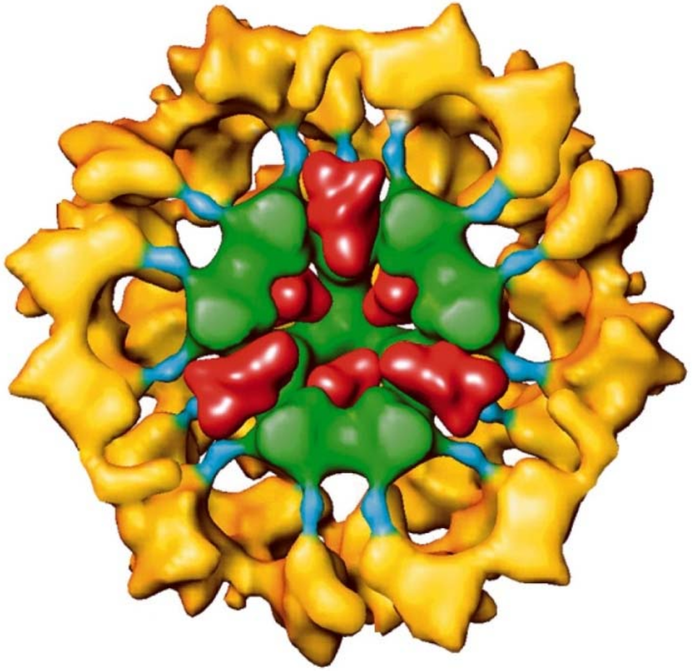
Chapter 16

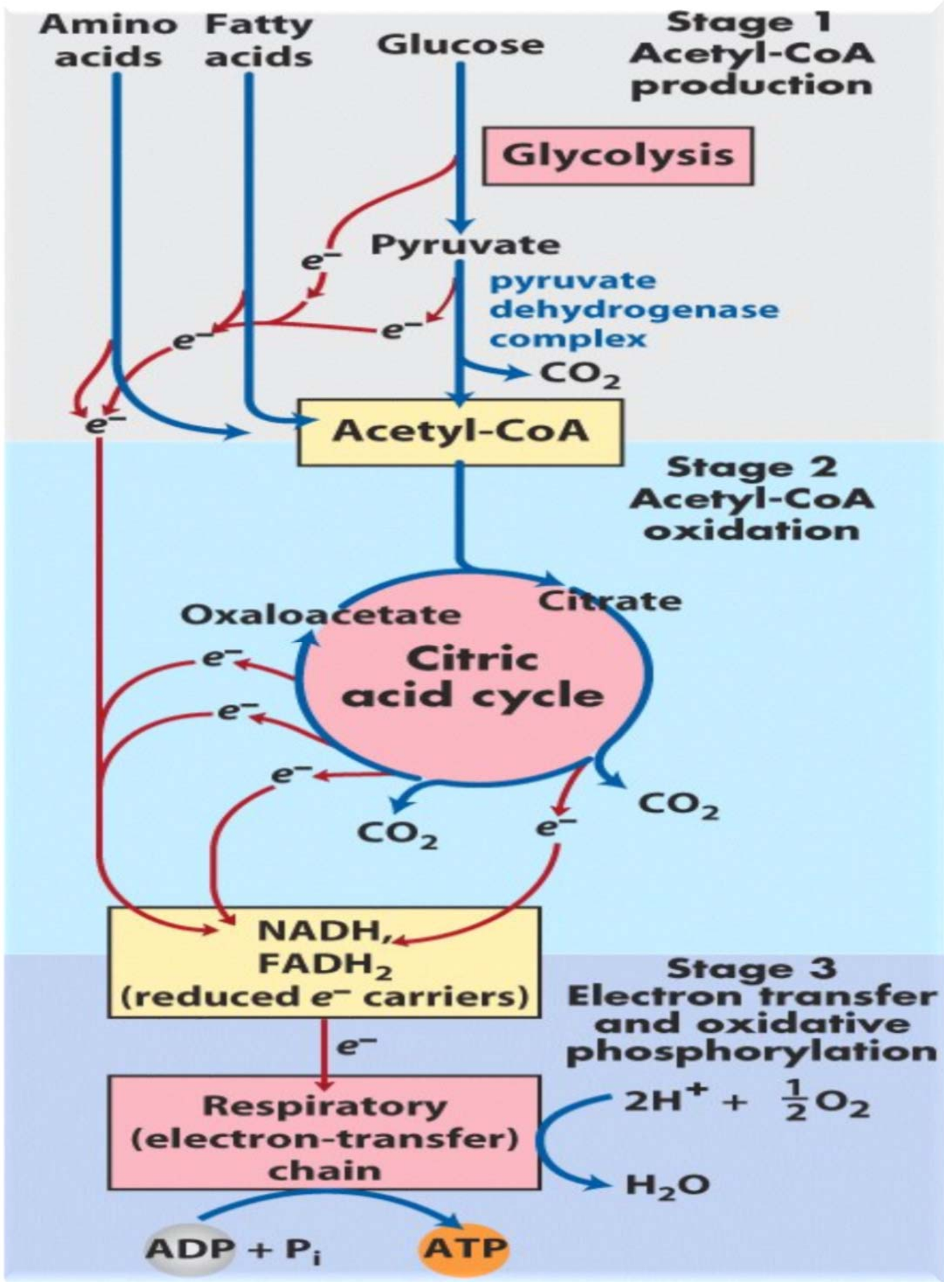
The Citric Acid Cycle

16.1 Production of Acetyl-CoA (Activated acetate)
16.2 Reaction of the Citric Acid Cycle
