Aerobic metabolism

6.1) Citric Acid Cycle

6.2) Electron transport

6.3) Oxidative phosphorylation

6.4) Oxidative stress

- Aerobic oxidation of glucose- greater amounth of energy then does fermentation

- Oxygen highly reactive

- Oxidative cell damage: enzymes, antioxidant molecules

The Citric Acid Cycle

The citric acid cycle is the final common pathway for the oxidation of fuel molecules: amino acids, fatty acids, & carbohydrates.

- Most fuel molecules enter the cycle as acetyl coenzyme A
- This cycle is the central metabolic hub of the cell
- It is the gateway to aerobic metabolism for any molecule that can be transformed into an acetyl group or dicarboxylic acid,
- It is also an important source of precursors for building blocks
- Also known as, Krebs Cycle, & Tricarboxylic Acid Cycle (TCA)

Chapter 17: Outline

17.1 The citric acid cycle oxidizes two-carbon units

17.2 Entry to the cycle and metabolism through it are controlled

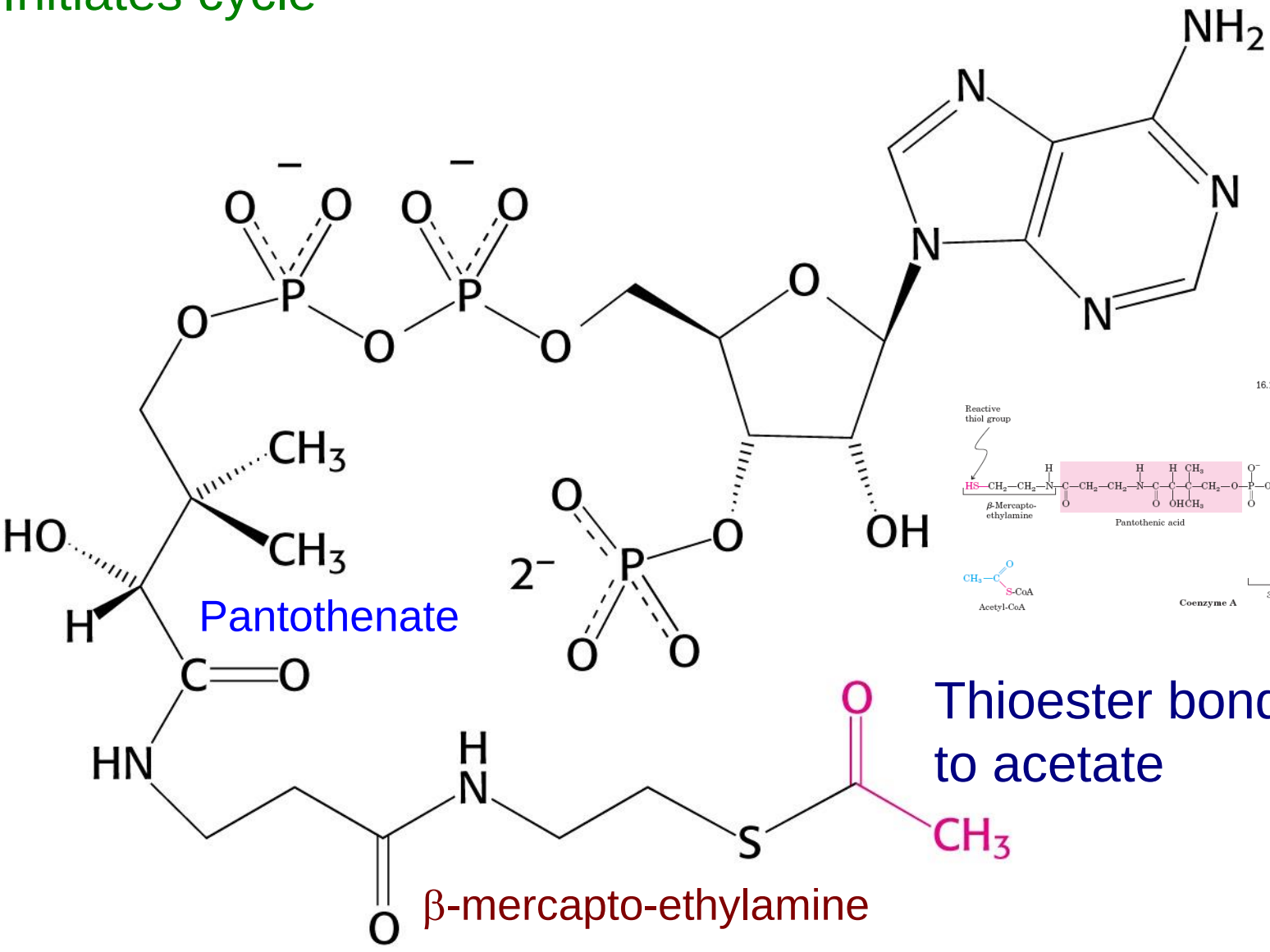
17.3 The cycle is a source of biosynthetic precursors

Overview of citric acid cycle

1. The function of the cycle is the harvesting of high-energy electrons from carbon fuels
2. The cycle itself neither generates ATP nor includes O_2 as a reactant
3. Instead, it removes electrons from acetyl CoA & uses them to form NADH & $FADH_2$ (high-energy electron carriers)
4. In oxidative phosphorylation, electrons from reoxidation of NADH & $FADH_2$ flow through a series of membrane proteins (electron transport chain) to generate a proton gradient
5. These protons then flow back through ATP synthase to generate ATP from ADP & inorganic phosphate
6. O_2 is the final electron acceptor at the end of the electron transport chain
7. The cytric acid cycle + oxidative phosphorylation provide > 95% of energy used in human aerobic cells

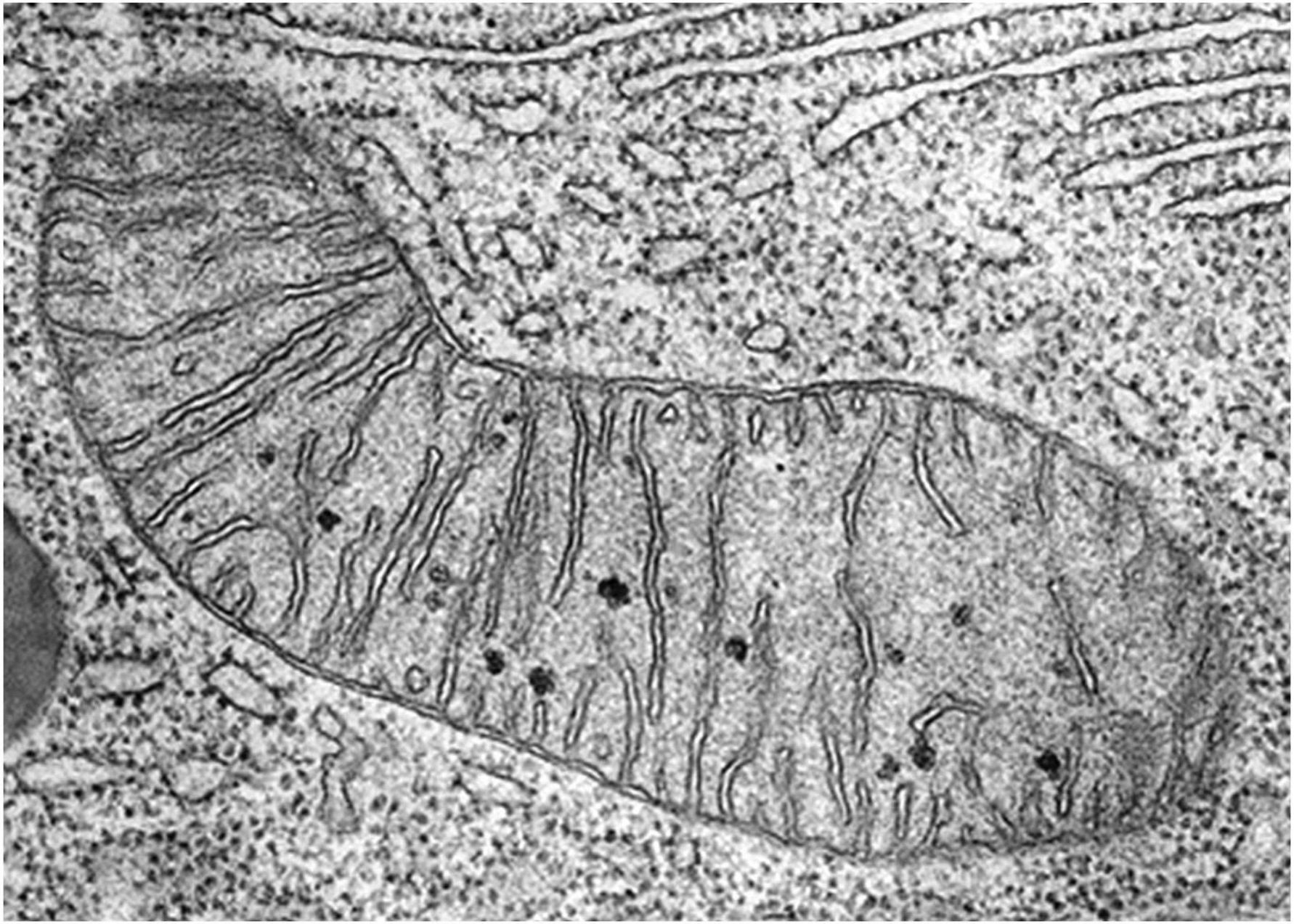
Fuel for the Citric Acid Cycle

Initiates cycle



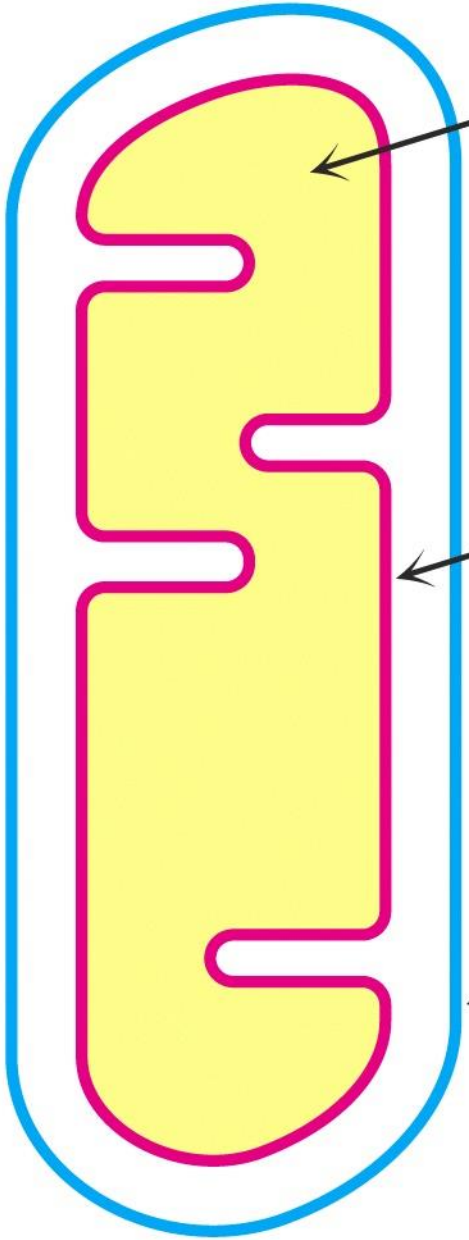
Acetyl coenzyme A (Acetyl CoA)

Mitochondrion



Double membrane, & cristae: invaginations of inner membrane

Mitochondrion



Matrix Oxidative decarboxilation of pyruvate, & citric acid cycle take place in matrix, along with fatty acid oxidation

