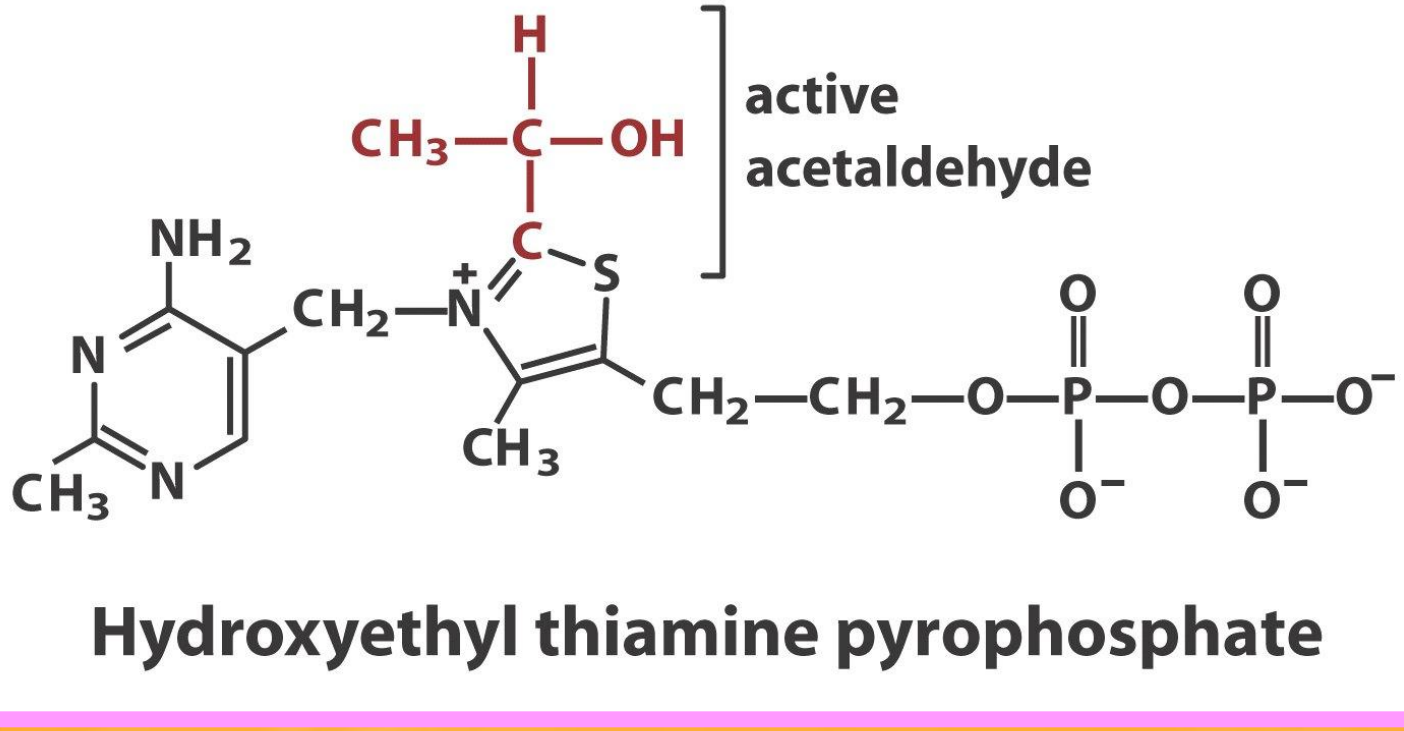
hence “fat cannot be converted to carbohydrate”

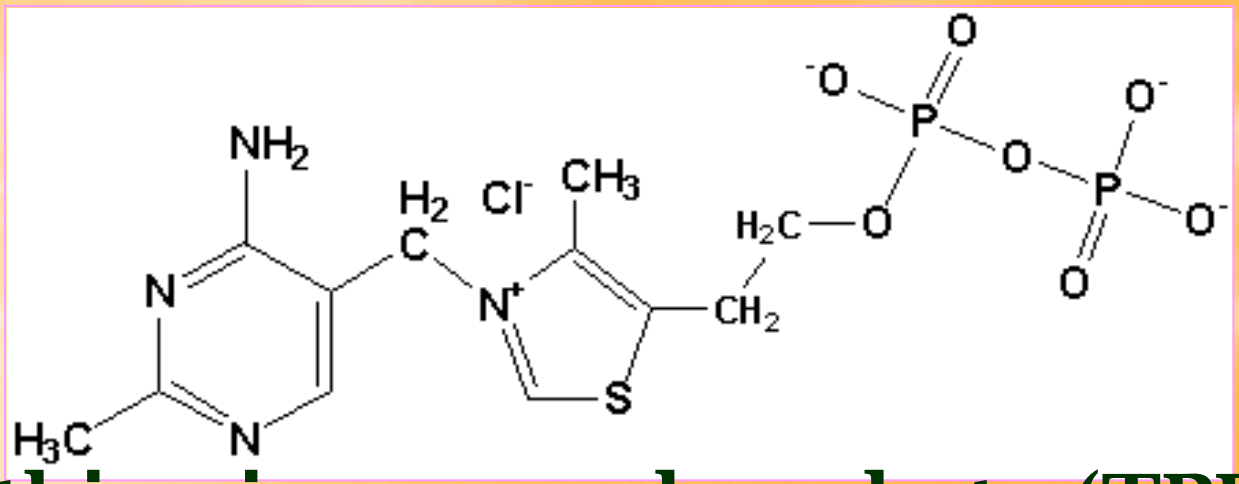
2. *Conversion of pyruvate to acetate*

- Multienzyme complex catalyzes oxidative decarboxylation of pyruvate to acetate. The overall reaction is:
- pyruvate + NAD+ + CoA → acetyl-CoA + NADH + H+ + CO2 DG'° = –33.5 kJ/mol
- Reaction requires five cofactors or prosthetic groups: thiamine pyrophosphate (TPP), flavin adenine dinucleotide (FAD), **coenzyme A (CoA)** , nicotinamide adenine dinucleotide (NAD), and **lipoate** .