16.3 Regulation of the Citric Acid Cycle
16.4 The Glyoxylate Cycle



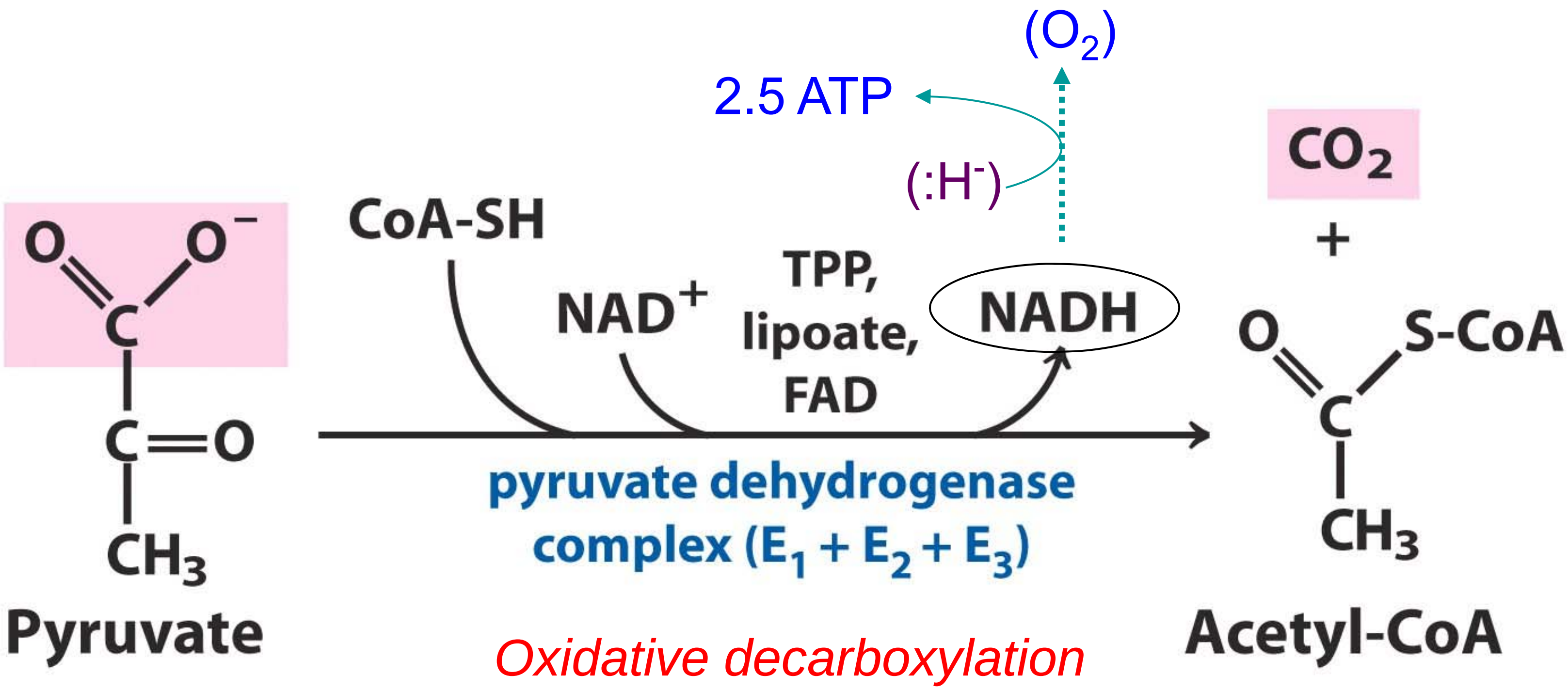
Catabolism of proteins, fats, and carbohydrates in the three stages of cellular respiration