Inner mitochondrial membrane

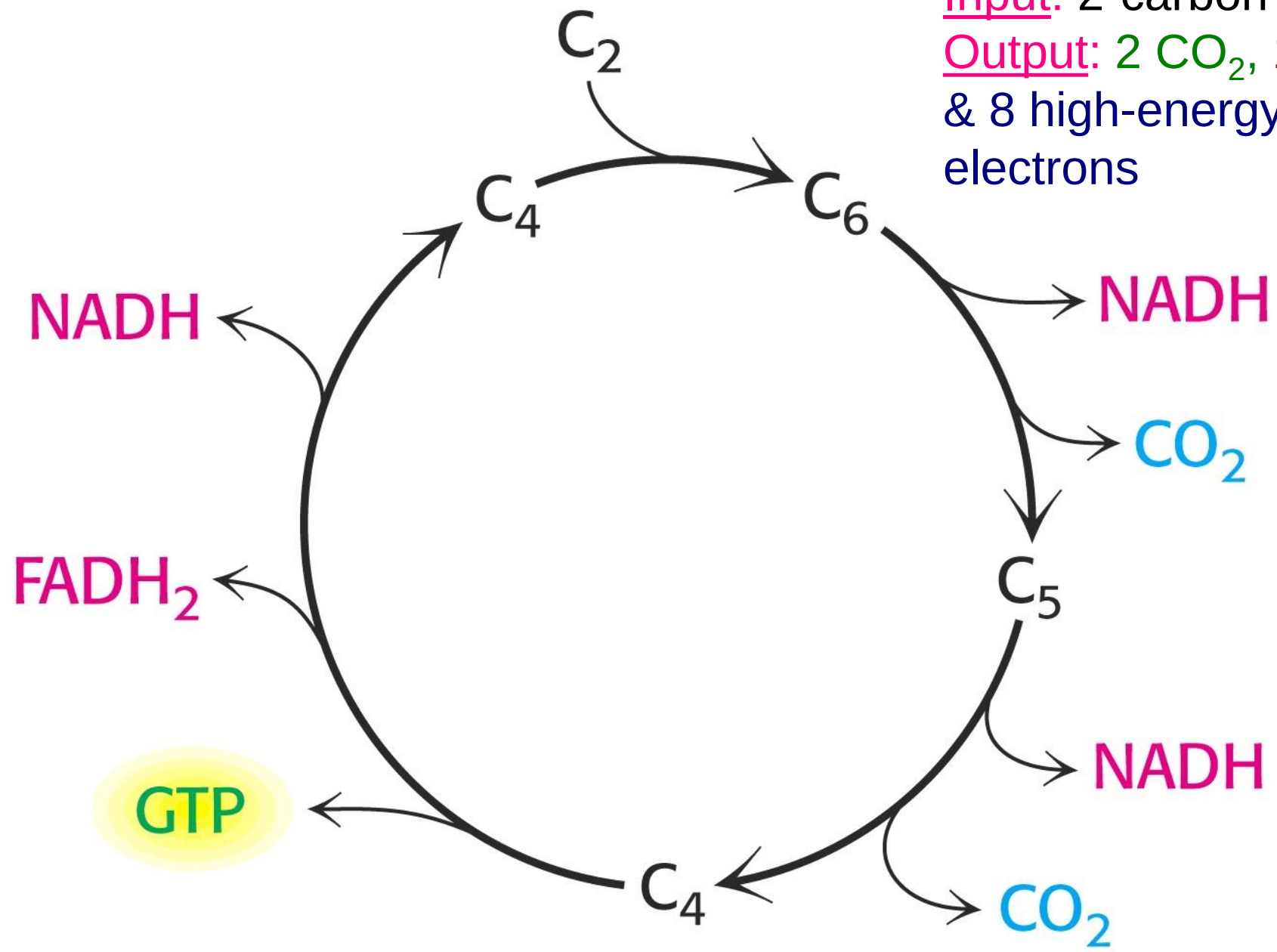
Site of oxidative phosphorylation

Outer mitochondrial membrane

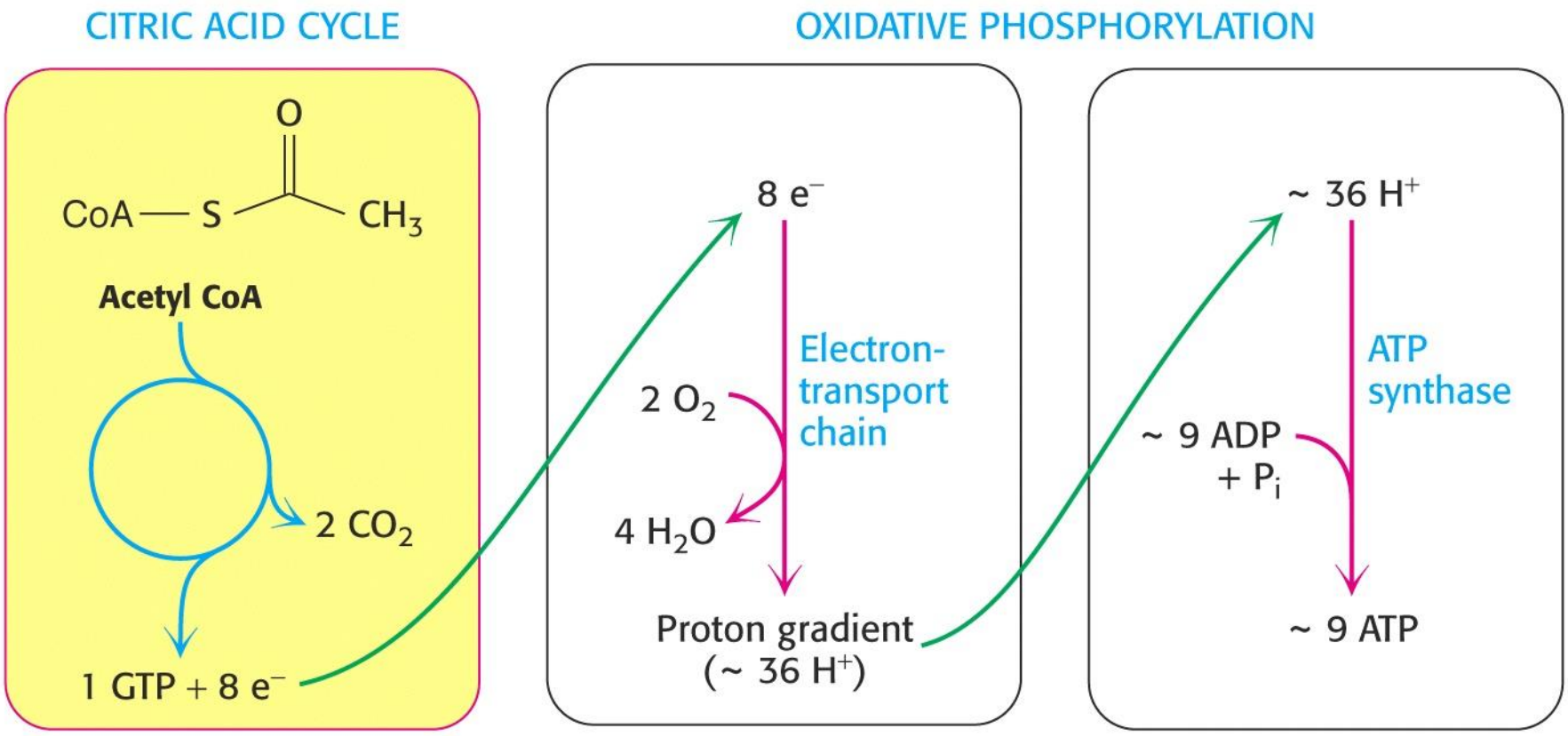
Permeable

Citric Acid Cycle: Overview

Input: 2-carbon units
Output: 2 CO_2, 1 GTP, & 8 high-energy electrons



Cellular Respiration

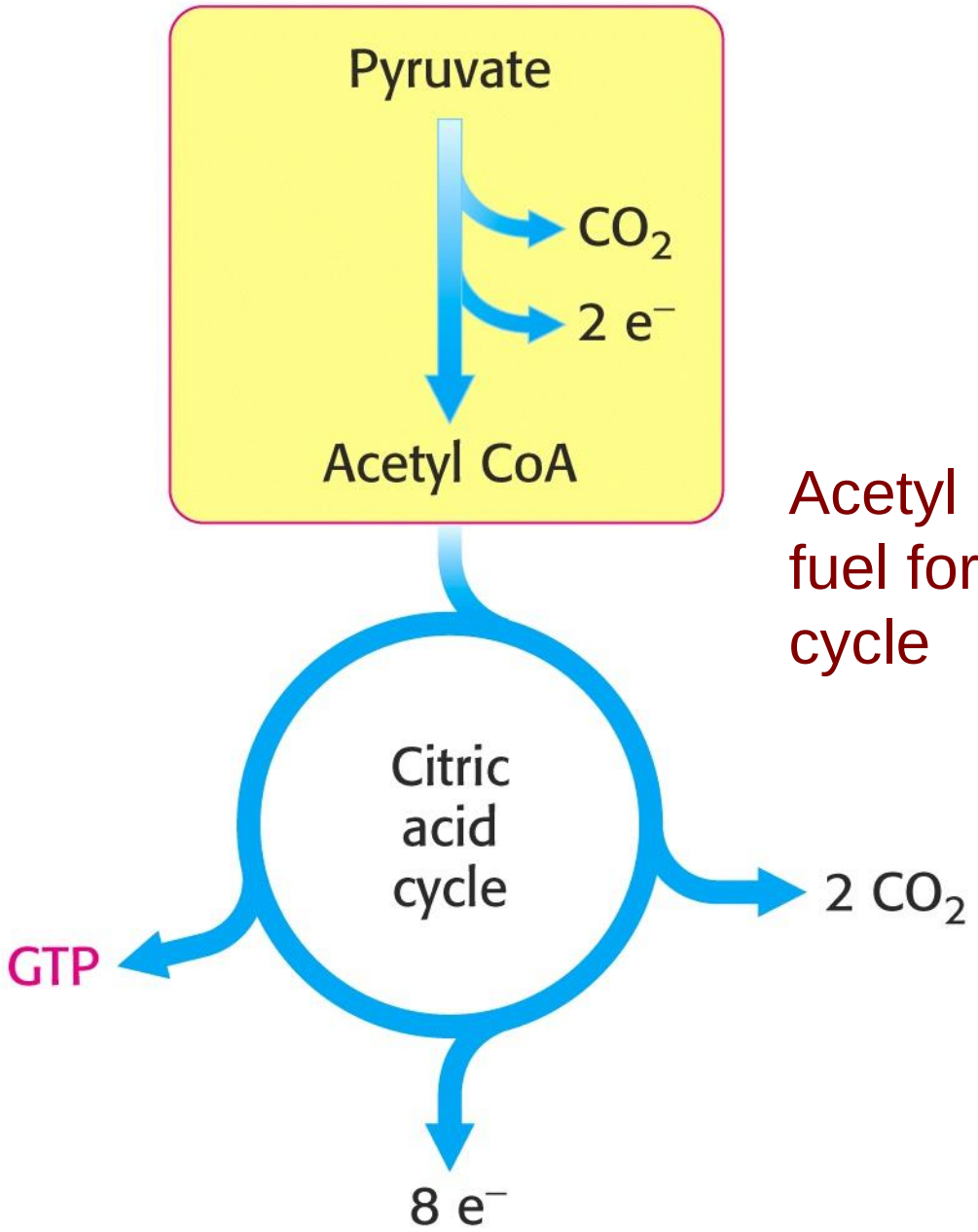


8 high-energy electrons from carbon fuels

Electrons reduce O_2 to generate a proton gradient

ATP synthesized from proton gradient

Glycolysis to citric acid cycle link

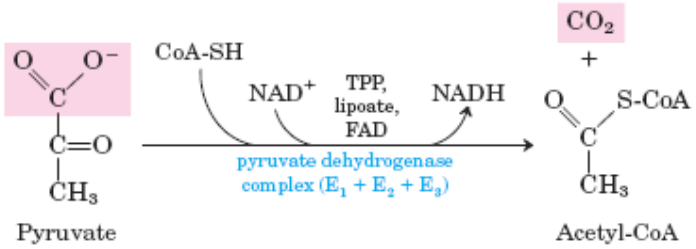


Acetyl CoA link is the fuel for the citric acid cycle

Oxidative decarboxylation

A large, highly integrated complex of three kinds of enzymes

Pyruvate + CoA + NAD^+ $\Rightarrow$ acetyl CoA + CO_2 + NADH



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

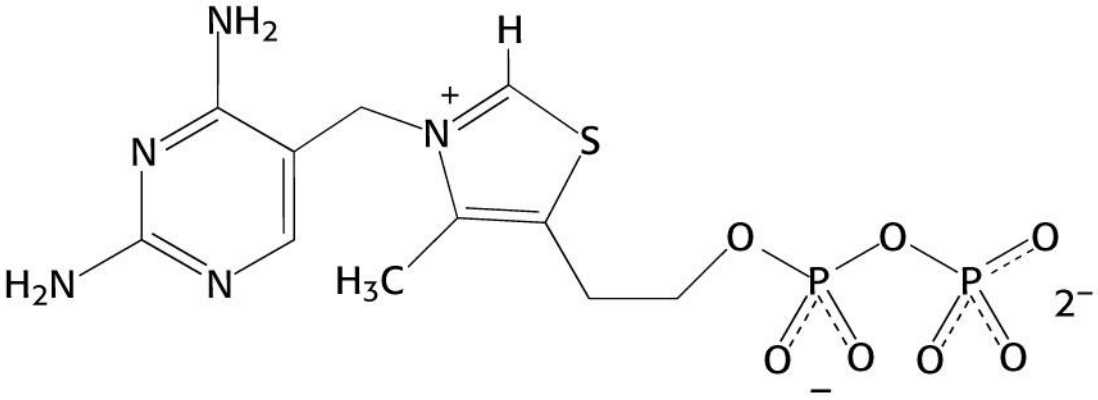
TABLE 17.1 Pyruvate dehydrogenase complex

Enzyme	Abbreviation	Number of chains	Prosthetic group	Reaction catalyzed
Pyruvate dehydrogenase component	E_1	24	TPP	Oxidative decarboxylation of pyruvate
Dihydrolipoyl transacetylase	E_2	24	Lipoamide	Transfer of the acetyl group to CoA
Dihydrolipoyl dehydrogenase	E_3	12	FAD	Regeneration of the oxidized form of lipoamide

Groups travel from one active site to another, connected by tethers to the core of the structure

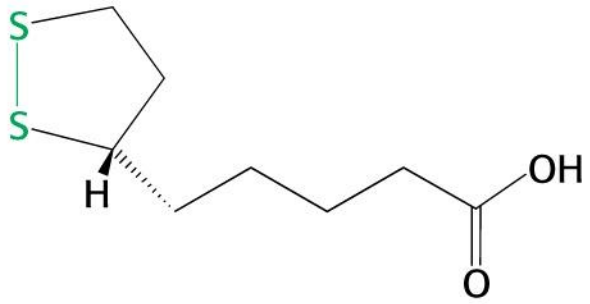
-irreversible oxidation, carboxyl group is removed from pyruvate as CO2 + AcCoA

3 enzymes
5 Coenzymes:



Thiamine pyrophosphate (TPP)

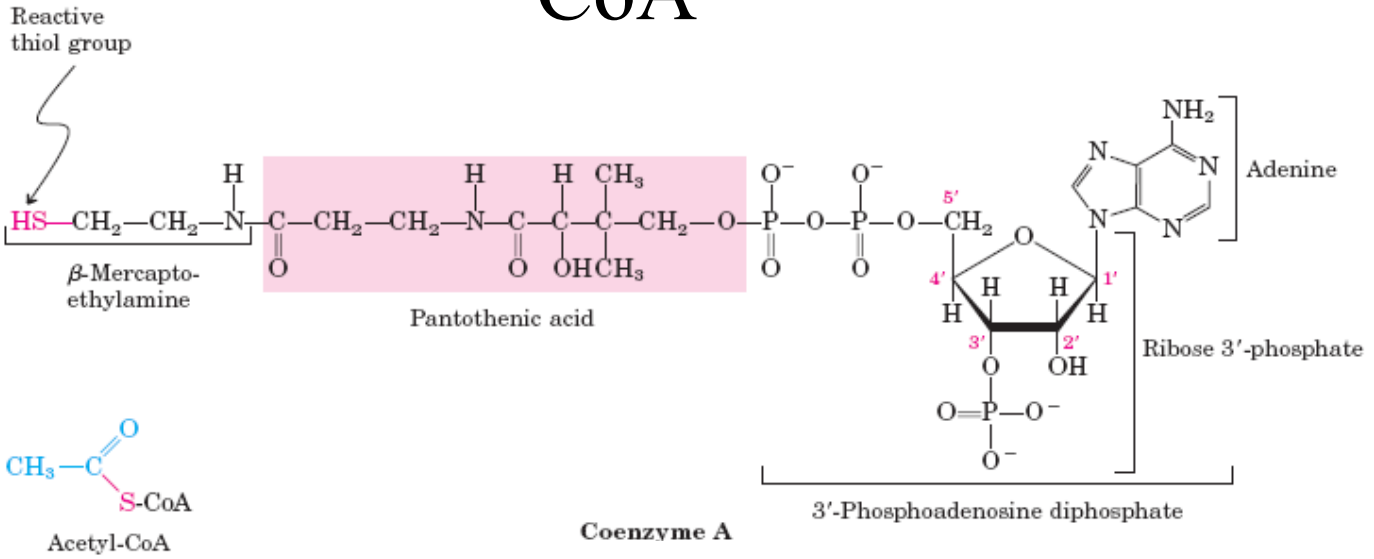
B_1 vitamin



Lipoic acid

Vitamines:thiamine, riboflavin (FAD), niacin (NAD), pantothenate (CoA)

CoA



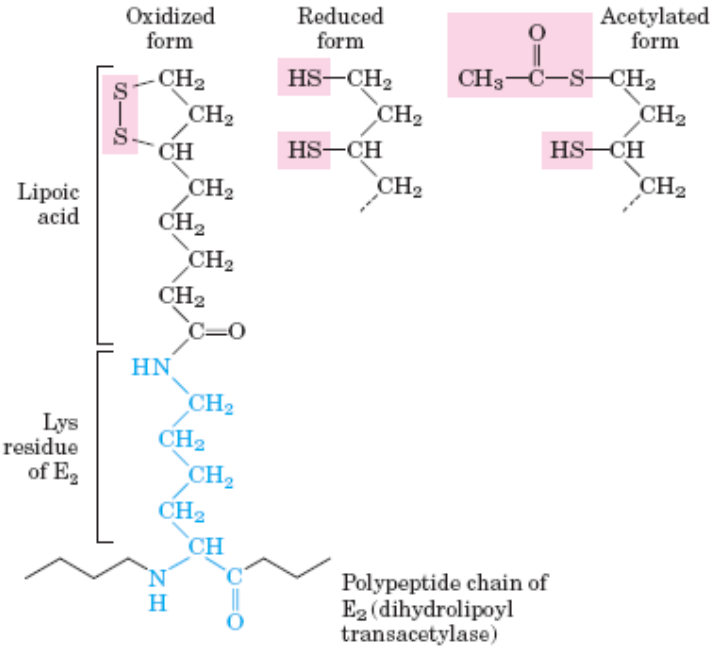
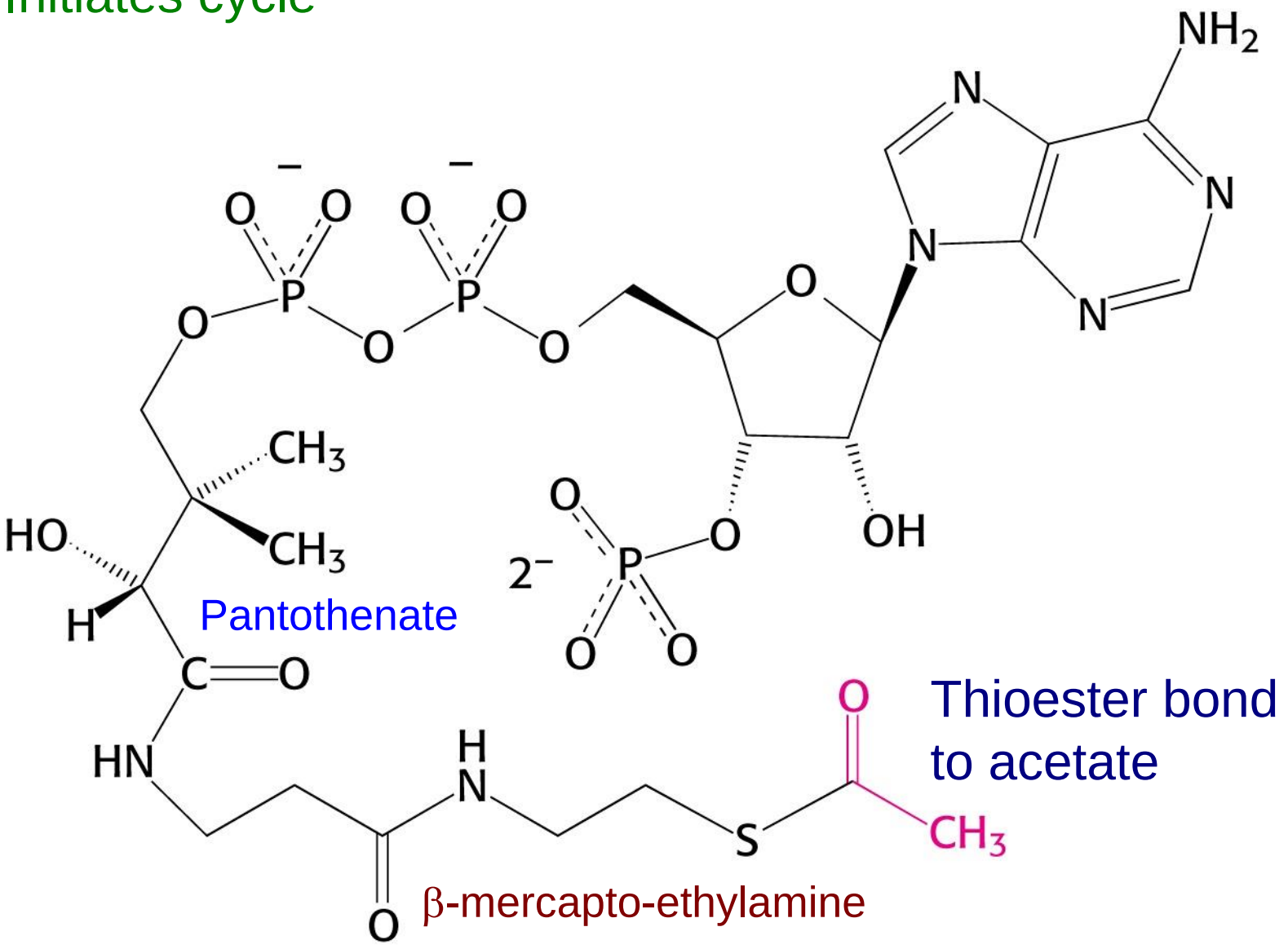