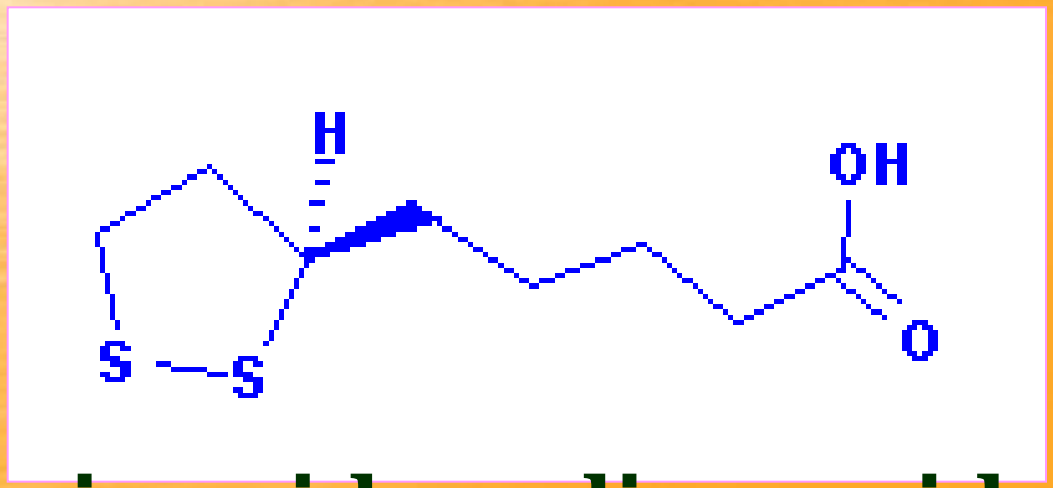
Thiamine pyrophosphate (TPP) and pyruvate decarboxylation



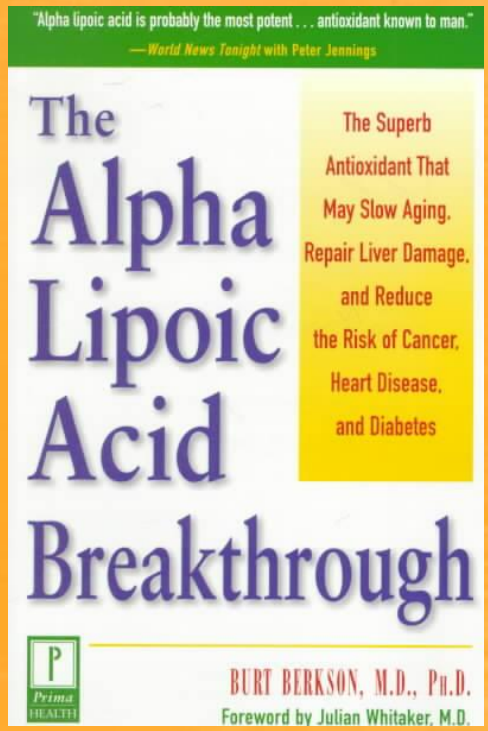




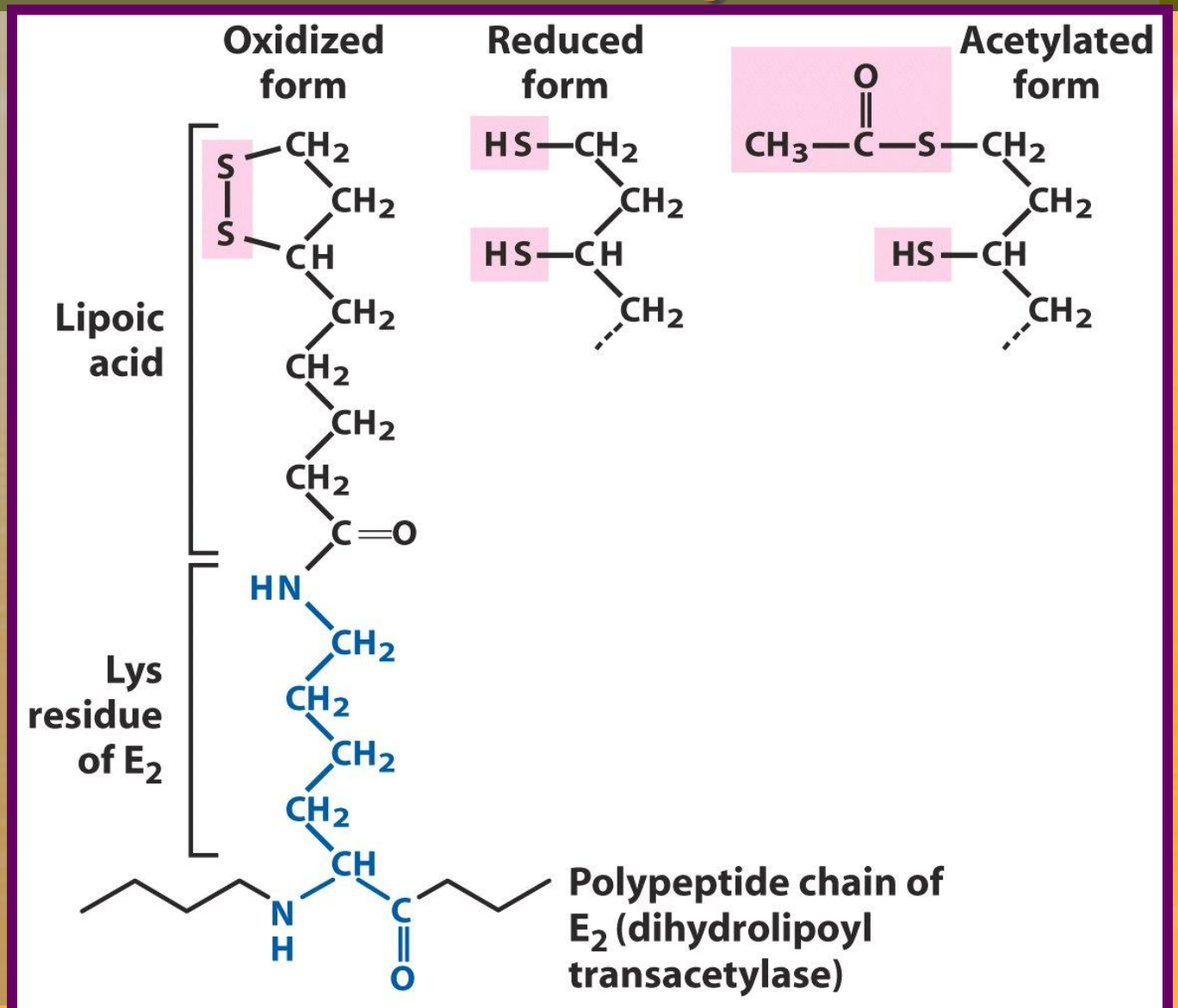
thiamine pyrophosphate (TPP)