Hans Krebs, 1900–1981



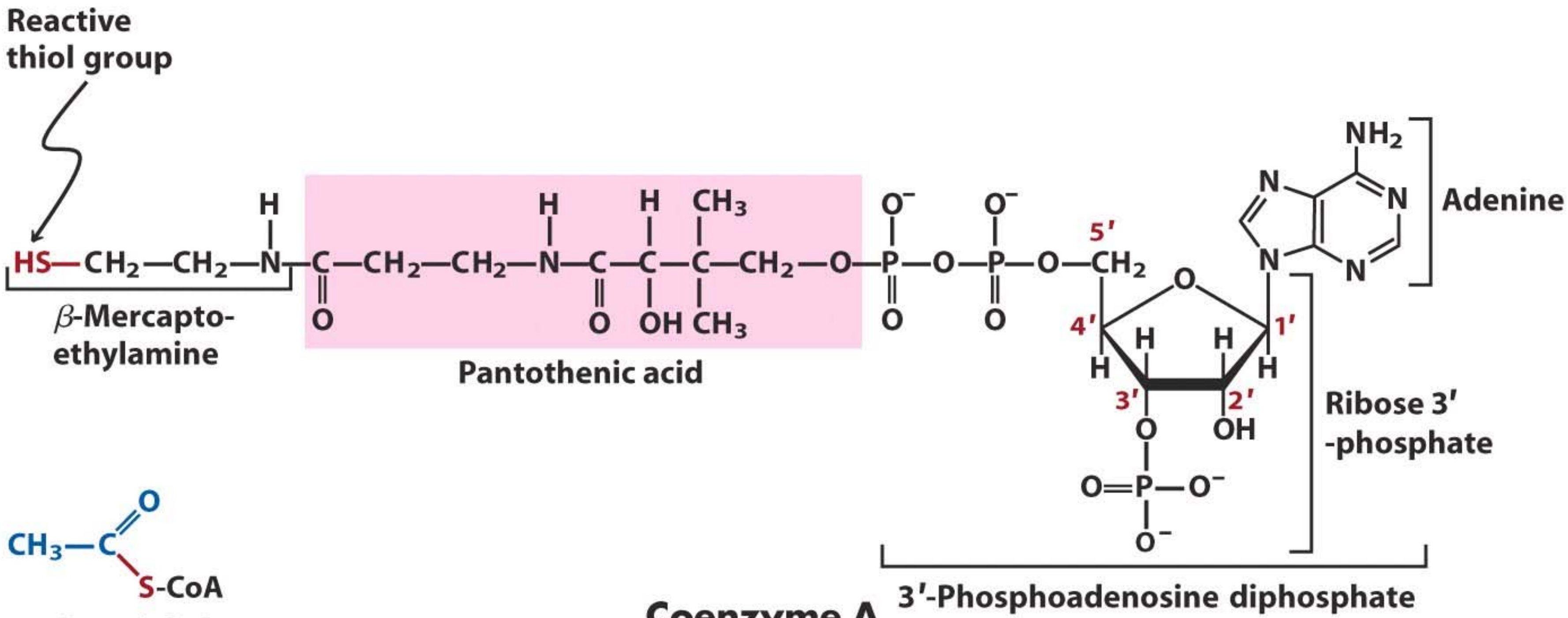
Production of Acetyl-CoA (Activated acetate)

Overall reaction catalyzed by the pyruvate dehydrogenase (PDH) complex



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

Coenzyme A (CoA)



Coenzyme A

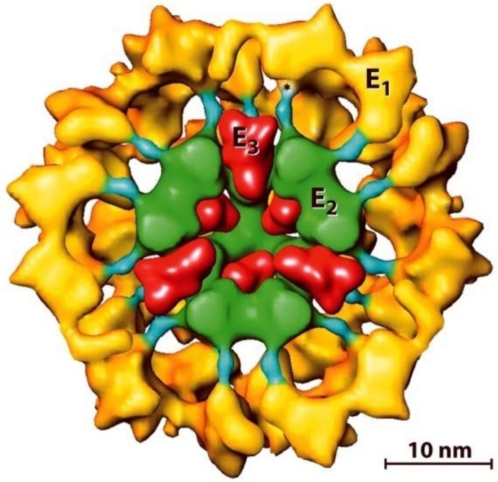
Acetyl-CoA

(acivated acetate)