FIGURE 16–4 Lipoic acid (lipoate) in amide linkage with a Lys residue. The lipoyllysyl moiety is the prosthetic group of dihydrolipoyl transacetylase (E_2 of the PDH complex). The lipoyl group occurs in

Fuel for the Citric Acid Cycle

Initiates cycle



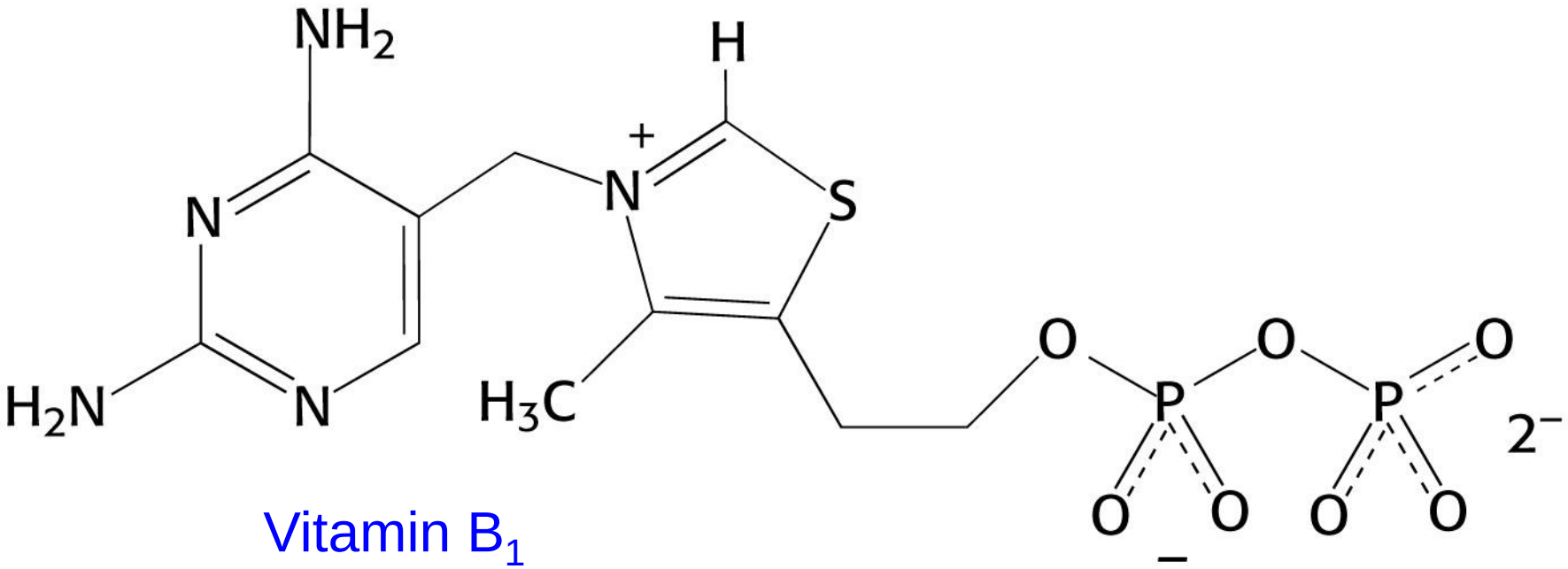
Pantothenate

Thioester bond to acetate

β-mercapto-ethylamine

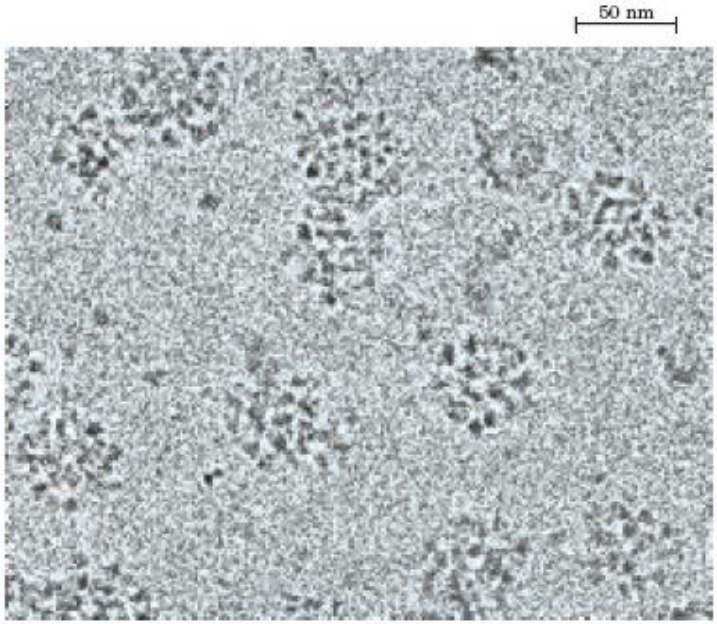
Acetyl coenzyme A (Acetyl CoA)

TPP

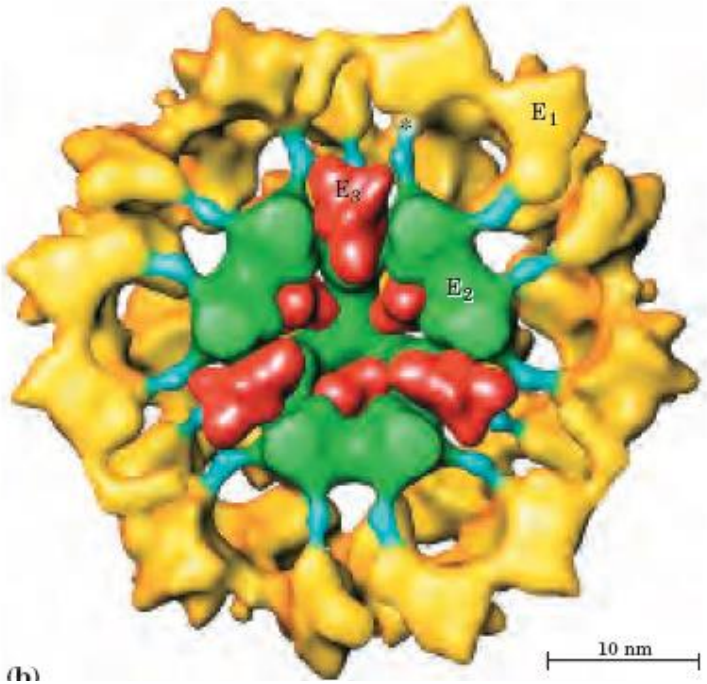


Vitamin B_1

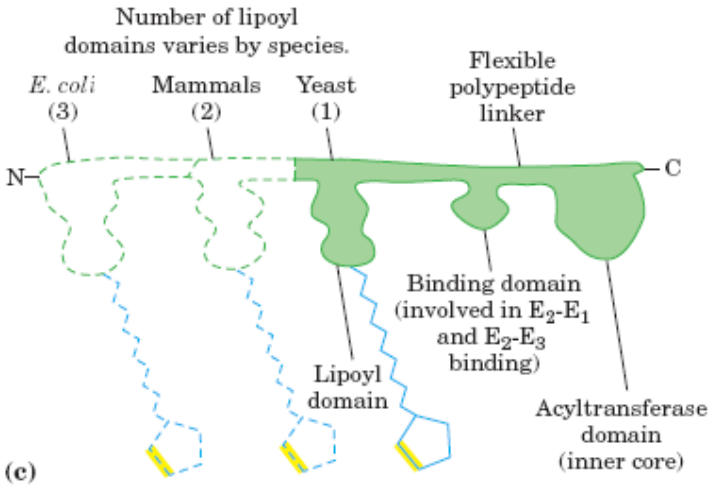
Thiamine pyrophosphate (TPP)


50 nm

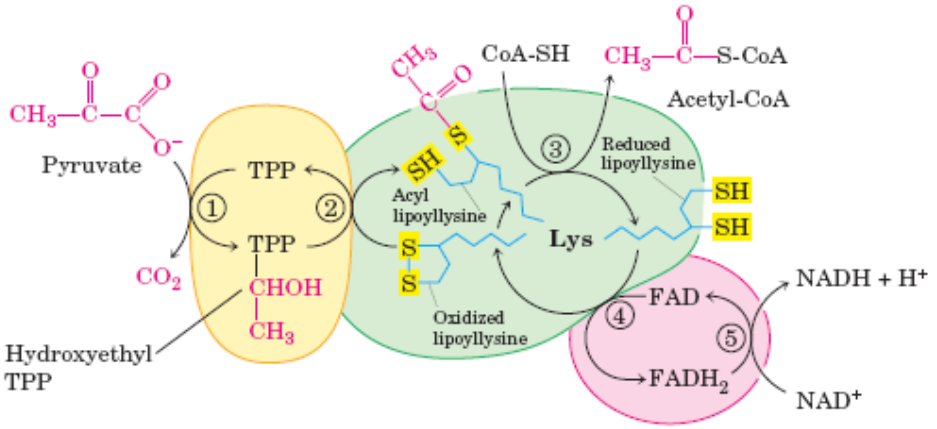
(a)

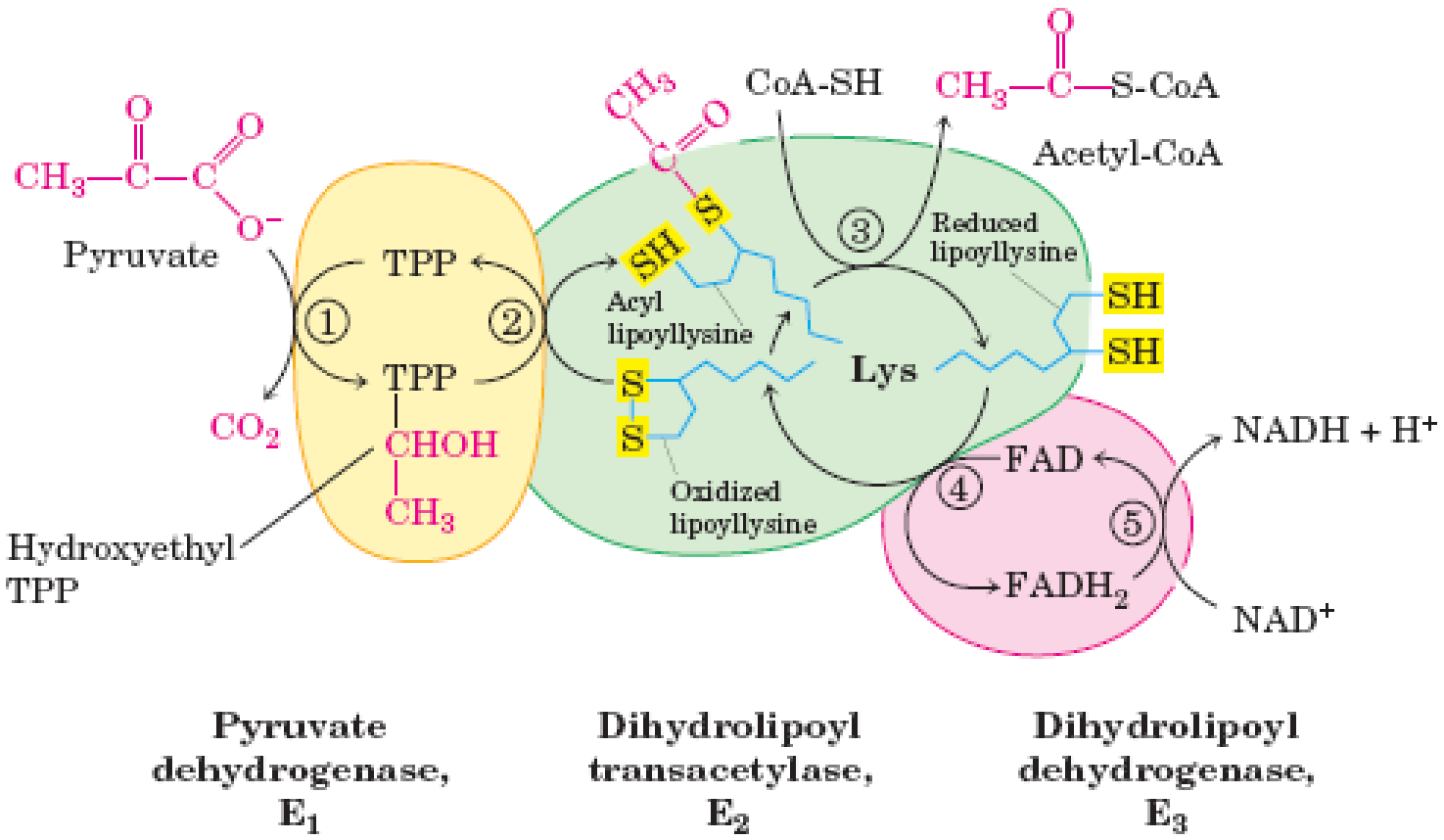

E1
E3
E2
10 nm

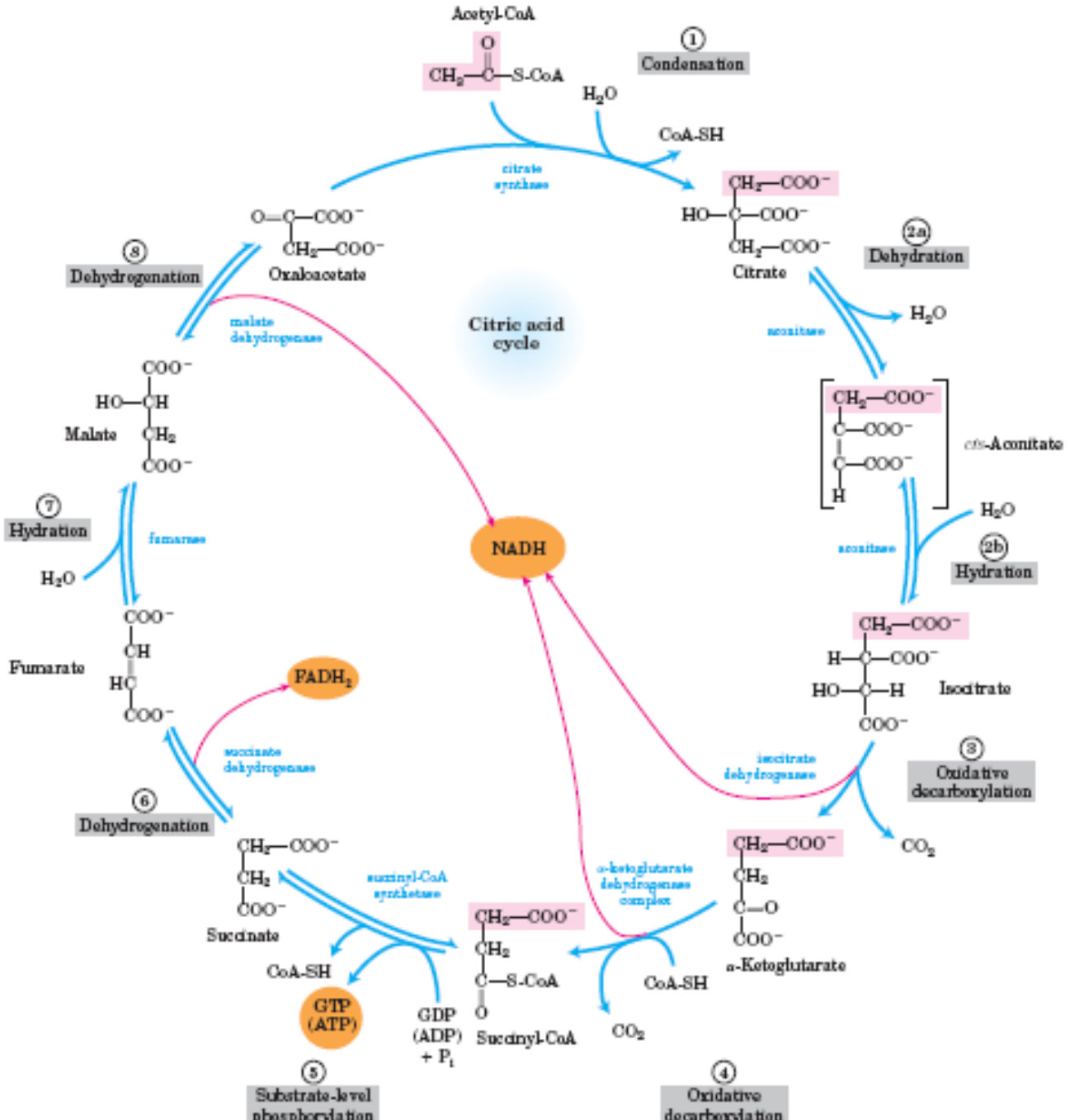
(b)