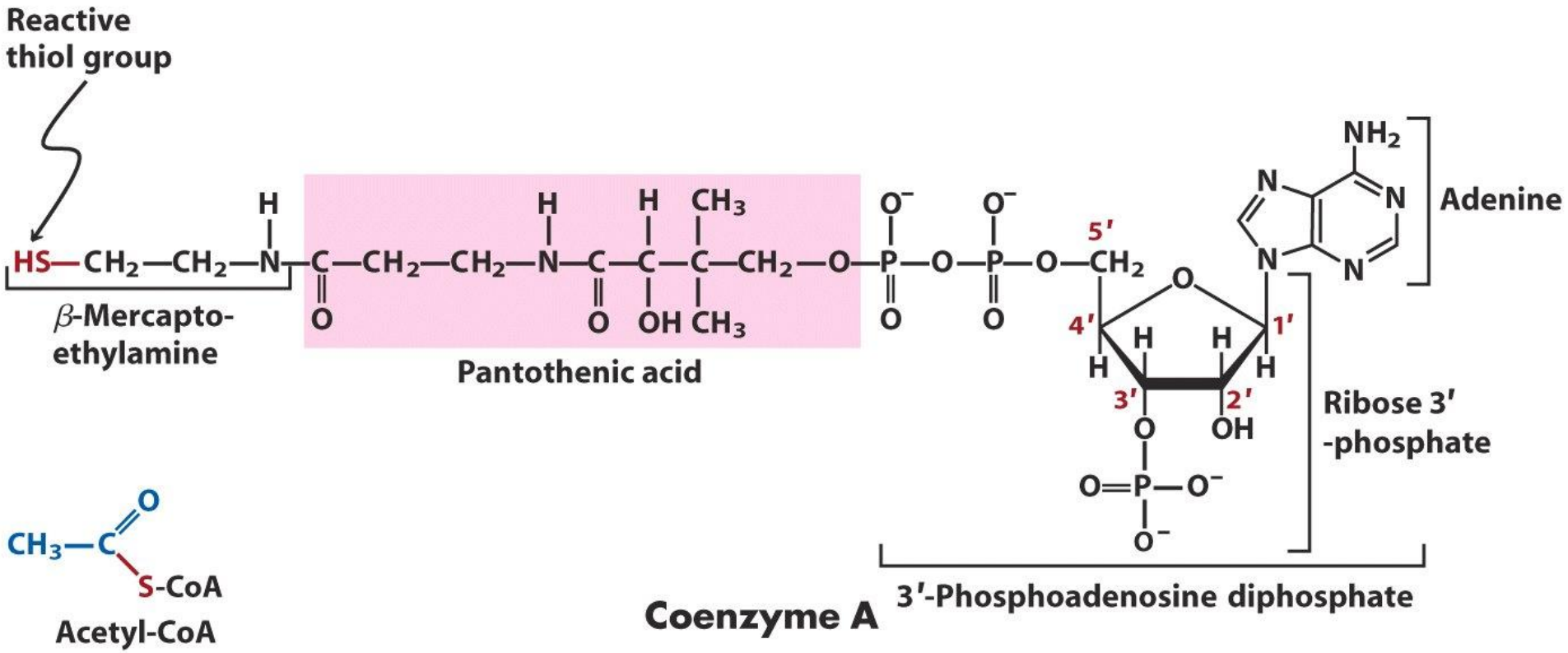
lipoic acid *aka* lipoamide



Lipoic Acid (Lipoate): Electron Carrier and Acyl Carrier

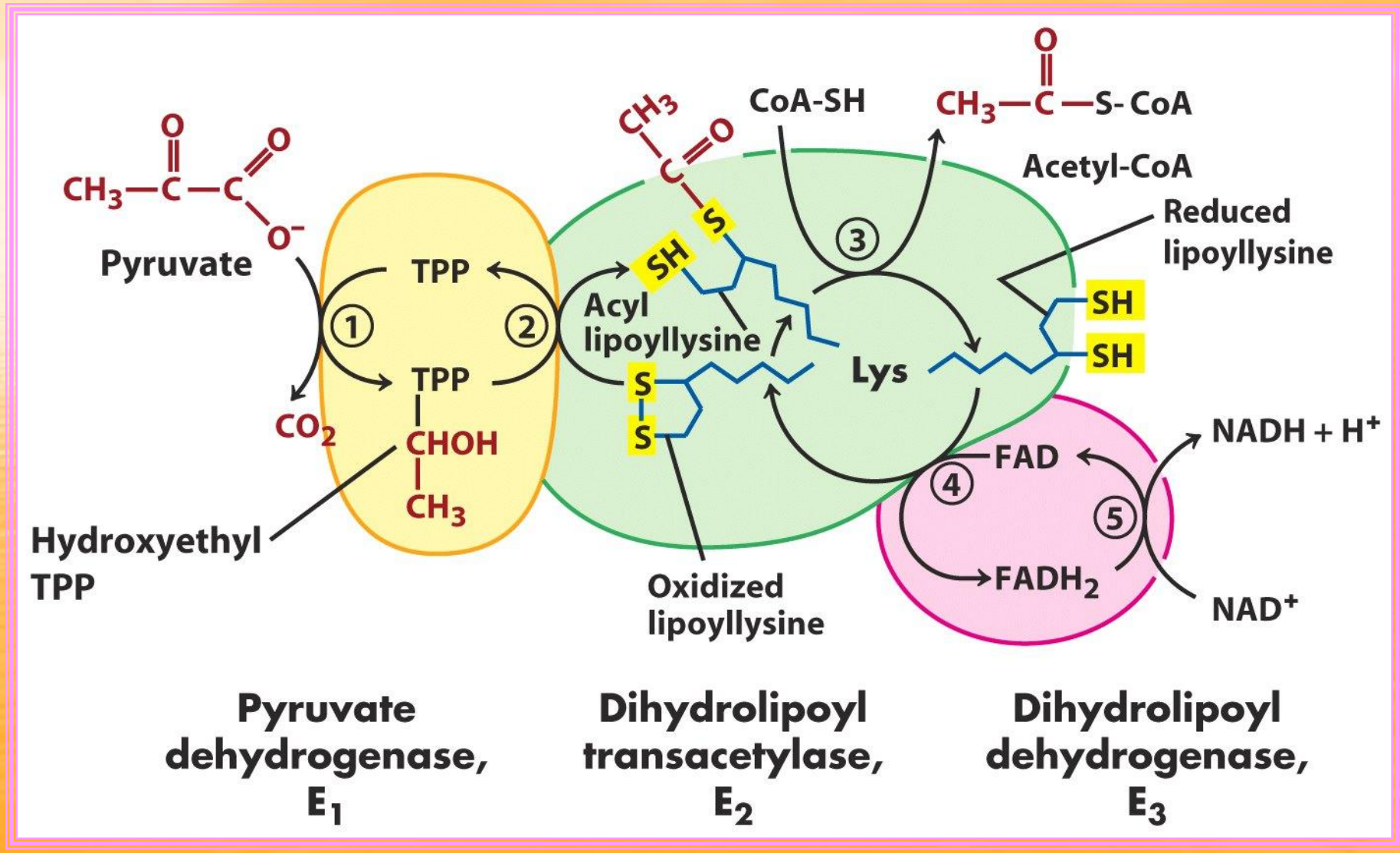


Structure of Coenzyme A



- Reaction is carried out by a complex consisting of three enzymes: pyruvate dehydrogenase (E1), dihydrolipoyl transacetylase (E2), and dihydrolipoyl dehydrogenase (E3).
- Each enzyme is present in multiple copies in this cluster, giving a complex that is variable in size for different organisms, but is universally very big: in *E. coli* its molecular weight is 4500 kDa, more than five times as large as the ribosome!

Oxidative Decarboxylation of Pyruvate to Generate Acetyl-CoA