Pyruvate dehydrogenase (PDH) complex

(in the mitochondria of eukaryotes and in the cytosol of prokaryotes)

Complex of three enzymes : pyruvate dehydrogenase (E1)
dihydrolipoyl transacetylase (E2)
dihydrolipoyl dehydrogenase (E3)

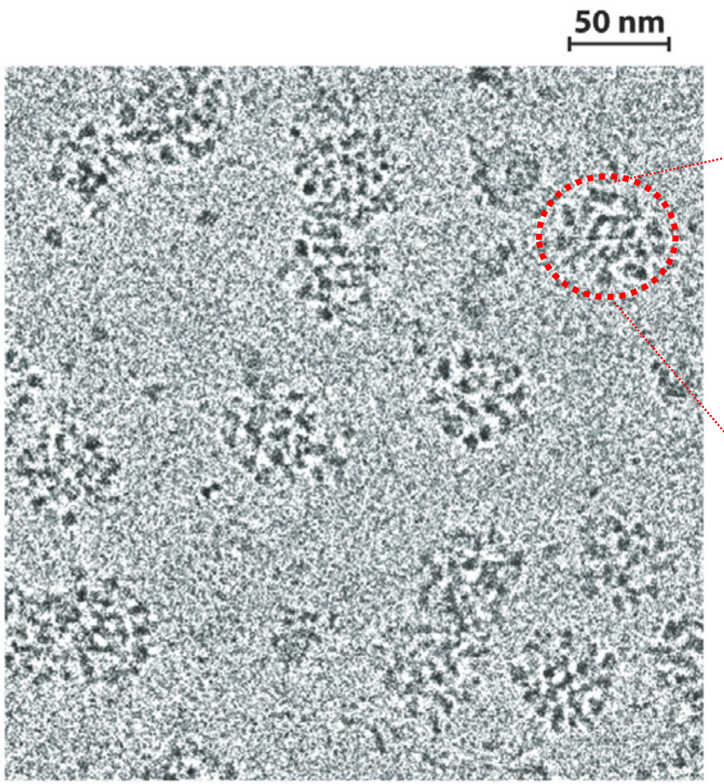


* requires 5 cofactors (prothetic groups)

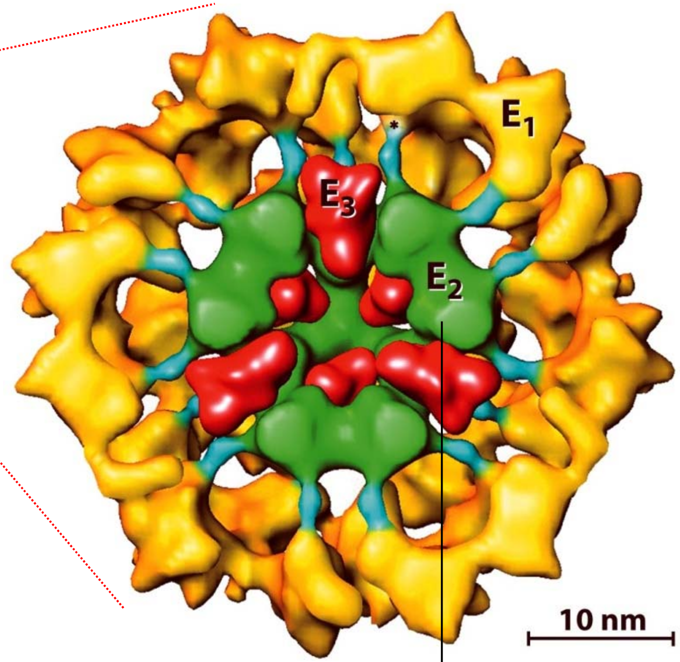
Thiamin pyrophosphate (TPP)	←	*thiamine (vit. B1)*
Flavin adenine dinucleotide (FAD)	←	*riboflavin*
Coenzyme A (CoA)	←	*pantothenate*
Nicotinamide adenine dinucleotide (NAD)	←	*niacin*
Lipoic acid (Lipoate)		