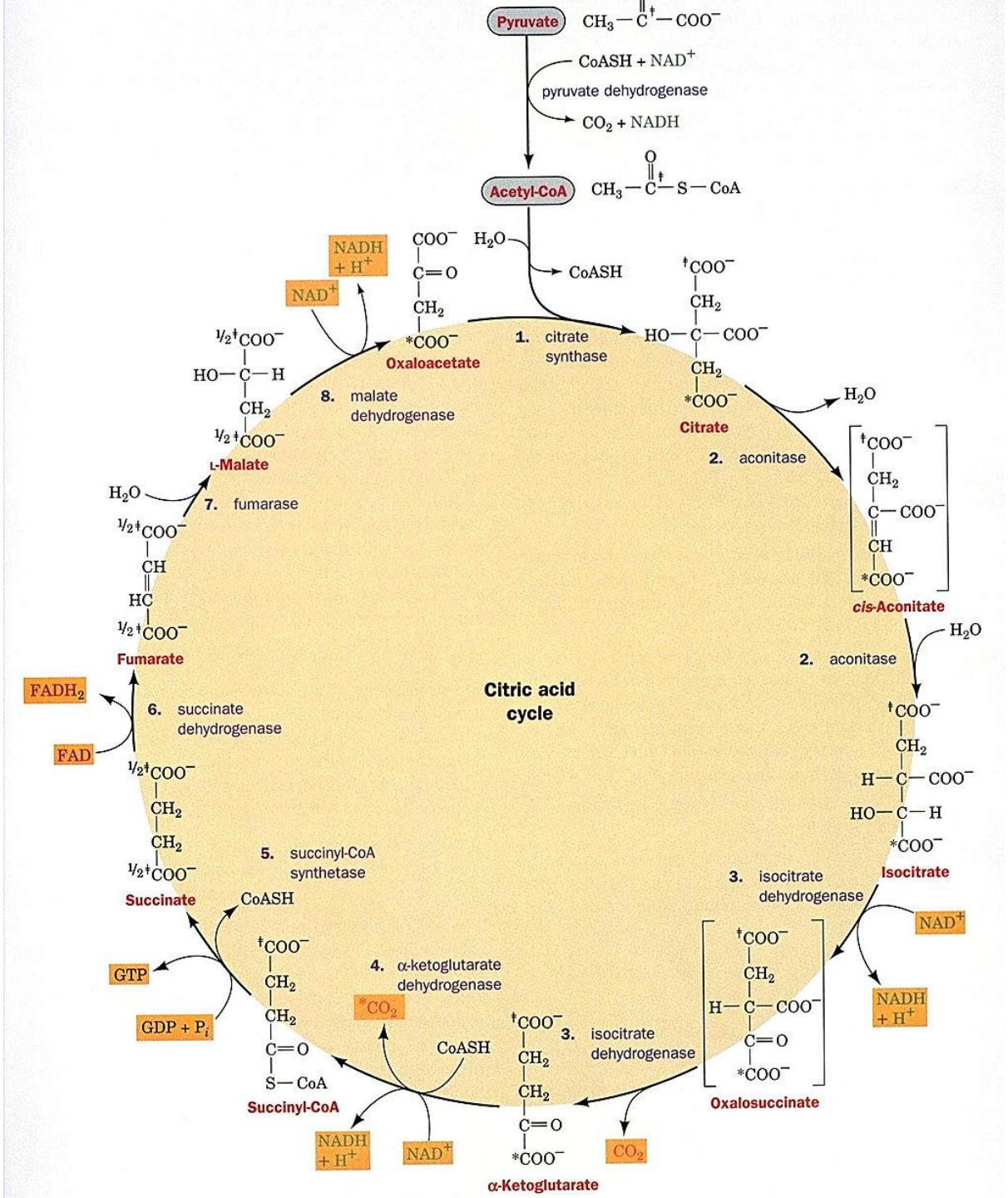

Number of lipoyl domains varies by species.
E. coli (3)
Mammals (2)
Yeast (1)
Flexible polypeptide linker
N
C
Binding domain (involved in E2-E1 and E2-E3 binding)
Lipoyl domain
Acyltransferase domain (inner core)

(c)


Pyruvate
TPP
TPP
CHOH
CH3
CO2
Hydroxyethyl TPP
CoA-SH
CH3—C—S-CoA
Acetyl-CoA
Reduced lipoyllysine
Acyl lipoyllysine
Lys
SH
SH
S
S
Oxidized lipoyllysine
FAD
FADH2
NADH + H+
NAD+
Pyruvate dehydrogenase, E1
Dihydrolipoyl transacetylase, E2
Dihydrolipoyl dehydrogenase, E3

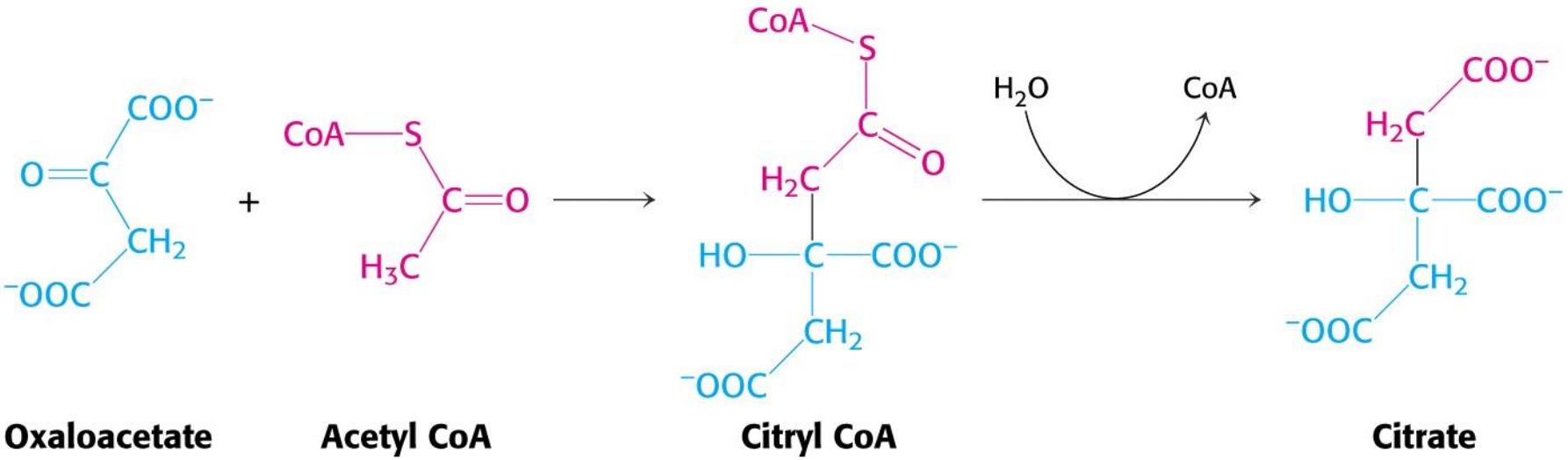

Pyruvate
TPP
TPP
CHOH
CH3
CO2
Hydroxyethyl TPP
CoA-SH
Acetyl-CoA
Acyl lipoyllysine
Reduced lipoyllysine
Lys
SH
SH
SH
S
S
Oxidized lipoyllysine
FAD
FADH2
NADH + H+
NAD+
1
2
3
4
5
Pyruvate dehydrogenase, E1
Dihydrolipoyl transacetylase, E2
Dihydrolipoyl dehydrogenase, E3


Acetyl-CoA
S-CoA
1
Condensation
H_2O
CoA-SH
citrate synthase
Oxaloacetate
Citrate
2a
Dehydration
H_2O
aconitase
Citric acid cycle
cis-Aconitate
H_2O
aconitase
2b
Hydration
Isocitrate
3
Oxidative decarboxylation
isocitrate dehydrogenase
CO_2
NADH
8
Dehydrogenation
malate dehydrogenase
Malate
7
Hydration
fumarase
H_2O
Fumarate
$FADH_2$
succinate dehydrogenase
6
Dehydrogenation
Succinate
succinyl-CoA synthetase
CoA-SH
GTP (ATP)
GDP (ADP) + P_i
5
Substrate-level phosphorylation
Succinyl-CoA
α-ketoglutarate dehydrogenase complex
CoA-SH
CO_2
α-Ketoglutarate
4
Oxidative decarboxylation


Pyruvate
CoASH + NAD+
pyruvate dehydrogenase
CO2 + NADH
Acetyl-CoA
CoASH
H2O
1. citrate synthase
Citrate
2. aconitase
cis-Aconitate
2. aconitase
Isocitrate
3. isocitrate dehydrogenase
NAD+
NADH + H+
Oxalosuccinate
3. isocitrate dehydrogenase
CO2
α-Ketoglutarate
4. α-ketoglutarate dehydrogenase
CO2
CoASH
NADH + H+
NAD+
Succinyl-CoA
5. succinyl-CoA synthetase
GTP
GDP + Pi
CoASH
Succinate
6. succinate dehydrogenase
FAD
FADH2
Fumarate
7. fumarase
H2O
L-Malate
8. malate dehydrogenase
NADH + H+
NAD+
Oxaloacetate
Citric acid cycle

Citrate Cycle: step 1 (citrate formation)