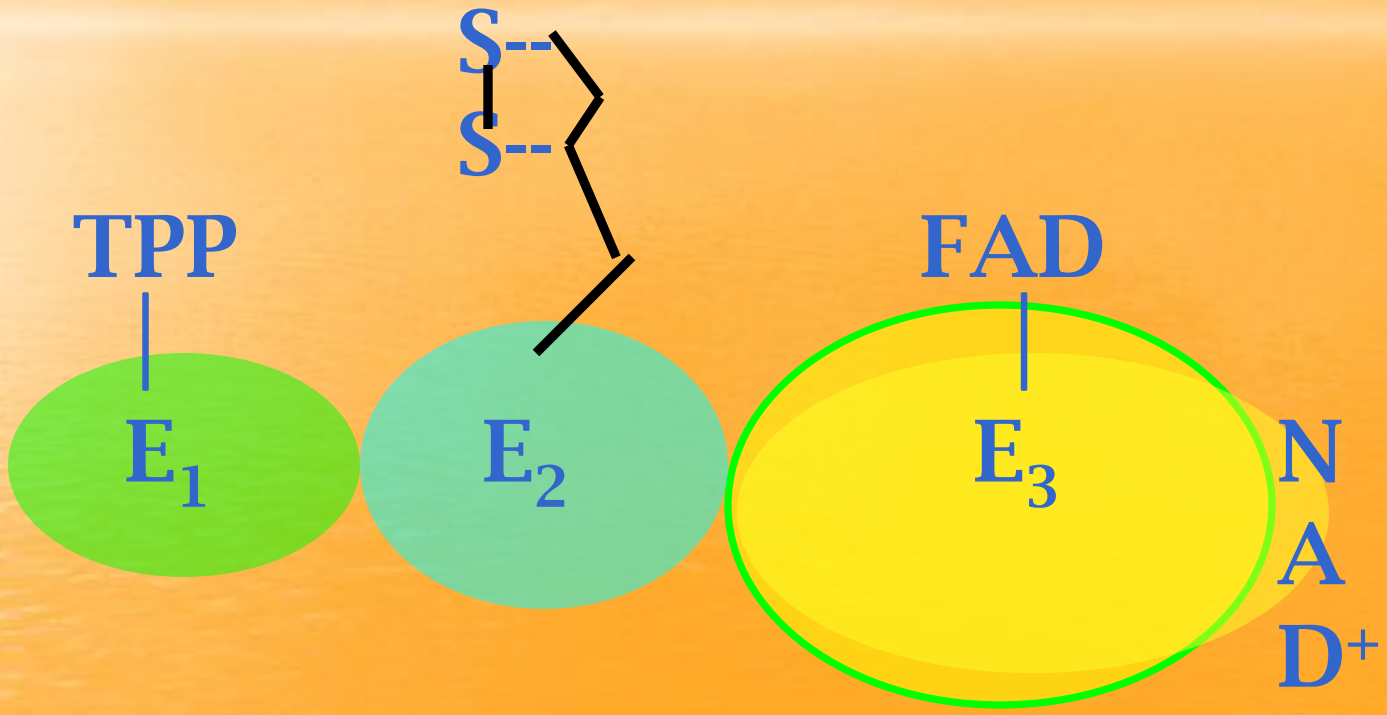


◆ **pyruvate dehydrogenase (=E_1)** has coenzyme = ***thiamine pyrophosphate*** (TPP)

-- TPP is coenzyme for all decarboxylations of α-keto acids.
-- lack of thiamine = beriberi

◆ **dihydrolipoyl transacetylase (=E_2)** has coenzymes ***lipoate*** and CoA

- dihydrolipoyl dehydrogenase (= E_3) has coenzymes FAD and NAD^+



- The core of the cluster is enzyme E2, to which the others are attached.
- In the *E. coli* complex there are 24 copies of E2.
- Each molecule of E2 has three molecules of covalently bound lipoate attached to lysine residues.
- The attachment of lipoate produces long ‘arms’ that carry acetyl groups from one active site to another within the complex.