Structure of the PDH complex

E1, E3 : 20 ~ 30 molecules

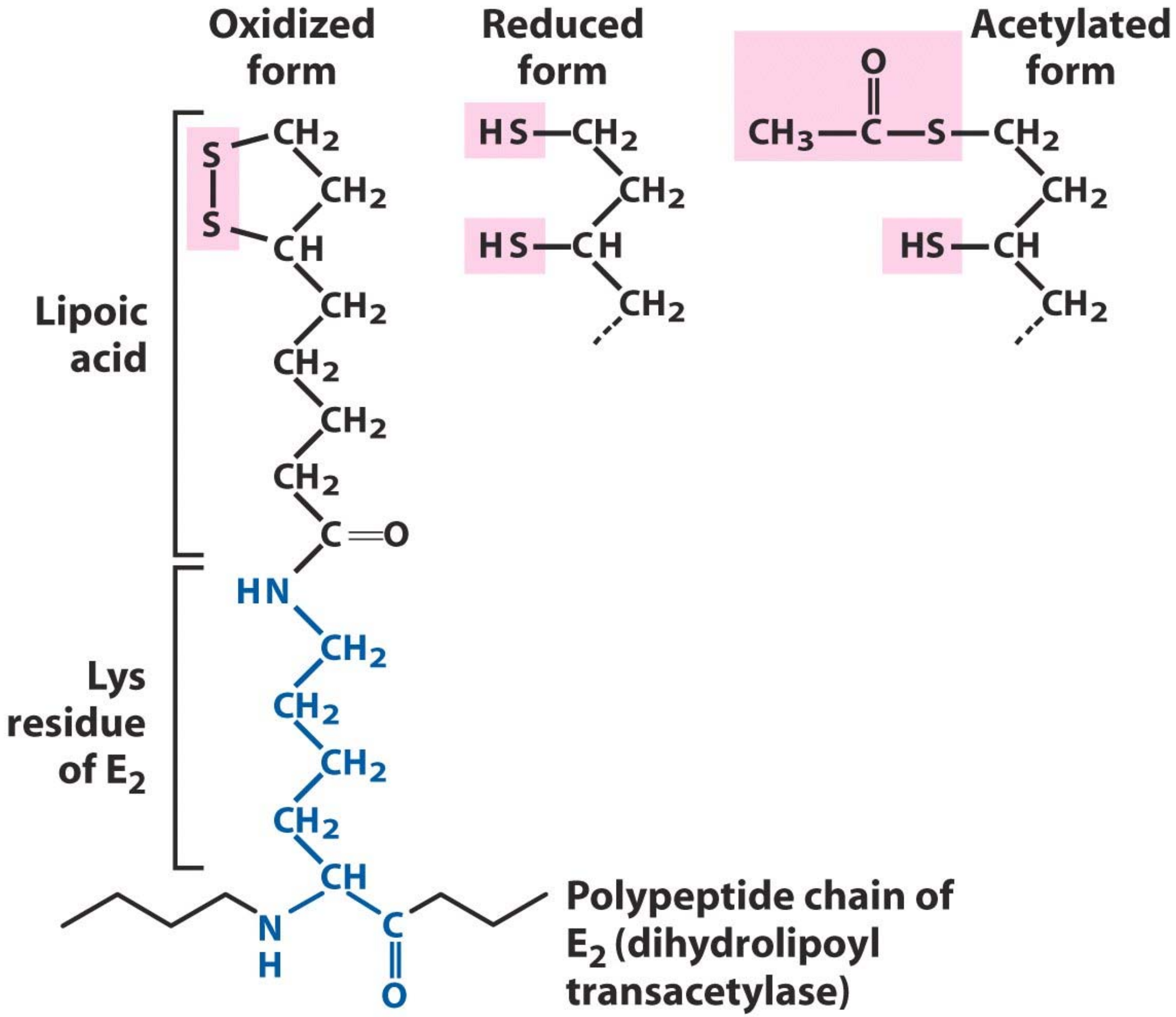


Cryoelectron micrograph of PDH complexes