Enzyme: Citrate synthase

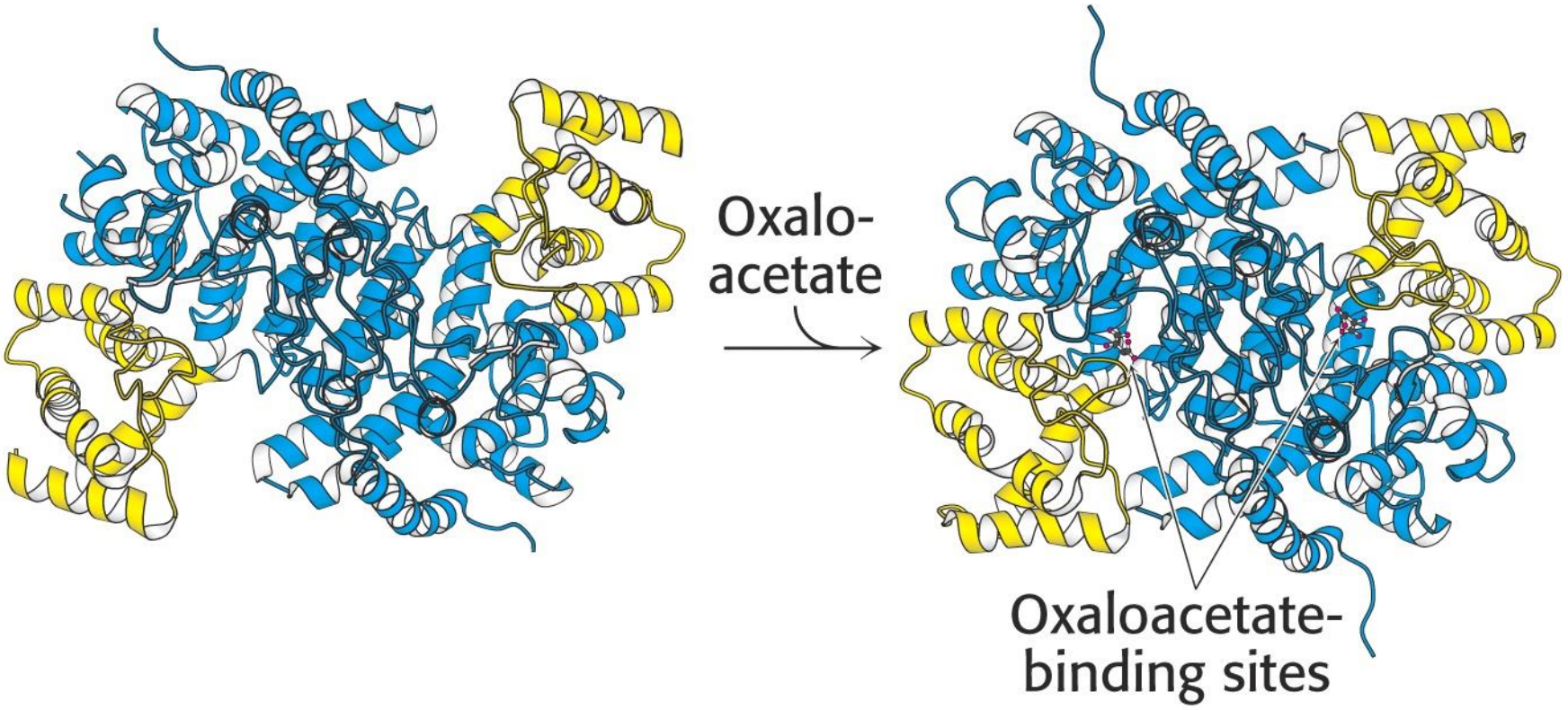


Condensation reaction

Hydrolysis reaction

Conformational changes in citrate synthase

Homodimer with large (blue) & small (yellow) domains

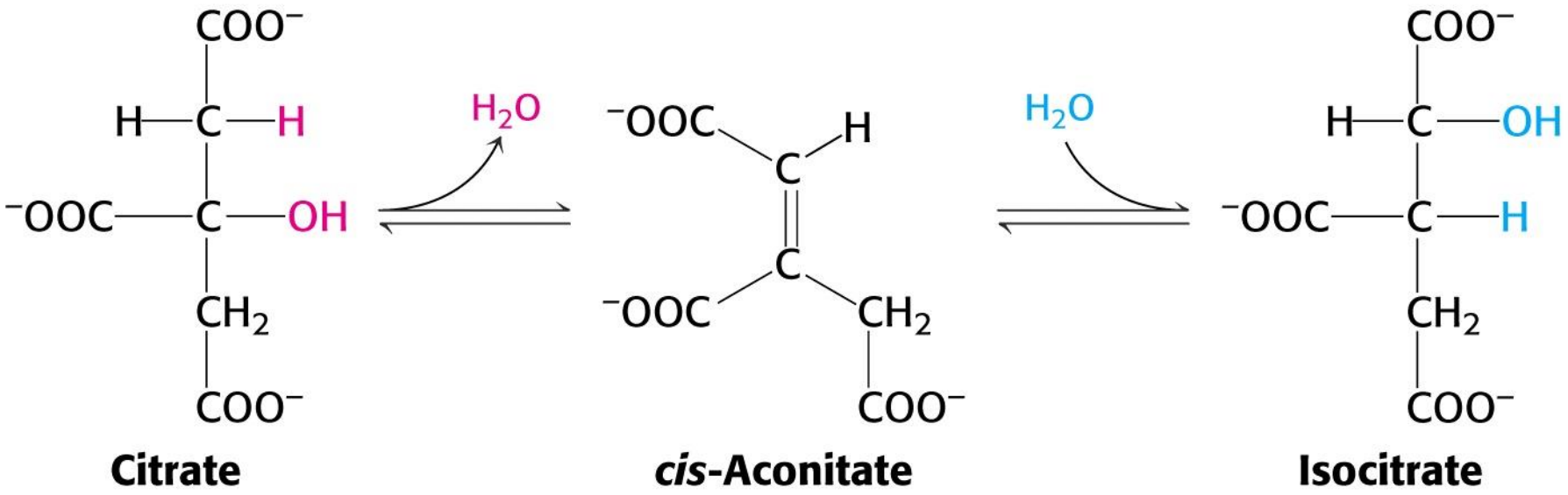


Open form

Closed form

Citrate isomerized to Isocitate: step 2

Enzyme: aconitase

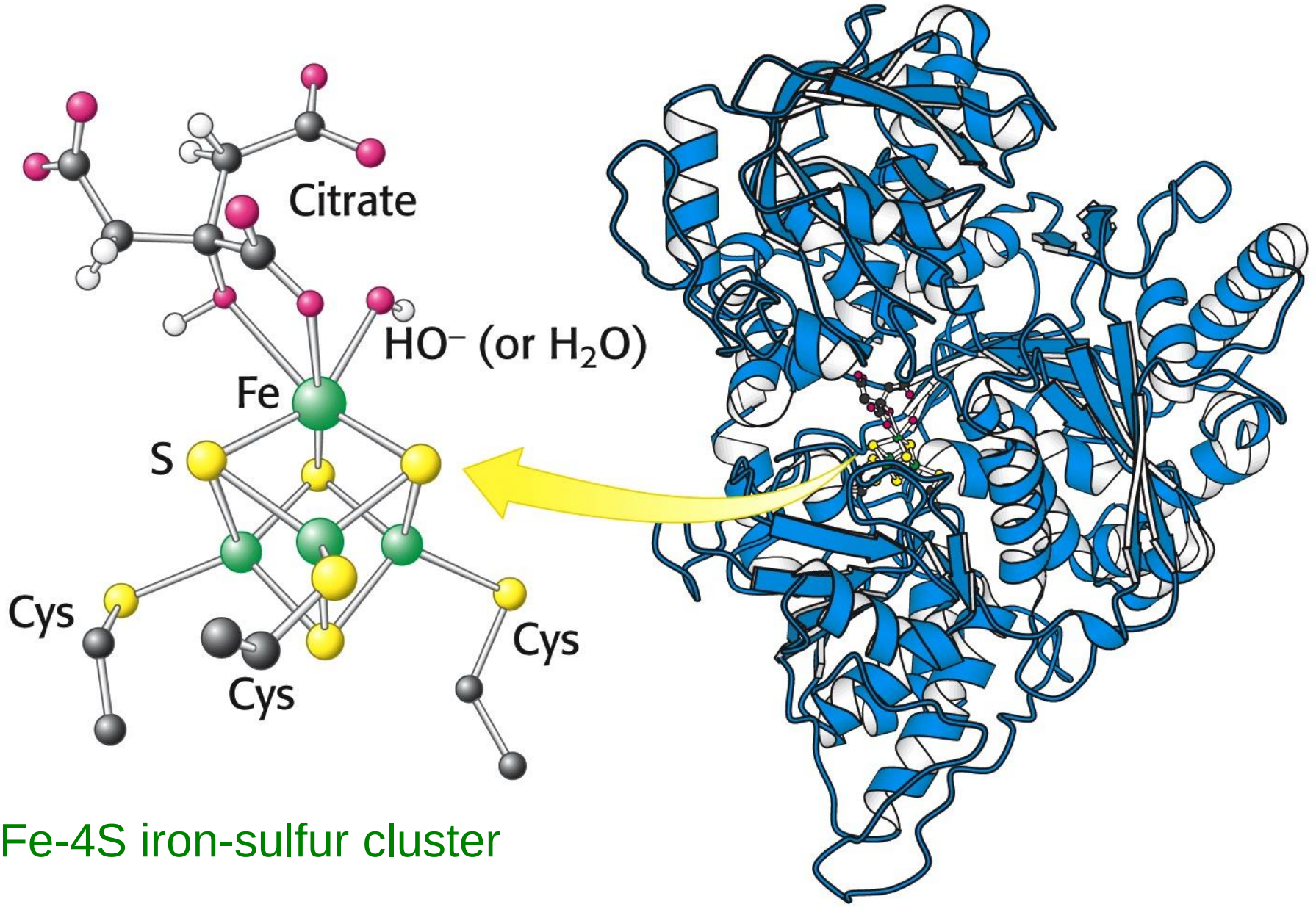


Citrate ***cis*-Aconitate** **Isocitrate**

Dehydration

Hydration

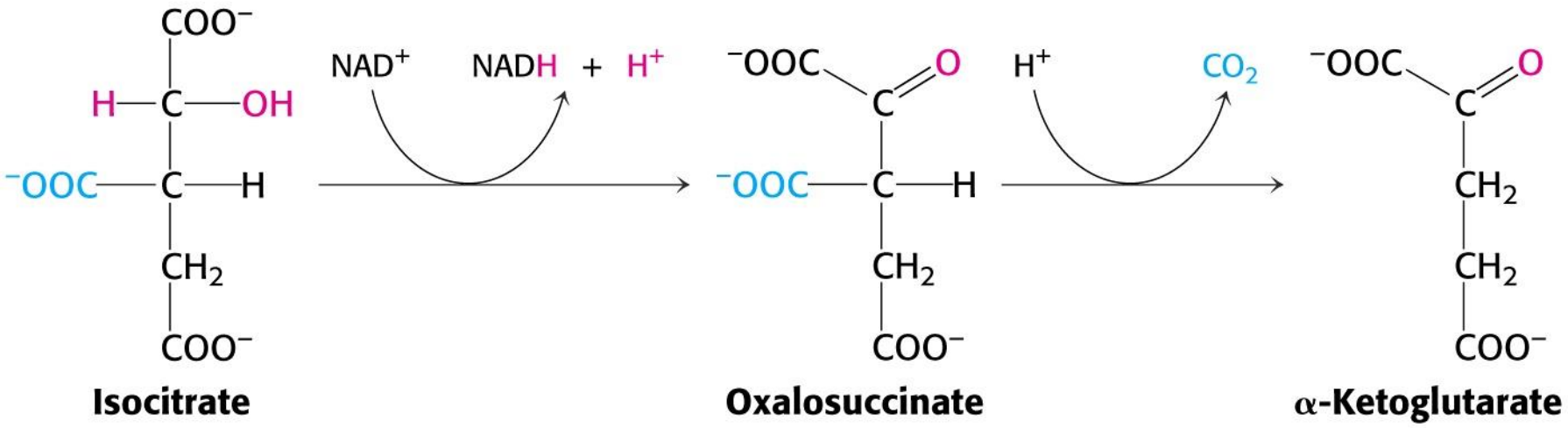
Aconitase: citrate binding to iron-sulfur cluster



4Fe-4S iron-sulfur cluster

Isocitrate to α-ketoglutarate: step 3

Enzyme: isocitrate dehydrogenase



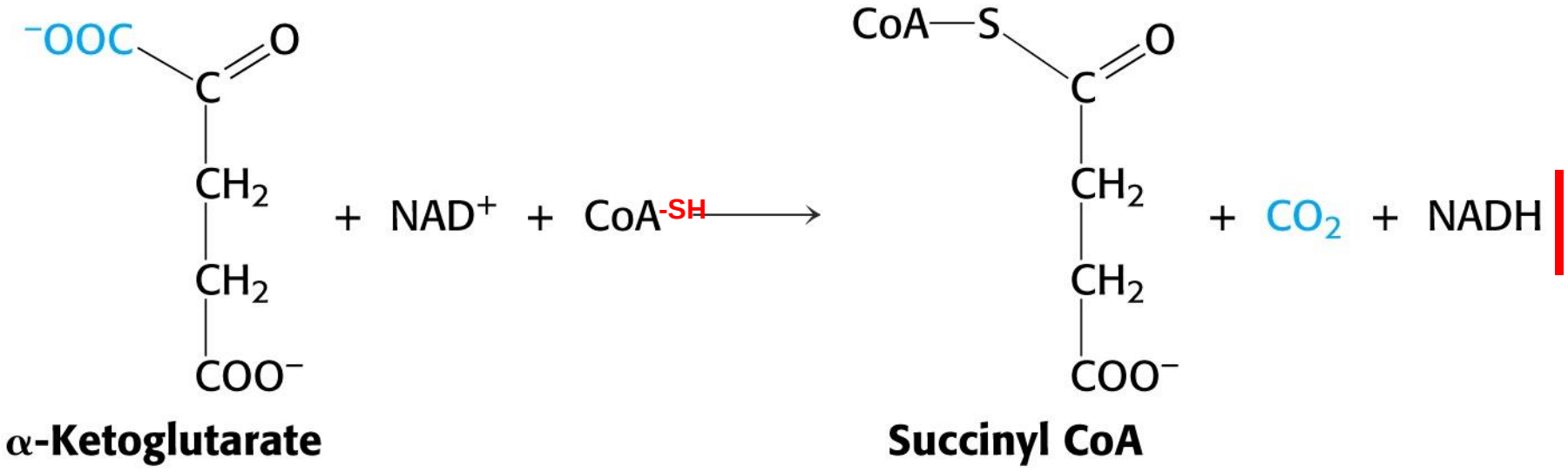
1st NADH produced

1st CO_2 removed

Oxidation of IC to aKG and CO2, Mn2+ in active site

Succinyl CoA formation: step4

Enzyme: α-ketoglutarate dehydrogenase



α-Ketoglutarate **Succinyl CoA**

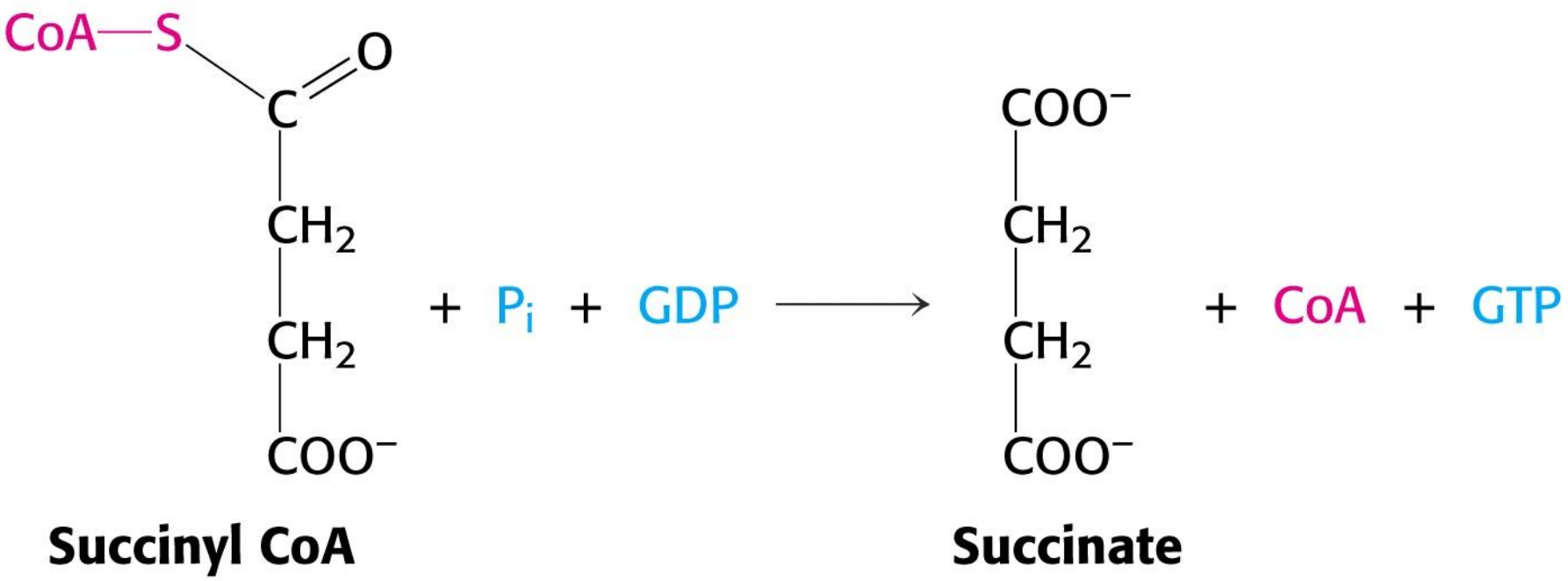
2nd NADH produced

2nd CO_2 removed

oxidation

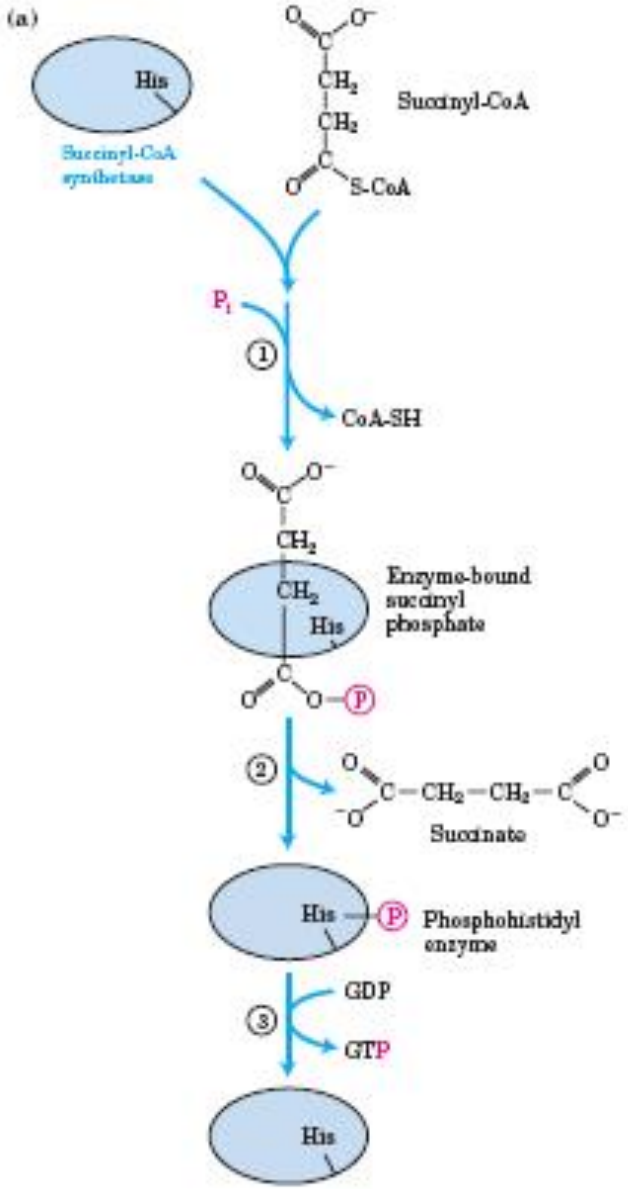
Succinate formation: step5

Enzyme: succinyl CoA synthetase

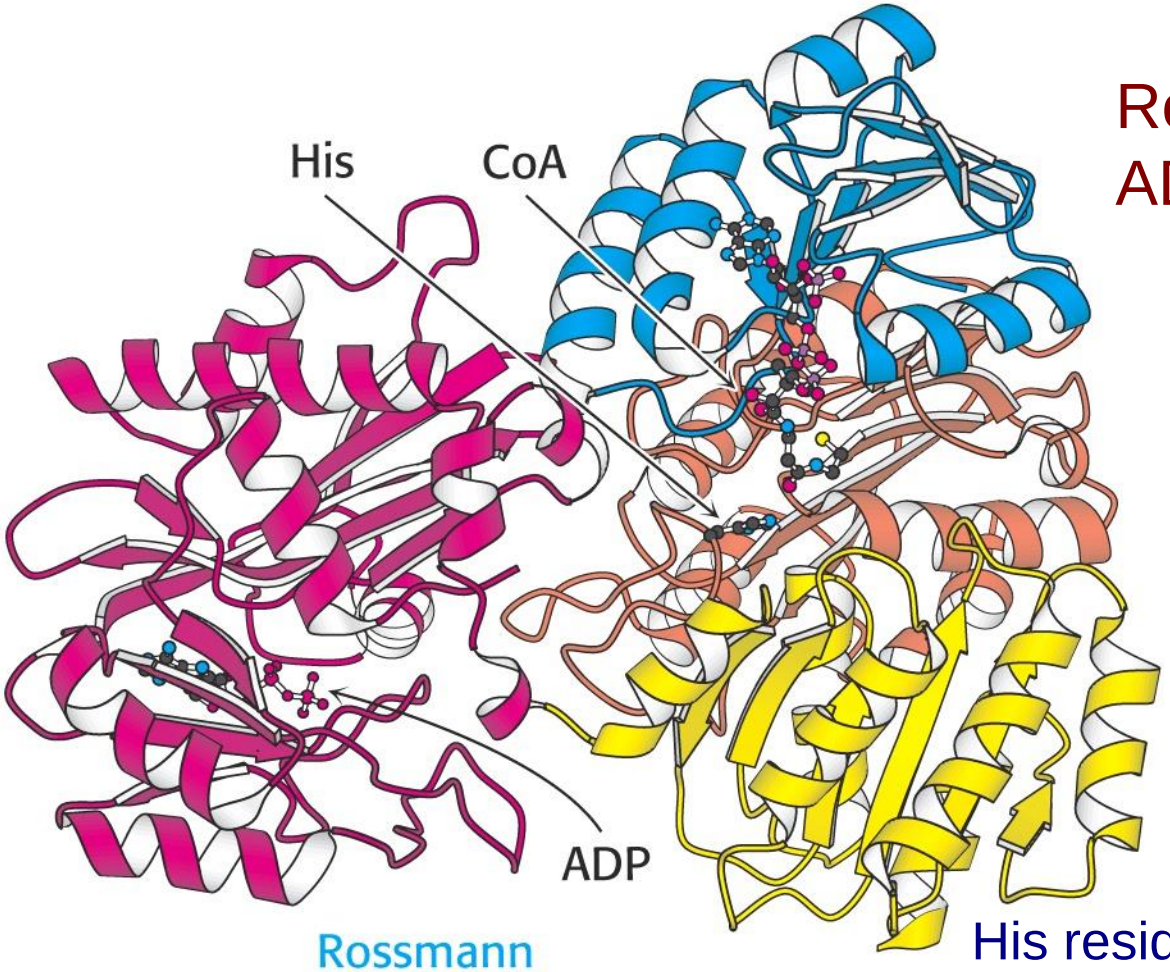


GTP produced

GTP + ADP ⇒ GDP + ATP (NPTase)


(a)
His
Succinyl-CoA synthetase
Succinyl-CoA
S-CoA
P_i
1
CoA-SH
Enzyme-bound succinyl phosphate
His
P
2
Succinate
His
P
Phosphohistidyl enzyme
GDP
3
GTP
His

Succinyl CoA synthetase



Rossman fold binds ADP component of CoA

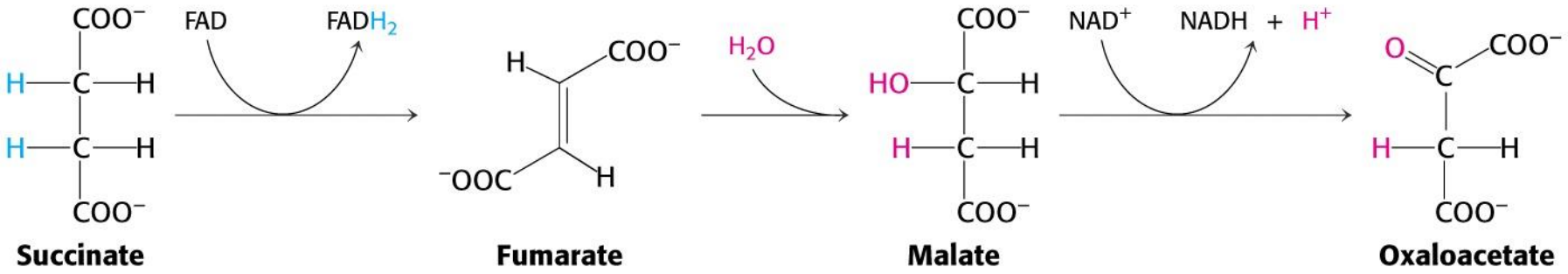
ATP-grasp domain is a nucleotide-activating domain, shown binding ADP.