- **The reaction steps are as follows:**

1. Decarboxylation of pyruvate (E1)

- Essentially the same reaction as catalyzed by pyruvate decarboxylase, but hydroxyethyl group remains bound to TPP

2. Oxidation of hydroxyethyl to acetate (E1)

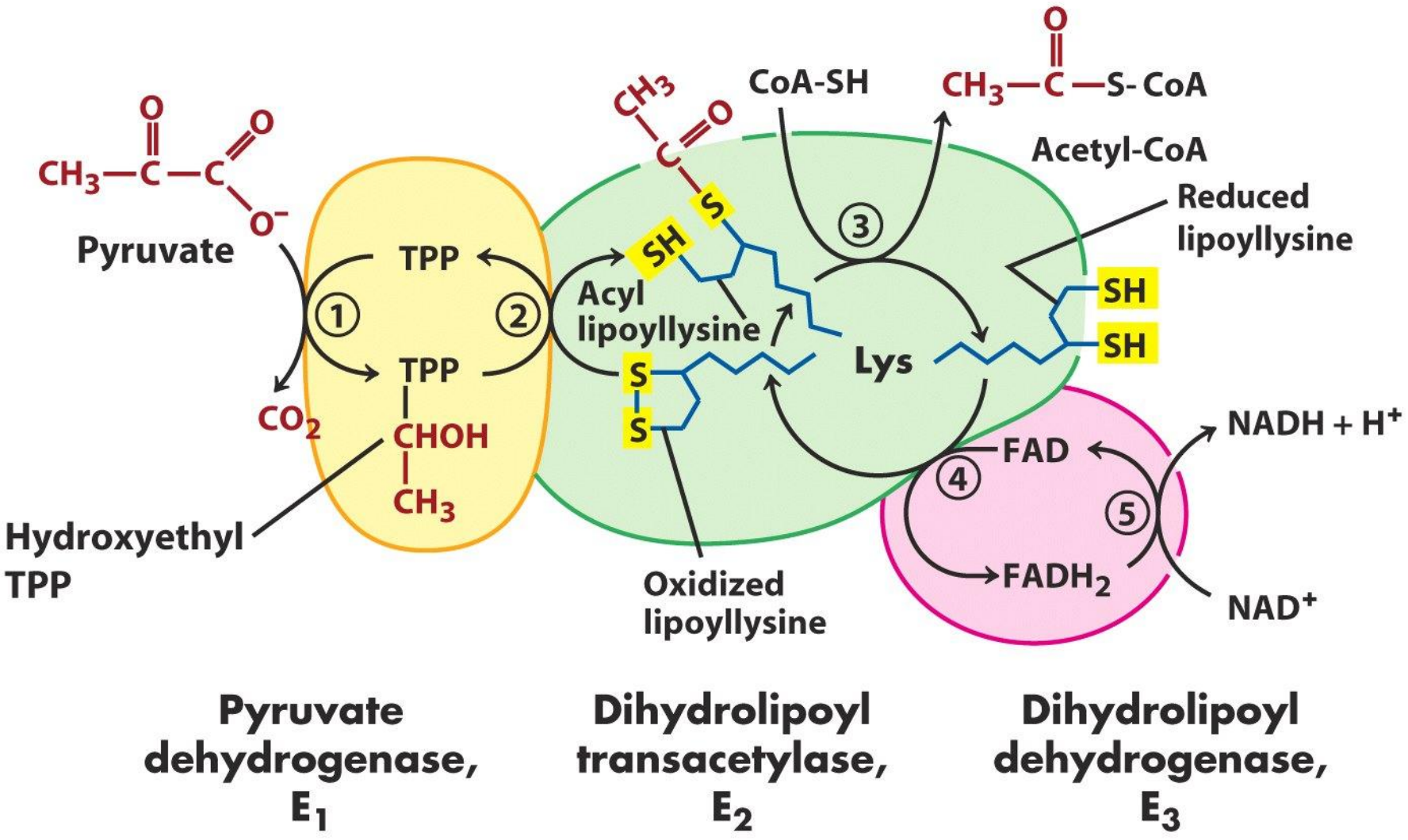
- Two electrons are removed in the oxidation reaction, and these reduce the disulfide bond of a lipoyllysine residue on E2 to two thiol groups.
- Acetate is transferred to one of the thiol groups .

3. Acetyl group transfer to CoA (E2)

- Arms of E2 move the acetyl group so that it can be transferred to CoA
- The bond to CoA is a thioester link – this is a ‘high-energy’ linkage, meaning that the acetyl group can be favorable transferred to another molecule (and we’ll see that it is again transferred to enter the citric acid cycle).