isolated from bovine kidney



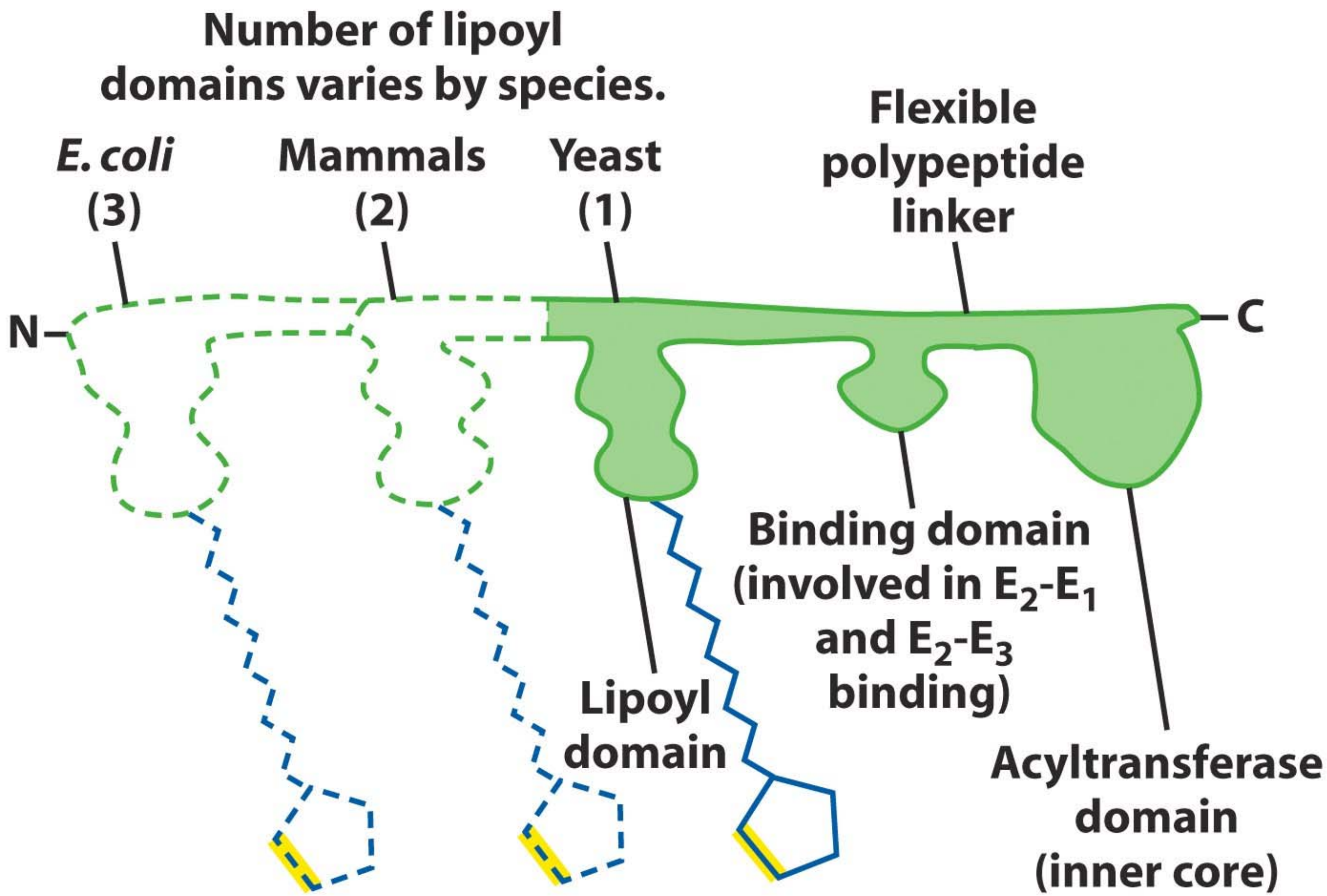
60 identical copies of **E2** form
a pentagonal dodecahedron (core)