His residue picks up phosphoryl group from near CoA, & swings over to transfer it to the nucleotide bound in the ATP-grasp domain

Oxaloacetate regenared by oxidation of succinate:

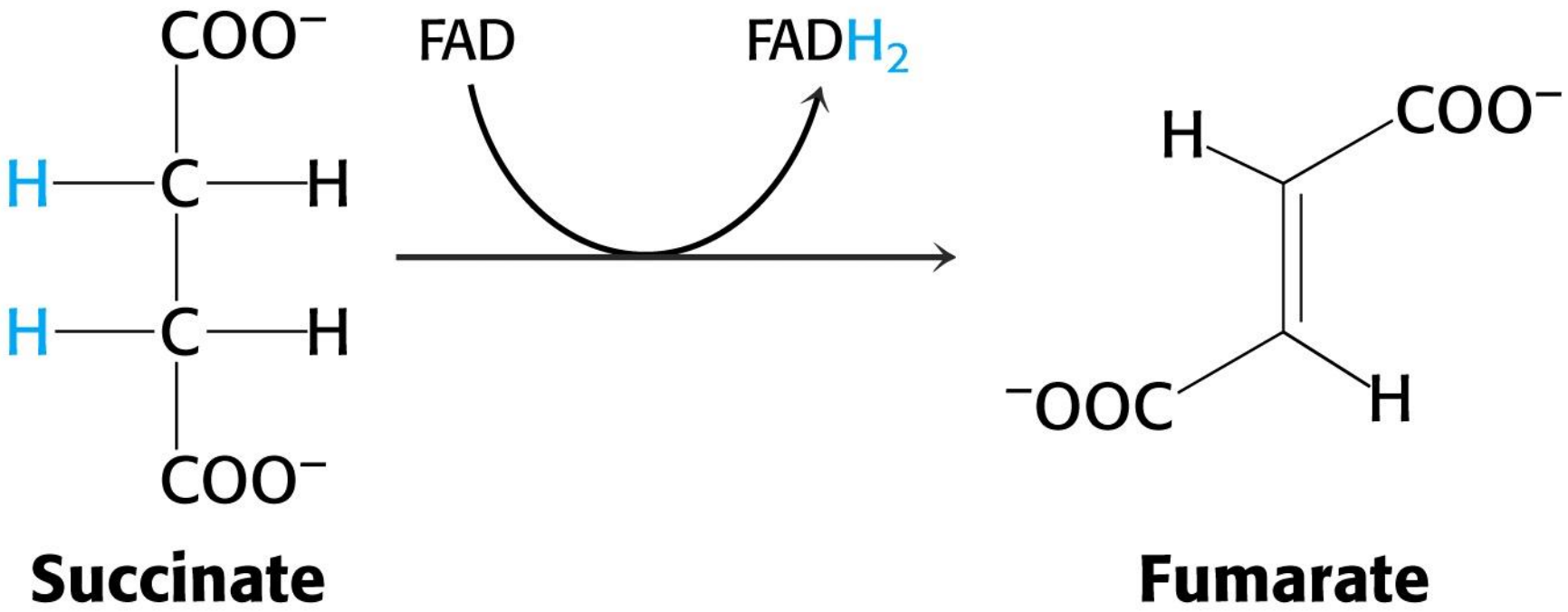
Steps 6 - 8

Oxidation, hydration, and oxidation



Succinate to Fumarate: step 6

Enzyme: succinate dehydrogenase

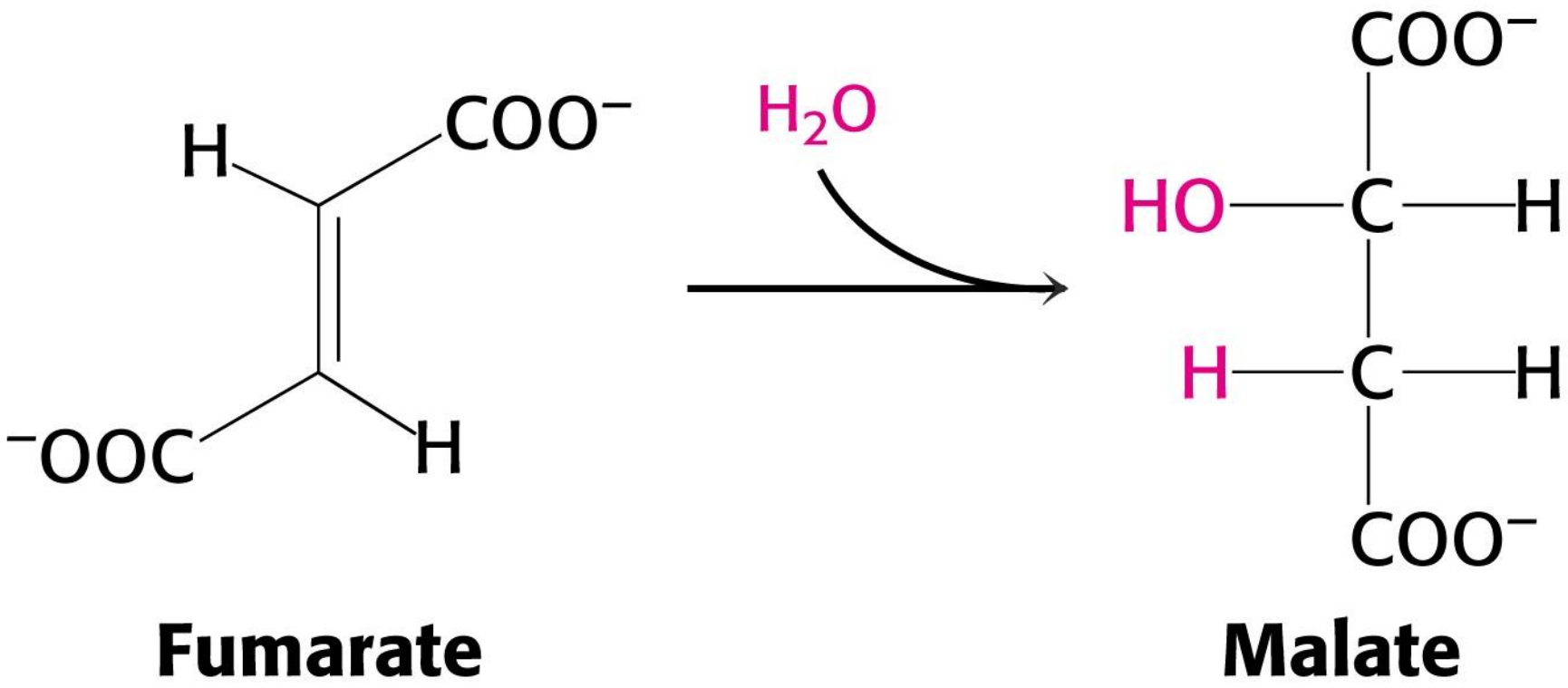


Succinate **Fumarate**

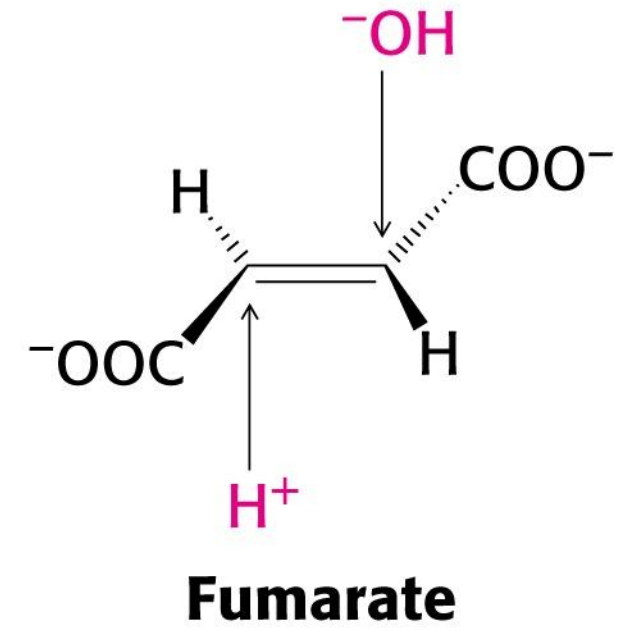
$FADH_2$ produced

Fumarate to Malate: step 7

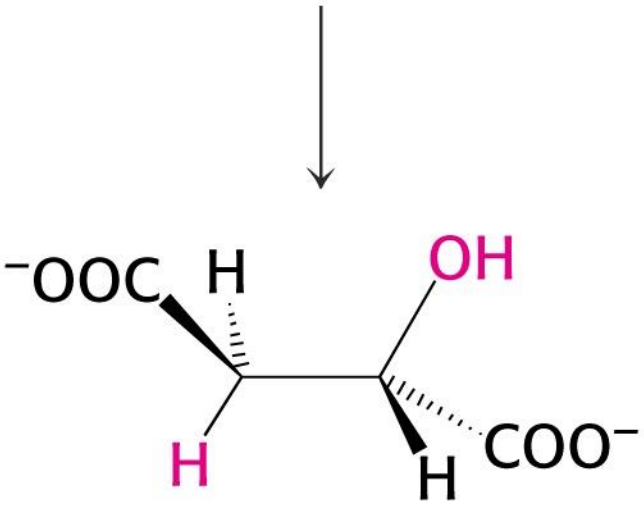
Enzyme: fumarase



Fumurate to L-Malate



Fumarate

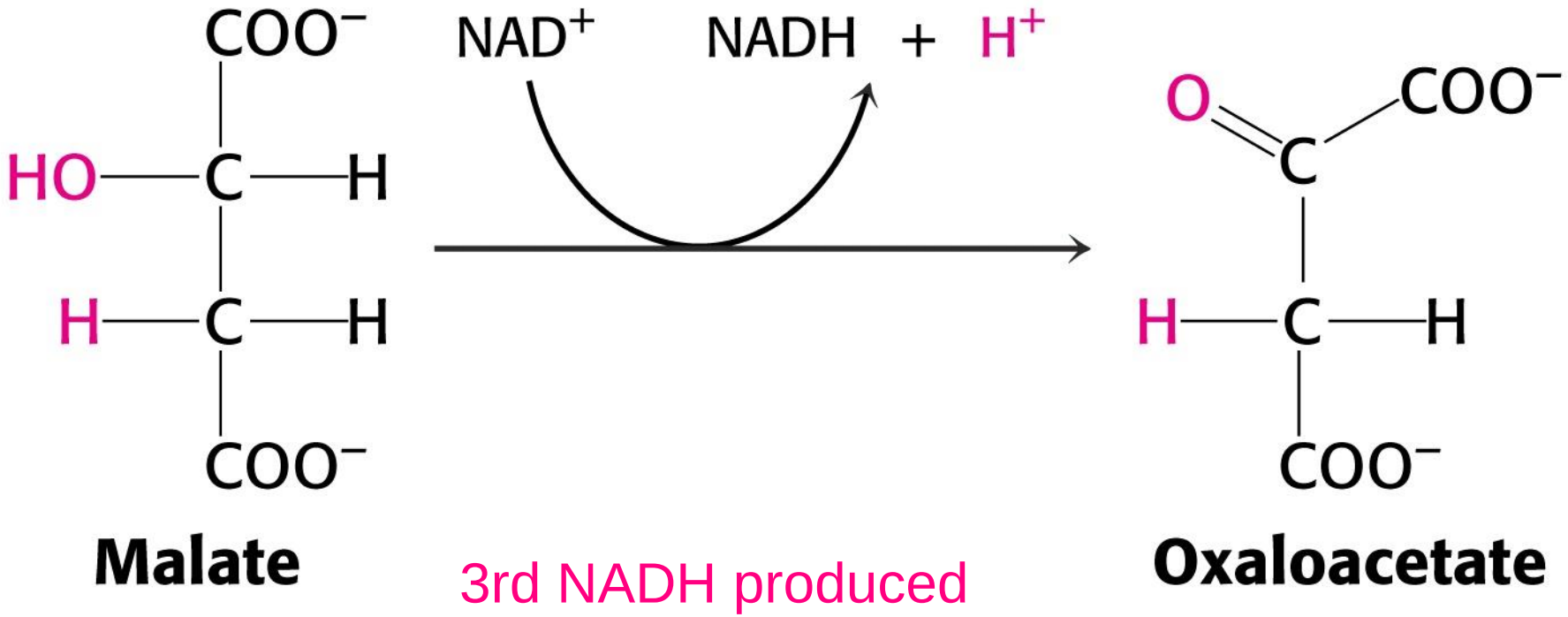


L-Malate

Hydroxyl group to one side only of fumarate double bond; hence, only L isomer of malate formed

Malate to Oxalate: step 8

Enzyme: malate dehydrogenase

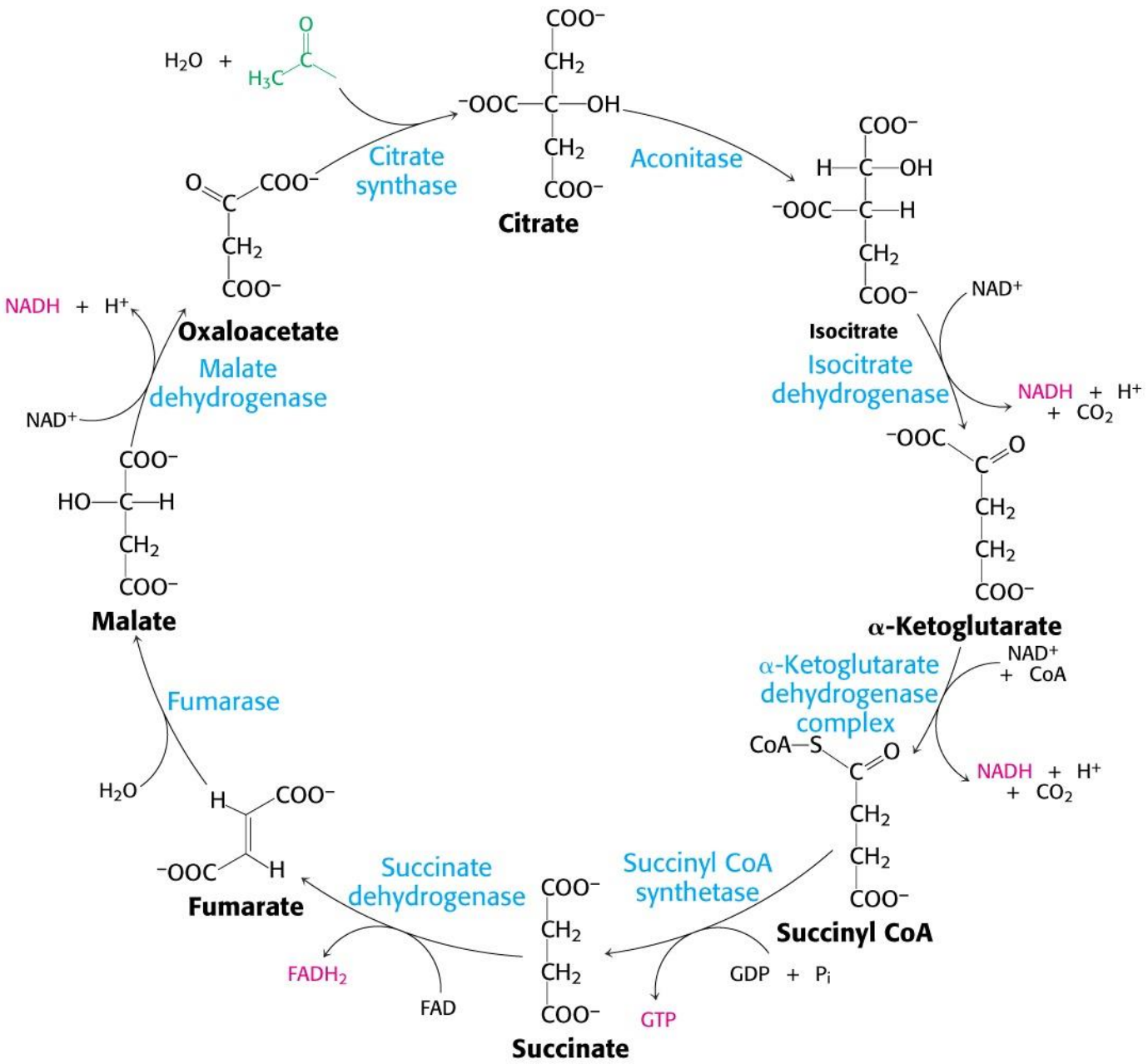


Malate

3rd NADH produced

Oxaloacetate

The citric acid cycle



Summary of 8 steps

TABLE 17.2 Citric acid cycle

Step	Reaction	Enzyme	Prosthetic group	Type*	$\Delta G^{\circ\prime}$ kcal mol^{-1}	$\Delta G^{\circ\prime}$ kJ mol^{-1}
1	Acetyl CoA + oxaloacetate + H_2O ⟶ citrate + CoA + H^+	Citrate synthase		a	−7.5	−31.4
2a	Citrate ⇌ *cis*-aconitate + H_2O	Aconitase	Fe–S	b	+2.0	+8.4
2b	*cis*-Aconitate + H_2O ⇌ isocitrate	Aconitase	Fe–S	c	−0.5	−2.1
3	Isocitrate + NAD^+ ⇌ α-ketoglutarate + CO_2 + NADH	Isocitrate dehydrogenase		d + e	−2.0	−8.4
4	α-Ketoglutarate + NAD^+ + CoA ⇌ succinyl CoA + CO_2 + NADH	α-Ketoglutarate dehydrogenase complex	Lipoic acid, FAD, TPP	d + e	−7.2	−30.1
5	Succinyl CoA + P_i + GDP ⇌ succinate + GTP + CoA	Succinyl CoA synthetase		f	−0.8	−3.3
6	Succinate + FAD (enzyme-bound) ⇌ fumarate + $FADH_2$ (enzyme-bound)	Succinate dehydrogenase	FAD, Fe–S	e	~0	0
7	Fumarate + H_2O ⇌ L-malate	Fumarase		c	−0.9	−3.8
8	L-Malate + NAD^+ ⇌ oxaloacetate + NADH + H^+	Malate dehydrogenase		e	+7.1	+29.7

*Reaction type: (a) condensation; (b) dehydration; (c) hydration; (d) decarboxylation; (e) oxidation; (f) substrate-level phosphorylation.