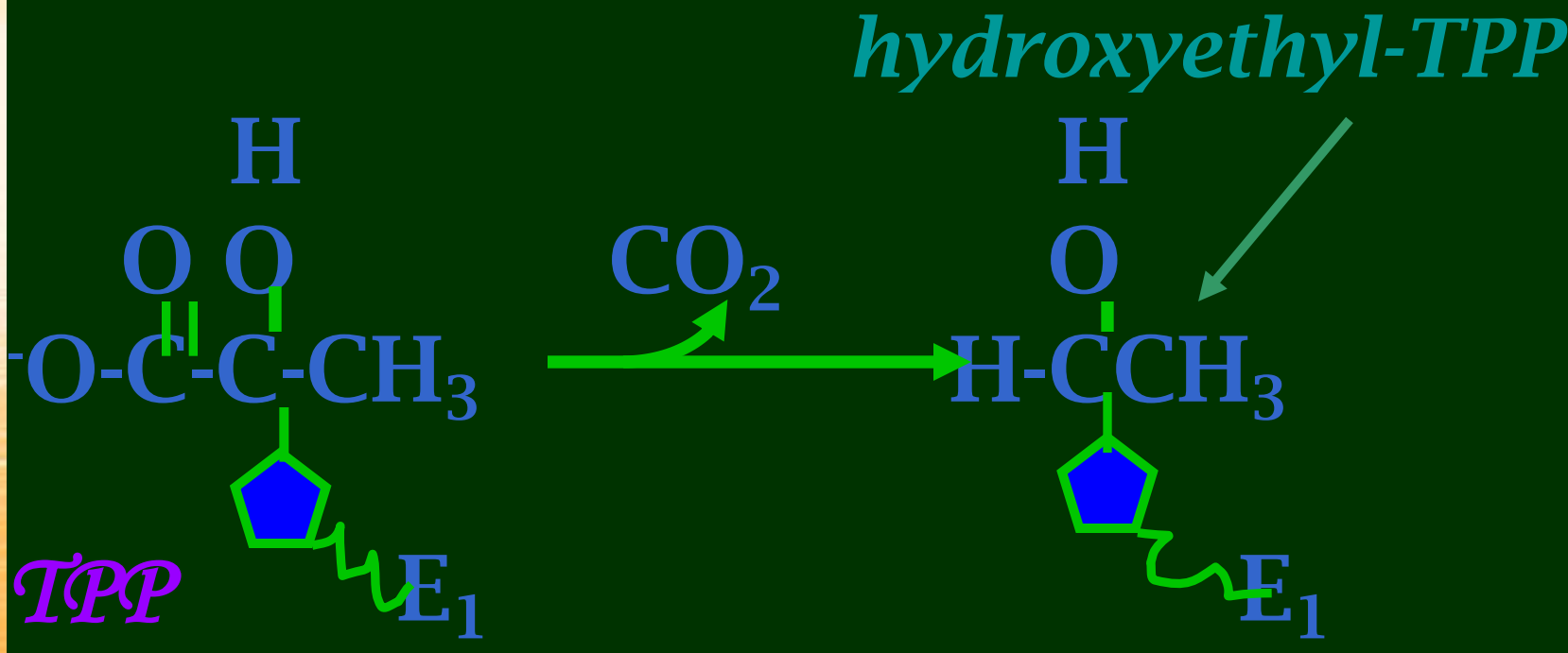
4. Reoxidation of the dithiol form of the lipoyl group of E2. (E3)
- Electrons are transferred to a FAD on E3 to give FADH2.
5. Transfer of electrons from FADH2 to NAD+
- NADH is released to solution, whereas the reoxidized FAD remains bound to E3.

Oxidative Decarboxylation of Pyruvate to Generate Acetyl-CoA



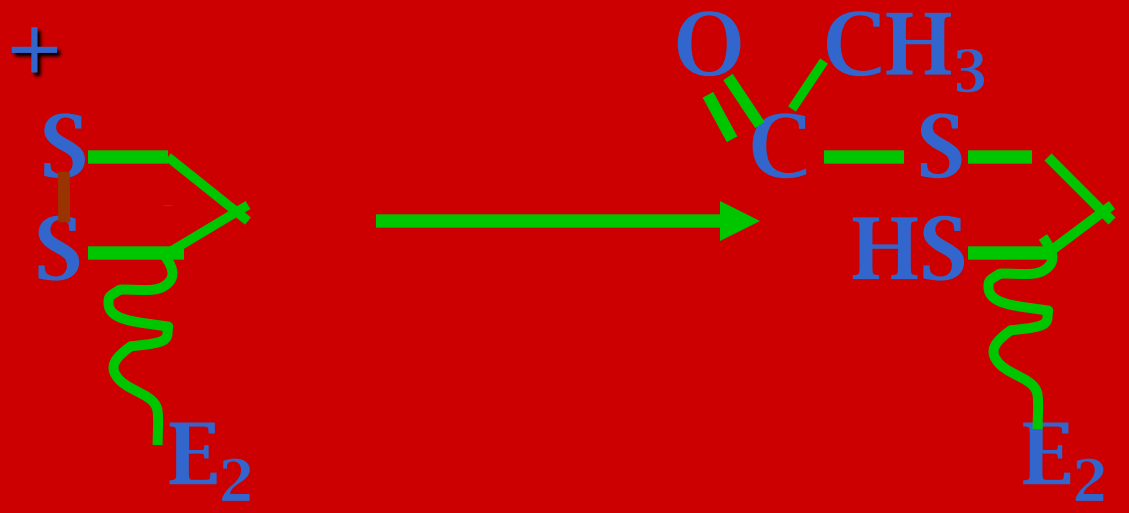
Partial reactions of PDH:

① introduction of *pyruvate onto TPP* in E_1



②Transfer to *lipoamide* of E_2

hydroxyethyl-TPP



lipoamide-E_2 **acetyl-lipoamide**-E_2
(disulfide,oxidized) (reduced)

- Note concurrent oxidation to acetyl and reduction of S

③ E_2 then *transfers* acetyl to CoA; acetyl-CoA *leaves*.