Lipoic acid (lipoate) in amide linkage with a Lys residue

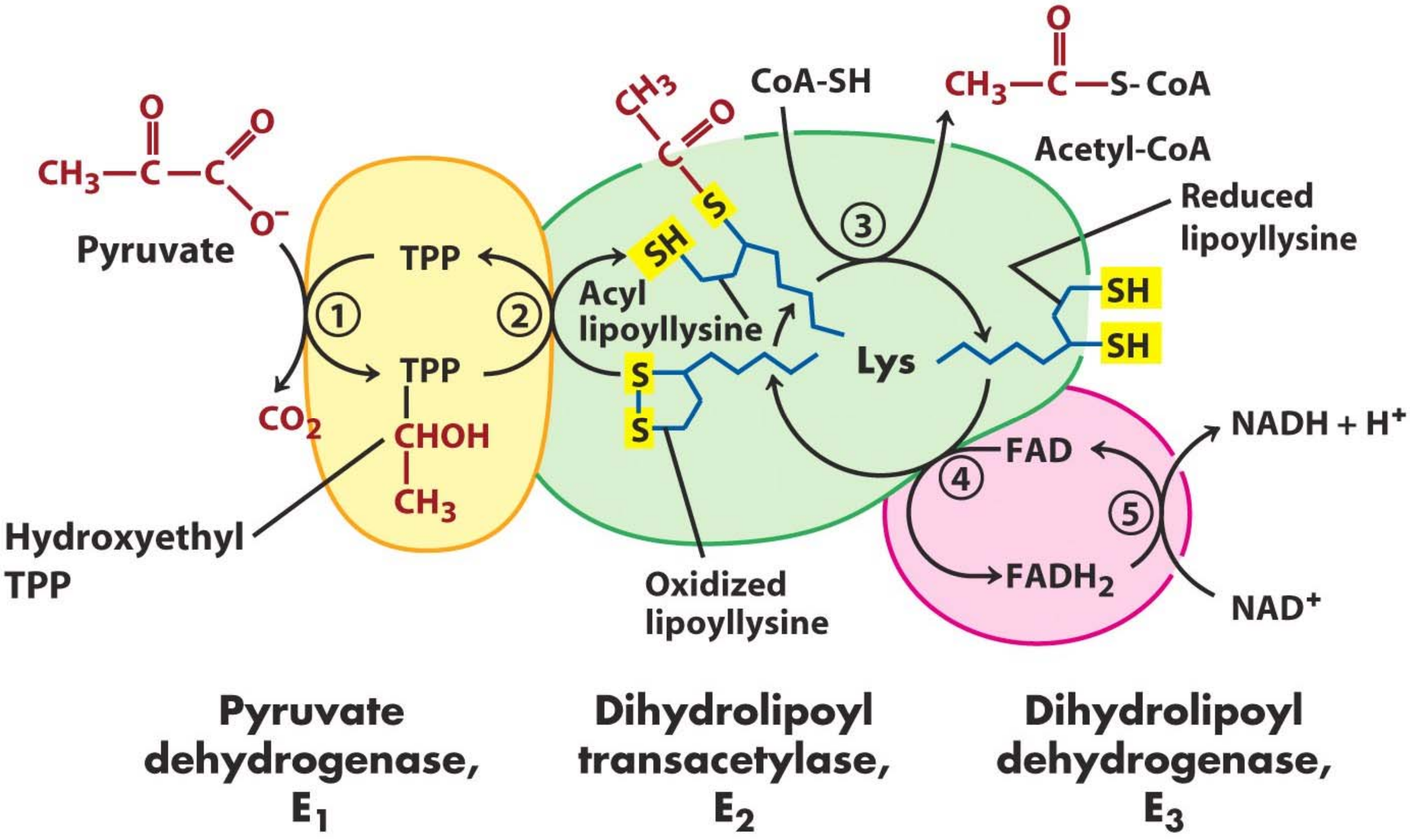


Structure of the PDH complex (E2)

(**E2** consists of three types of domains linked by short polypeptide linkers)



Oxidative decarboxylation of pyruvate to acetyl-CoA by the PDH complex



Substrate channeling

Beriberi

Multienzyme complex help to increase the rate of cell metabolism

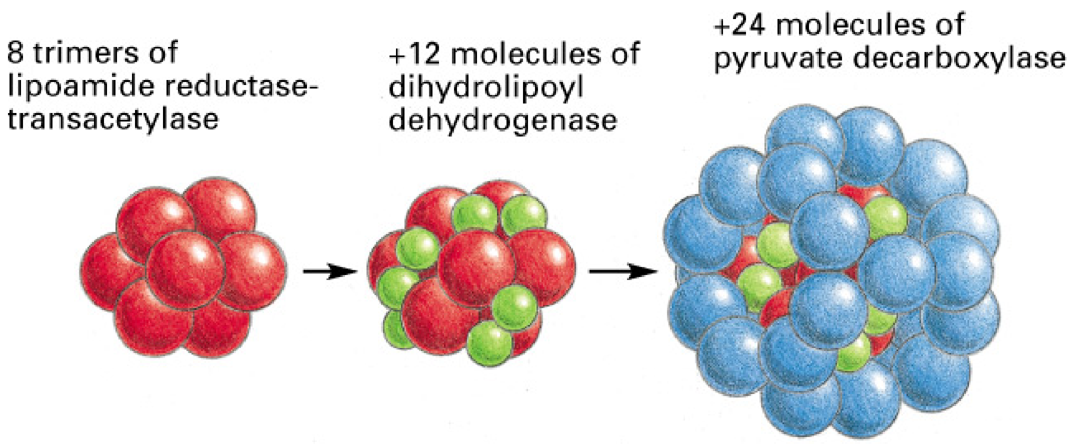
Rxn rate : enzyme's intrinsic speed of action

frequency with which the enzyme collides with its substrates

→ *diffusion-limited* rxn (conc.-dependent)

most metabolites in cell : ~ 10^{-6} M conc.
most enzyme conc. : << [metabolite]