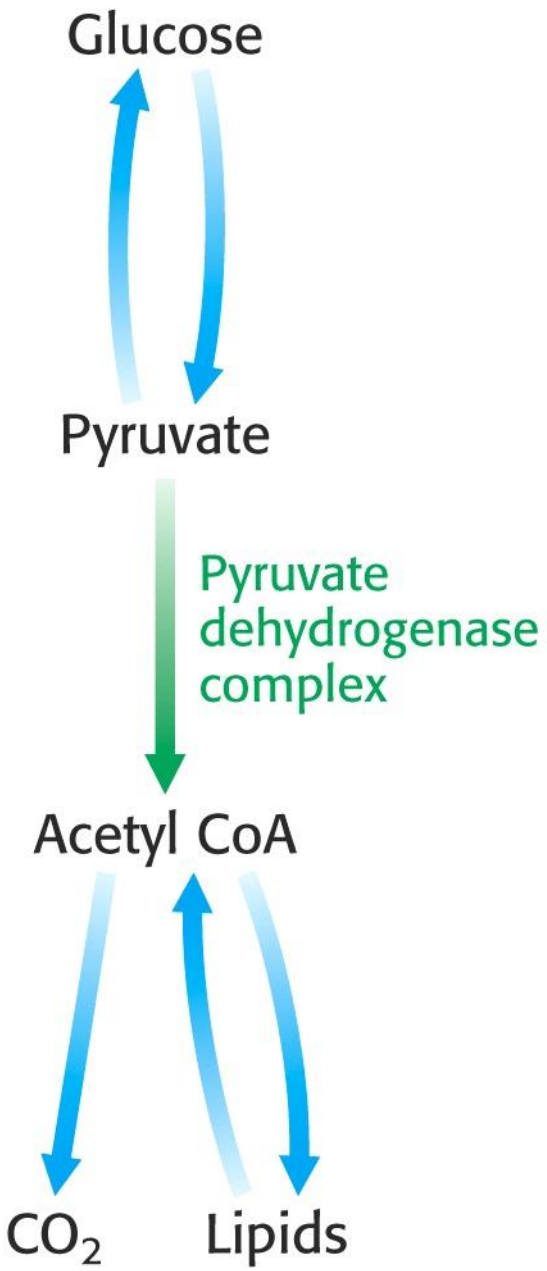
Proton gradient generates 2.5 ATP per NADH, & 1.5 per $FADH_2$

9 ATP from 3 NADH + 1 FADH2. Also, 1 GTP

Thus, 1 acetate unit generates equivalent of 10 ATP molecules.

In contrast, 2 ATP per glucose molecule in anaerobic glycolysis

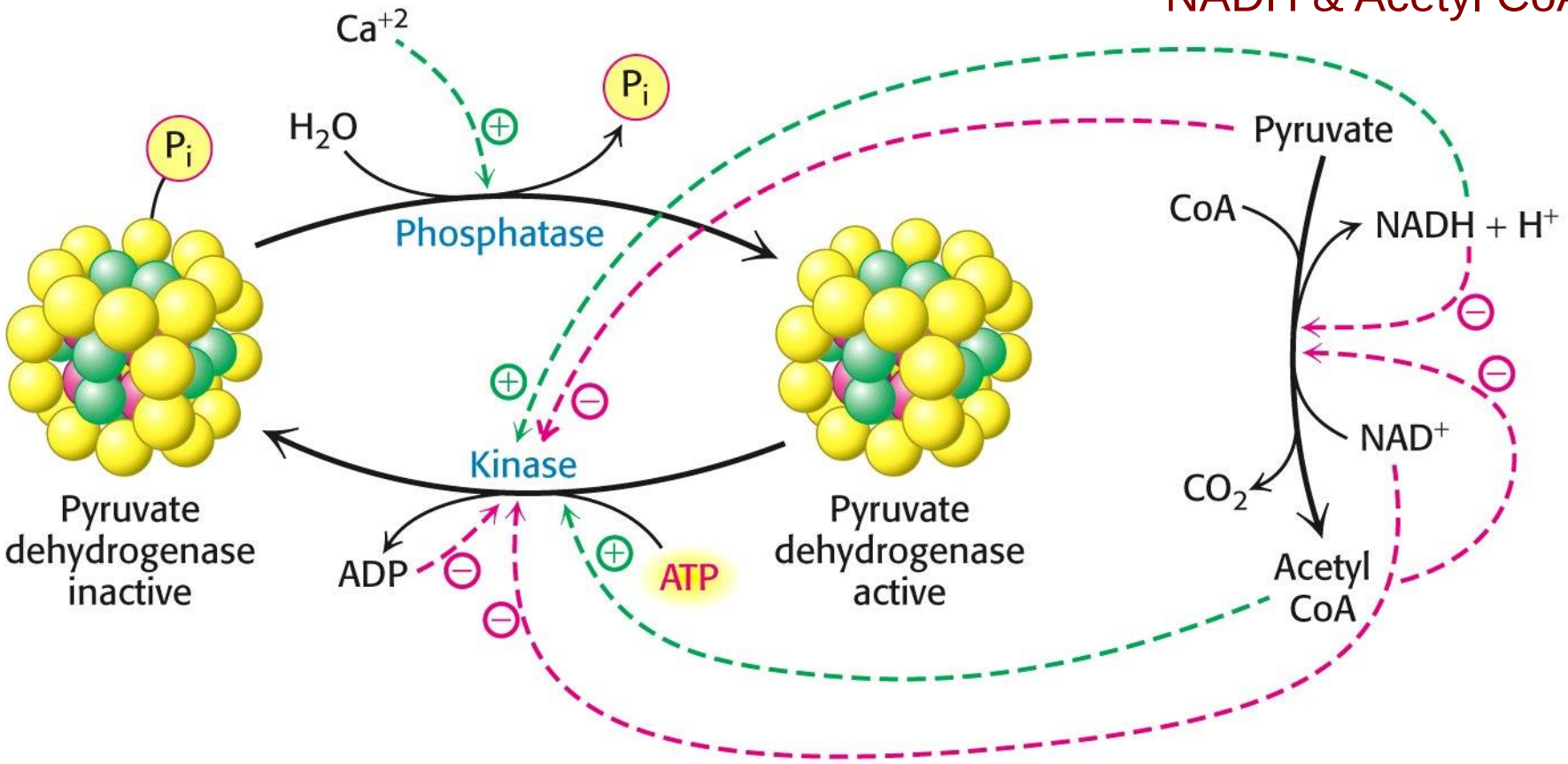
Pyruvate to Acetyl CoA, irreversible



Key irreversible step in the metabolism of glucose

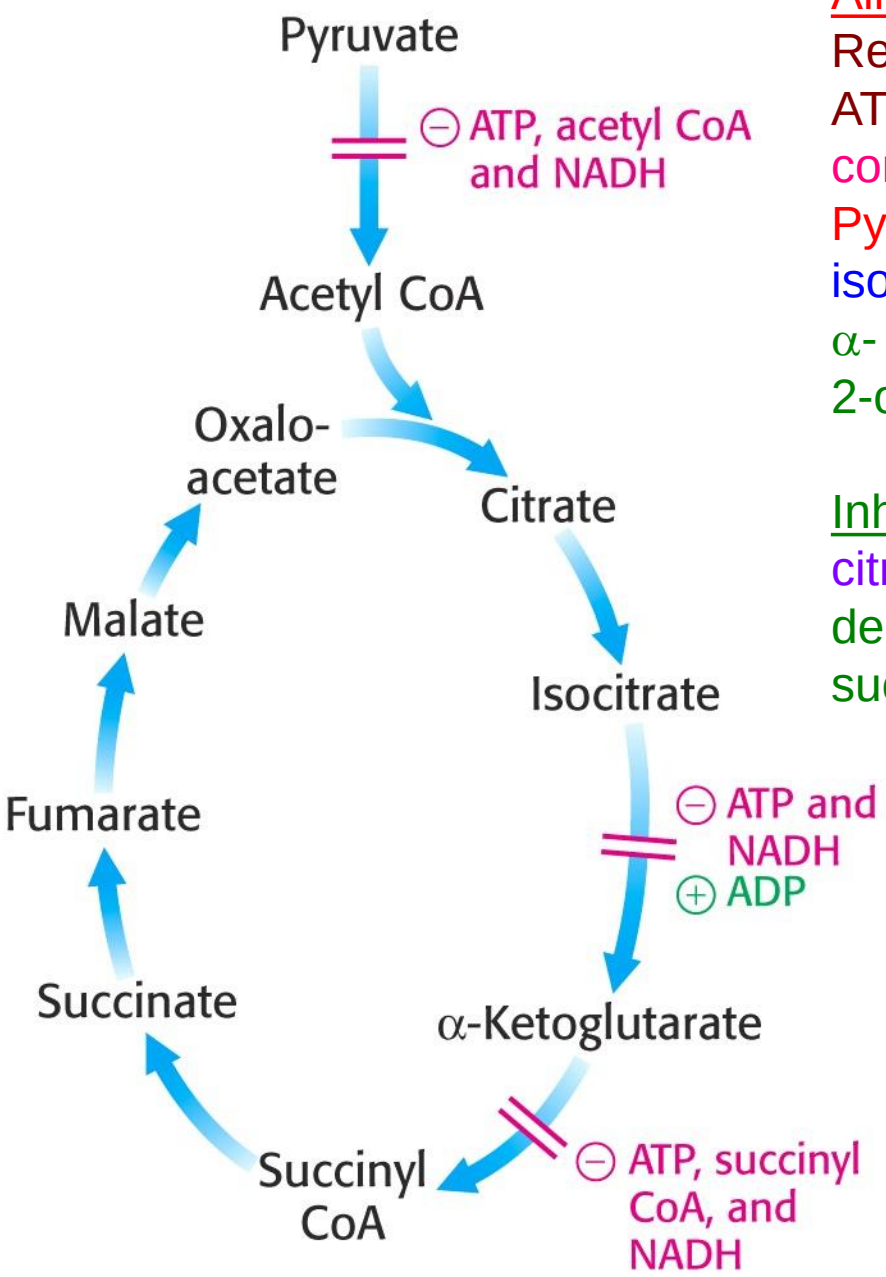
Regulation of pyruvate dehydrogenase

Inhibited by products,
NADH & Acetyl CoA



Also regulated by covalent modification,
the kinase & phosphatase also regulated

Control of citric acid cycle



Allosteric regulation

Regulated primarily by ATP & NADH concentrations,

control points:

Pyruvate dehydrogenase

isocitrate dehydrogenase &

α- ketoglutarate dehydrogenase

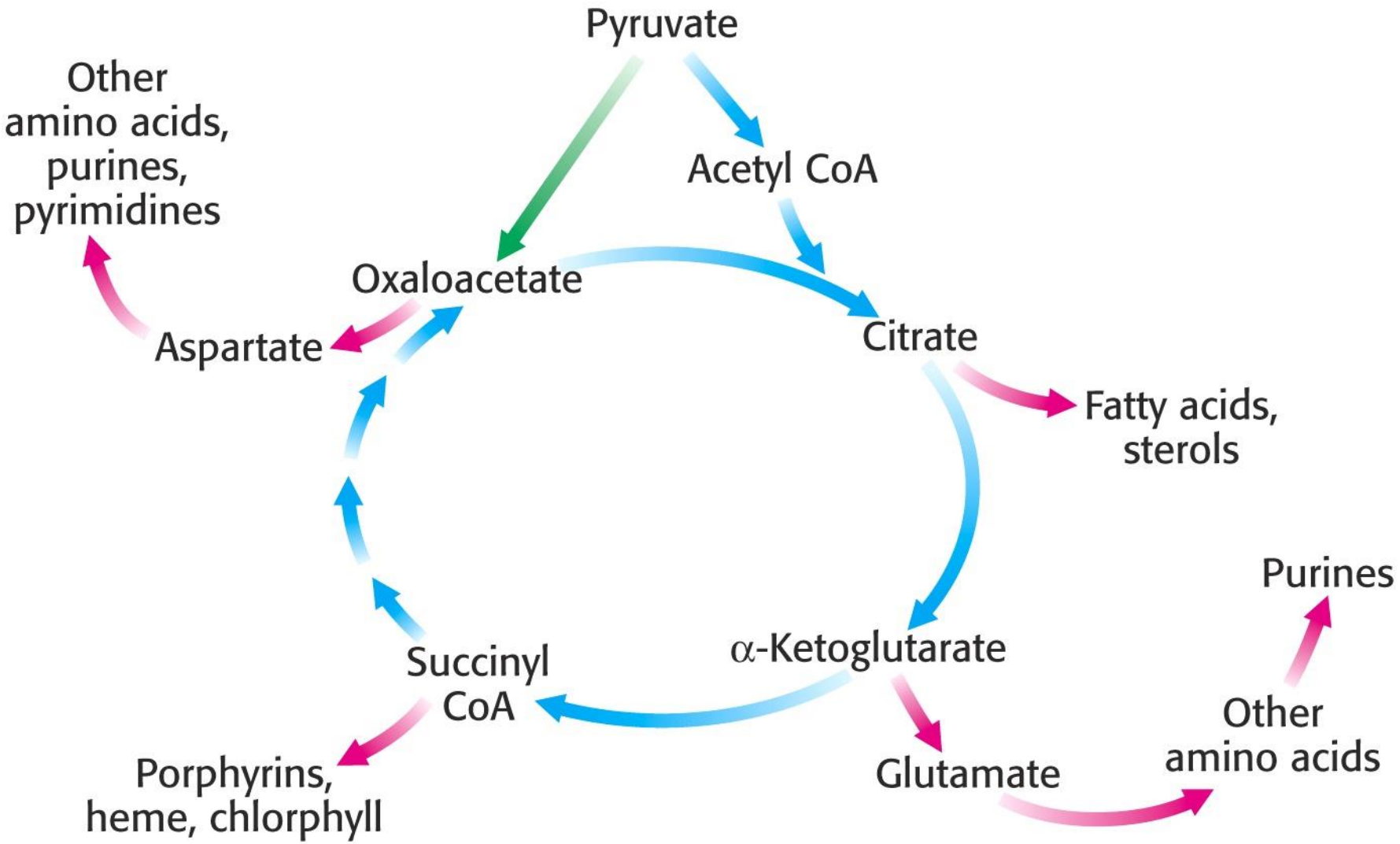
2-oxo-glutarate dehydrogenase)