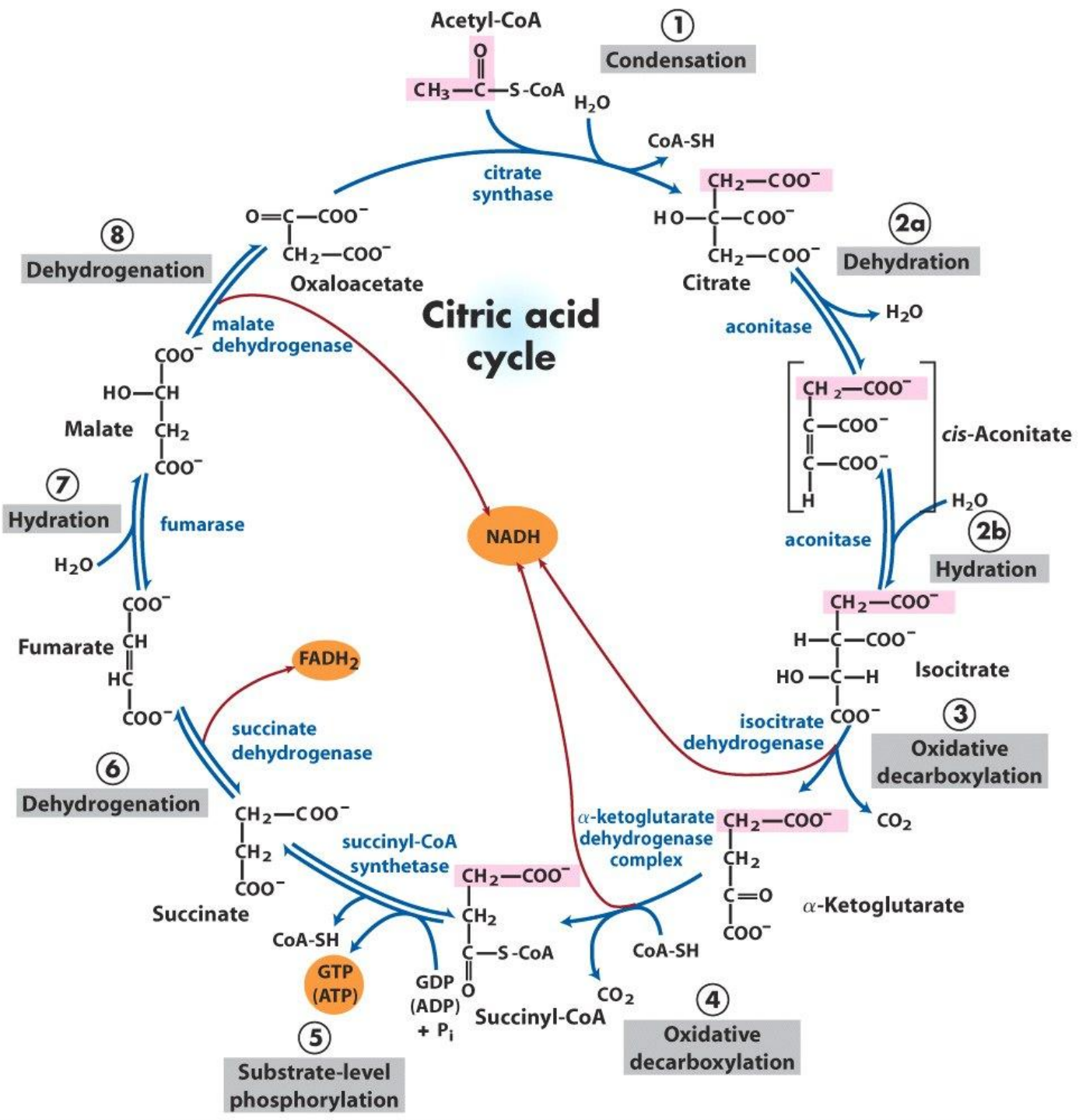
④ E_3 uses its bound coenzyme FAD to *oxidize* lipoamide back to disulfide and generating $FADH_2$.

⑤ FAD is recovered from $FADH_2$ via *reducing* NAD to NADH.

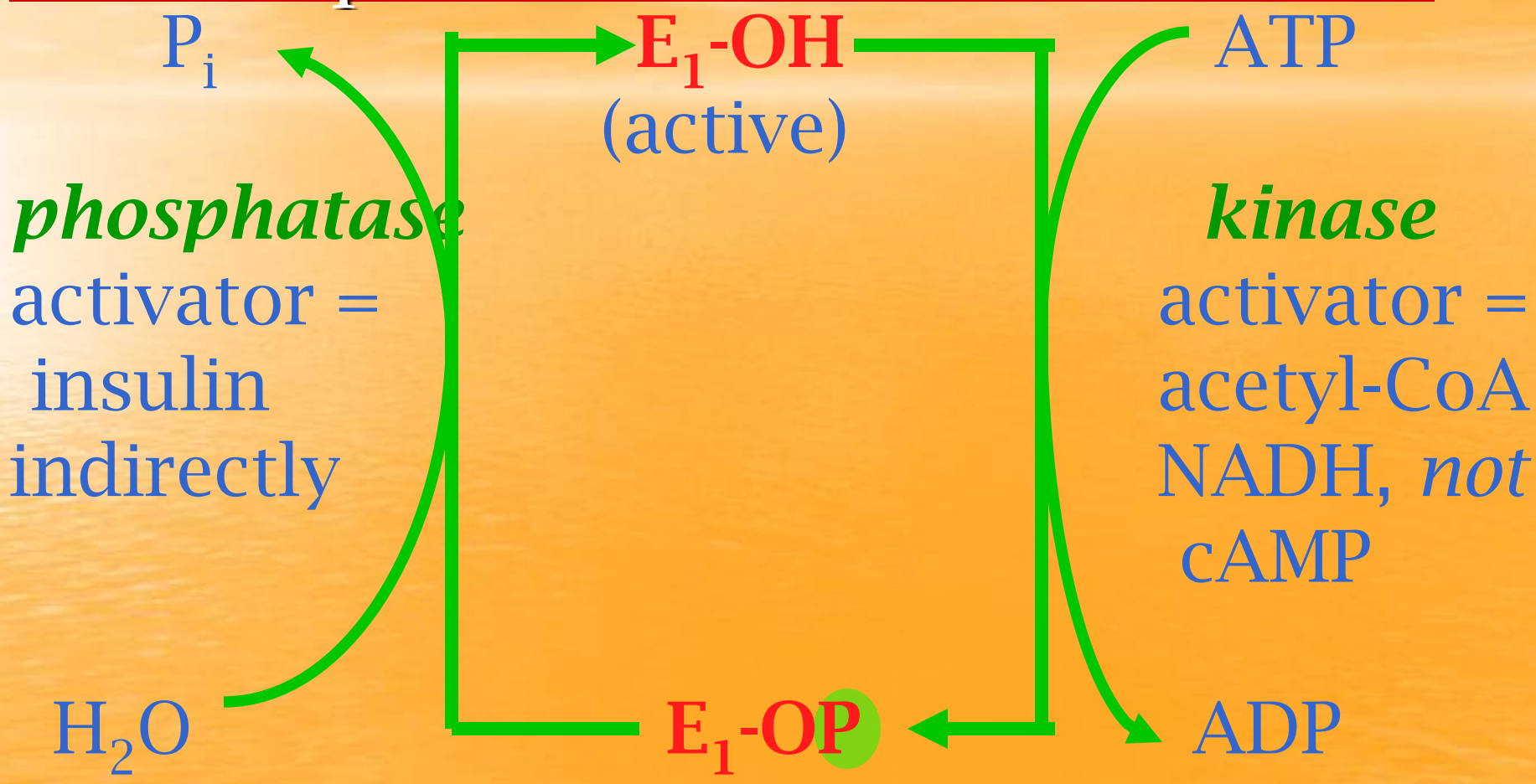
☛ An NADH is generated.

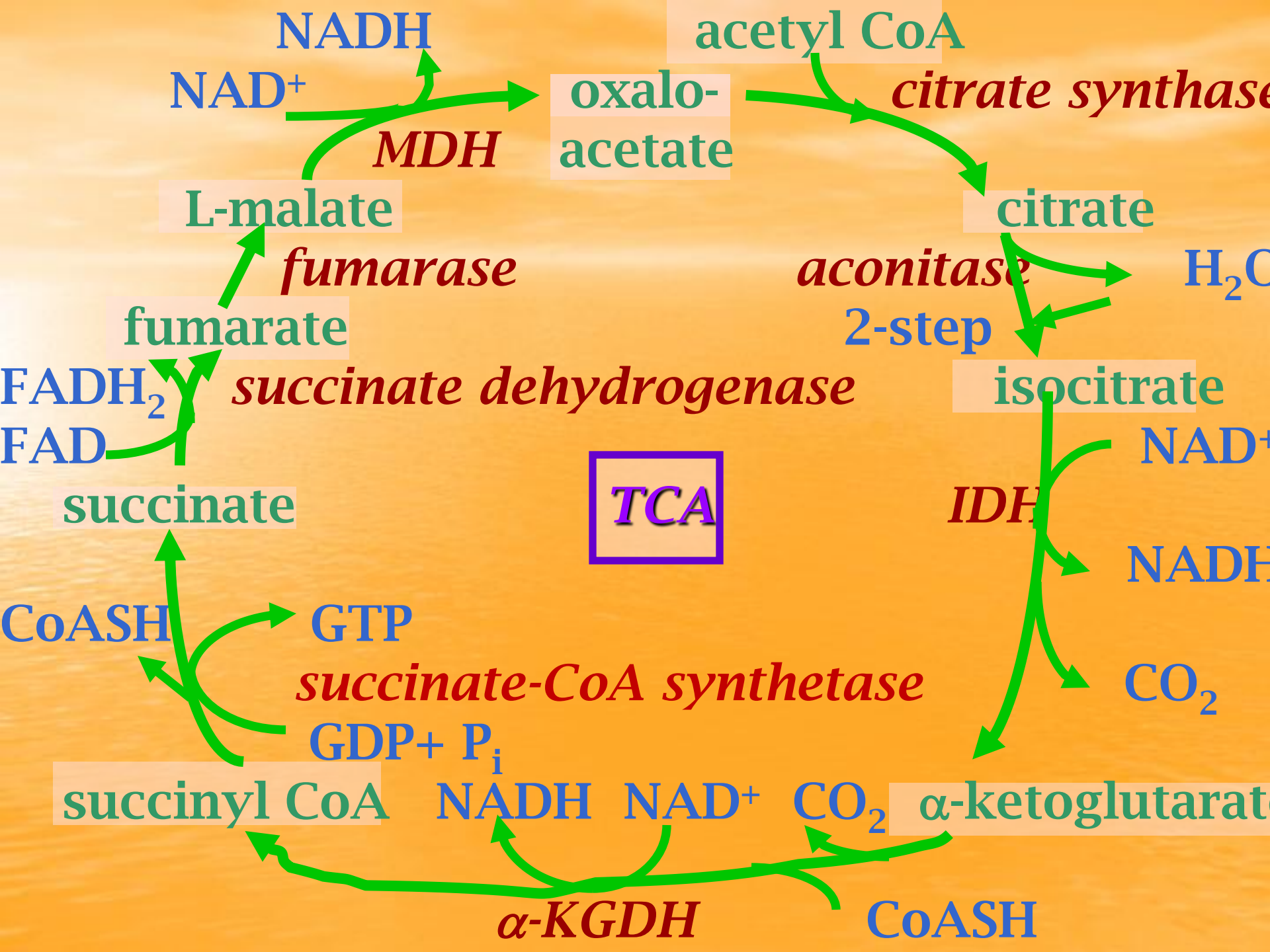
Regulation of pyruvate dehydrogenase complex

- Product inhibited by ATP, acetyl-CoA, and NADH
- Allosterically activated by AMP, CoA, and NAD+
- In vertebrates, there is another level of regulation: covalent protein modification.
- The complex is inhibited by reversible phosphorylation of a specific Ser residue on E1.
- The kinase responsible for the phosphorylation is allosterically activated by ATP, so that when ATP is in high concentration E1 becomes phosphorylated and the complex is inhibited.


Acetyl-CoA
1
Condensation
citrate synthase
CoA-SH
Oxaloacetate
Citrate
2a
Dehydration
aconitase
cis-Aconitate
2b
Hydration
Isocitrate
3
Oxidative decarboxylation
isocitrate dehydrogenase
α-ketoglutarate dehydrogenase complex
α-Ketoglutarate
4
Oxidative decarboxylation
Succinyl-CoA
5
Substrate-level phosphorylation
succinyl-CoA synthetase
GTP (ATP)
GDP (ADP) + Pi
Succinate
6
Dehydrogenation
succinate dehydrogenase
Fumarate
7
Hydration
fumarase
Malate
8
Dehydrogenation
malate dehydrogenase
Citric acid cycle
NADH
FADH2
H2O
CO2

Phosphorylation/dephosphorylation of E_1 subunit




NADH
NAD+
MDH
oxalo-
acetate
acetyl CoA
citrate synthase
L-malate
fumarase
citrate
aconitase
2-step
H2O
fumarate
FADH2
succinate dehydrogenase
isocitrate
FAD
NAD+
succinate
TCA
IDH
NADH
CoASH
GTP
succinate-CoA synthetase
CO2
GDP+ Pi
succinyl CoA
NADH
NAD+
CO2
α-ketoglutarate
α-KGDH
CoASH

3.Reactions of the citric acid cycle

- The citric acid cycle is the first example of a cyclic pathway .
- In each turn of the cycle, a molecule of acetyl-CoA donates its acetyl group to the four-carbon molecule oxaloacetate to form the six-carbon molecule citrate.
- In the citric acid cycle two carbons of citrate are oxidized to CO2, conversing energy in the form of NADH and FADH2.
- At the end of the cycle the remaining four carbons regenerate oxaloacetate, ready to pick up another acetyl group and begin the cycle again.