Inhibition by product:

citrate synthase- citrate- 2-oxo-glutarate dehydrogenase-

succinyl CoA

Biosynthetic roles of the citric acid cycle



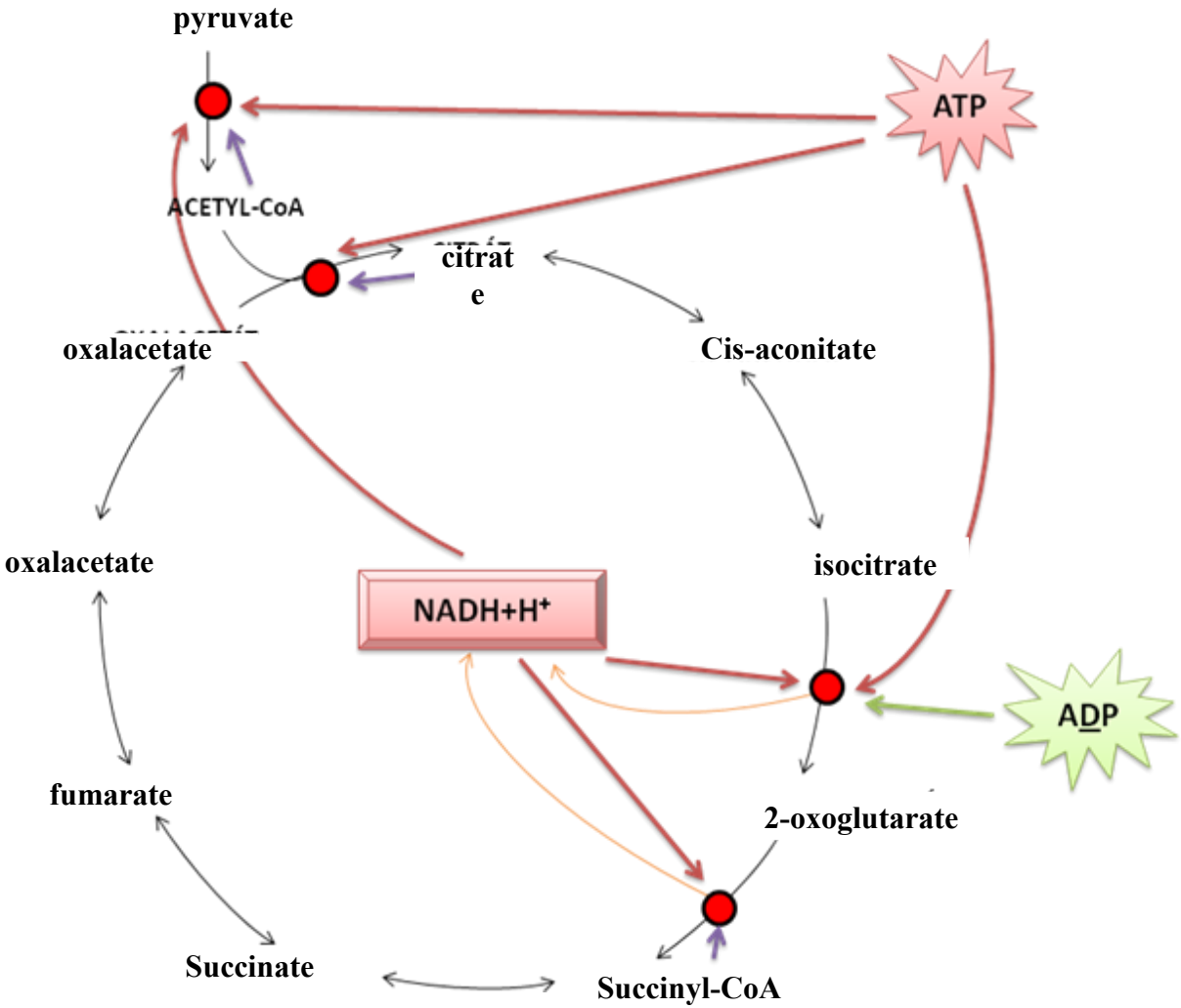
Key Enzymes for regulation of CAC, inhibitors and activators

Enzyme	ATP[a]	NADH[a]	different
Pyruvate dehydrogenase	-	-	- acetyl-CoA (inh. prod.)
Citrate syntethase	-		- citrate (inhibition by product)
Isocitrate dehydrogenase	-	-	+ ADP (allosteric activation)
2-OG-dehydrogenase		-	- sukcinyl-CoA (inh. prod.)

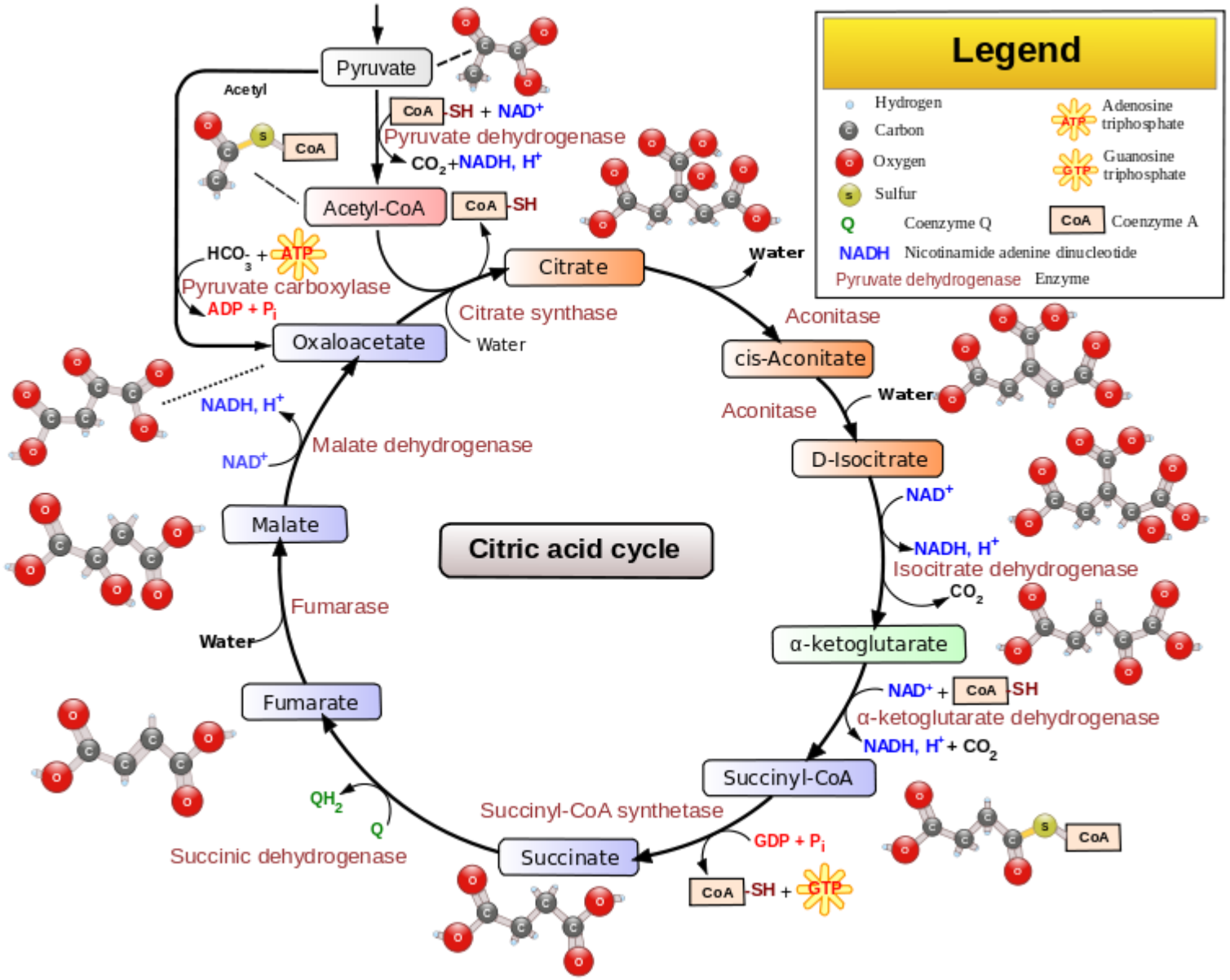
[a] allosteric inhibitor
[b] feedback inhibitor (inhibition by reaction product)
[c] allosteric activator

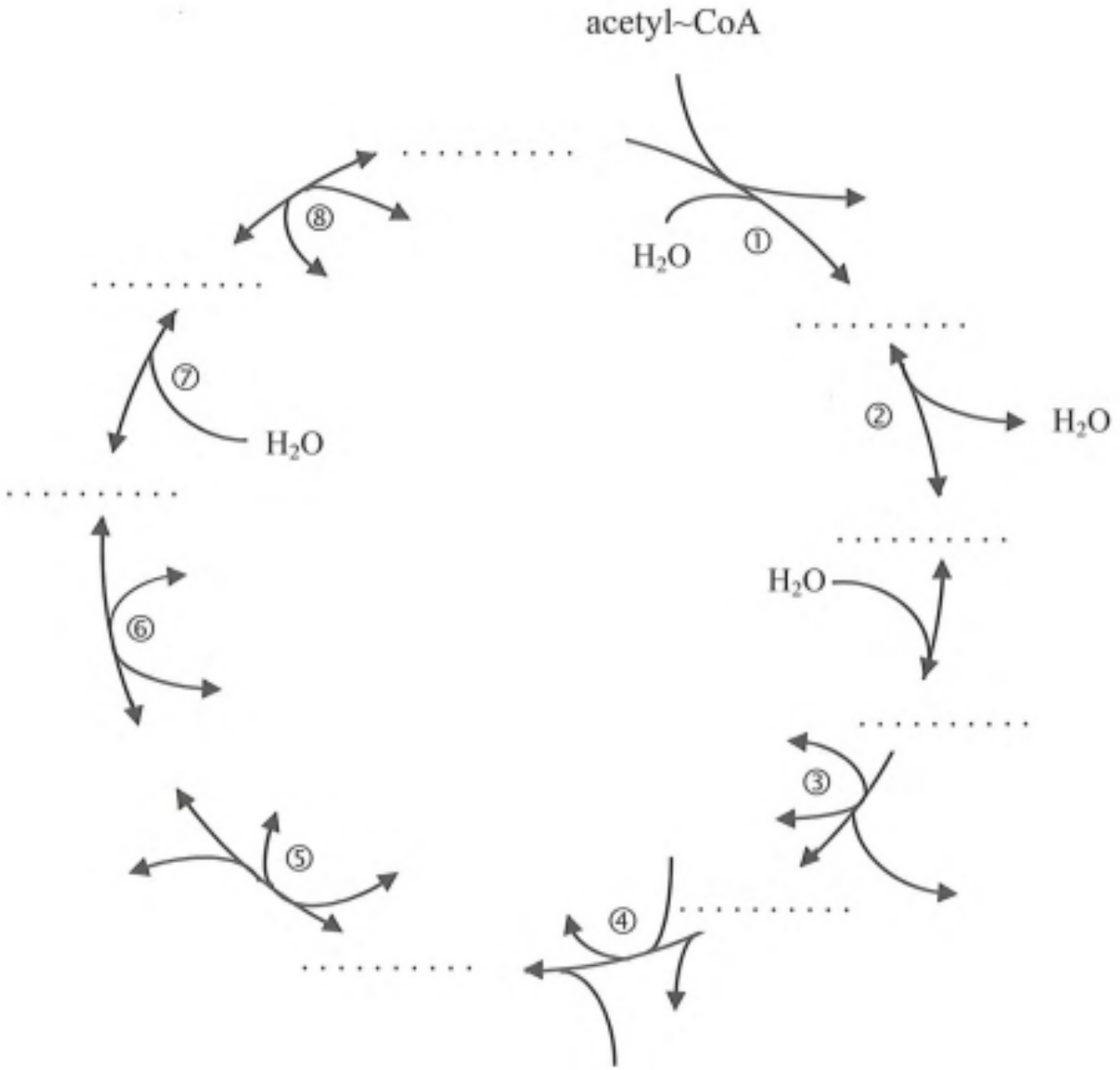
NOVÁK, Jan. *Biochemie I*. Brno: Muni, 2009, s. 237.

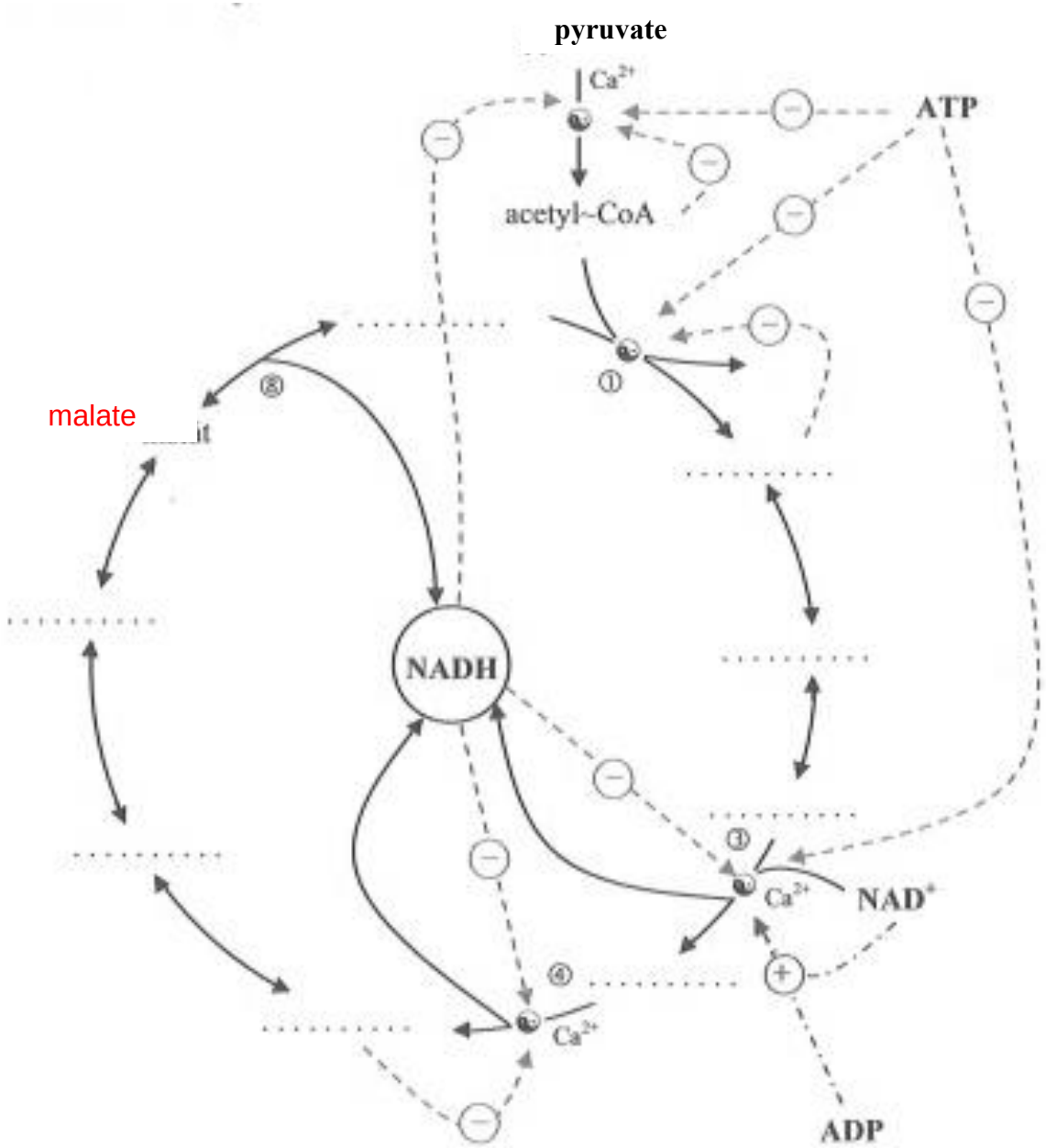
Regulation of CAC



NOVÁK, Jan. *Biochemie I*. Brno: Muni, 2009, s. 238.


Legend
Hydrogen
Carbon
Oxygen
Sulfur
Coenzyme Q
Nicotinamide adenine dinucleotide
Enzyme
Adenosine triphosphate
Guanosine triphosphate
Coenzyme A
Pyruvate
Acetyl
CoA
SH + NAD+
Pyruvate dehydrogenase
CO2+NADH, H+
Acetyl-CoA
HCO3- + ATP
Pyruvate carboxylase
ADP + Pi
Oxaloacetate
Citrate
Citrate synthase
Water
Aconitase
cis-Aconitate
Water
Aconitase
D-Isocitrate
NAD+
NADH, H+
Isocitrate dehydrogenase
CO2
α-ketoglutarate
NAD+ + CoA SH
α-ketoglutarate dehydrogenase
NADH, H+ + CO2
Succinyl-CoA
Succinyl-CoA synthetase
GDP + Pi
CoA SH + GTP
Succinate
QH2
Q
Succinic dehydrogenase
Fumarate
Water
Fumarase
Malate
NADH, H+
NAD+
Malate dehydrogenase
Citric acid cycle


acetyl~CoA
H2O
①
②
H2O
H2O
③
④
⑤
⑥
⑦
H2O
⑧


pyruvate
Ca2+
ATP
acetyl~CoA
①
malate
⑧
NADH
③
Ca2+
NAD+
④
Ca2+
ADP