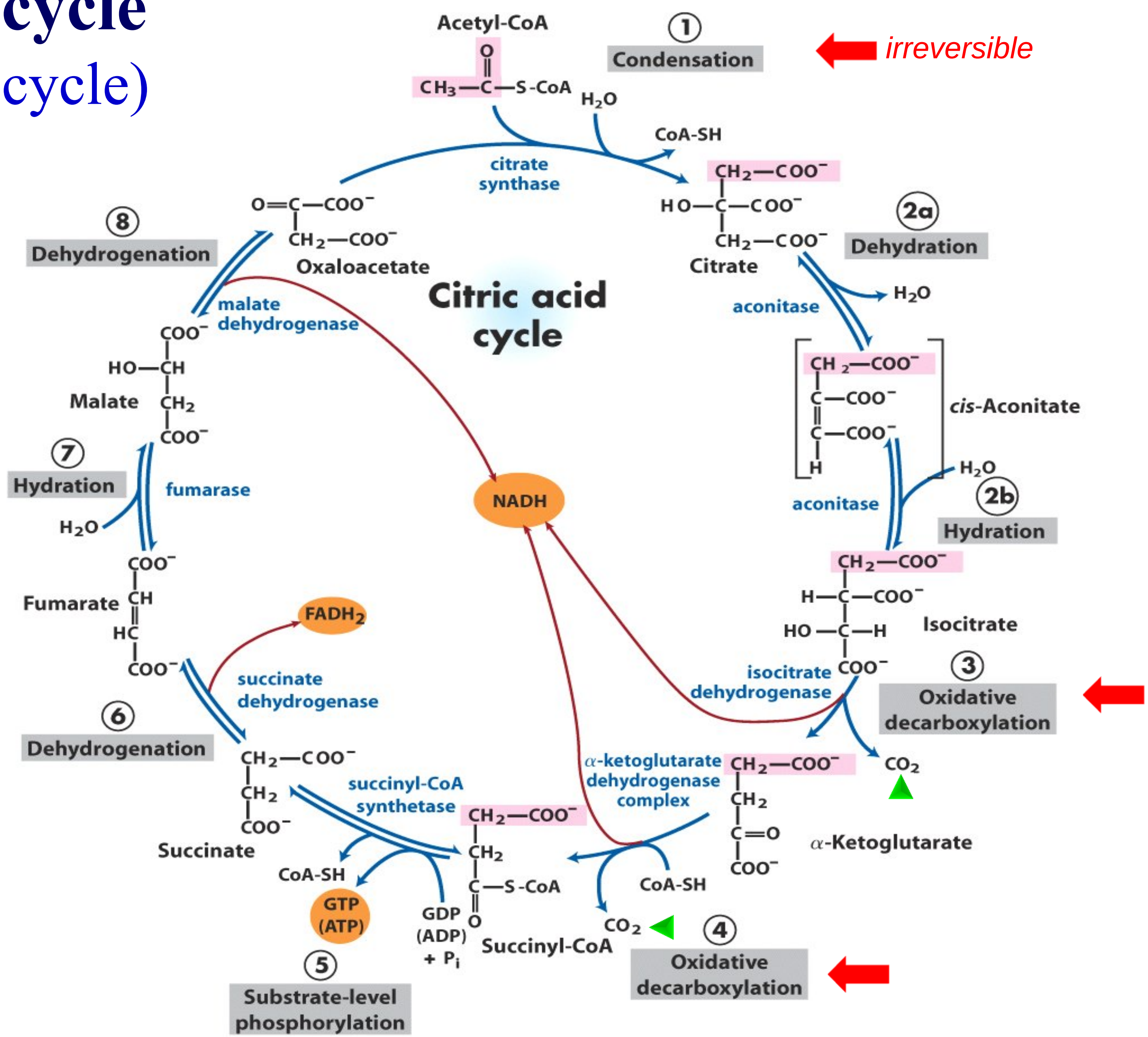
⇒ Multienzyme complex
Compartmentalization



Pyruvate dehydrogenase

Citric acid cycle

(TCA, Krebs cycle)



Synthase or **Synthetase**

catalyze condensation reaction

Synthase : no nucleoside triphosphate (ATP, GTP …) is required as an energy source.

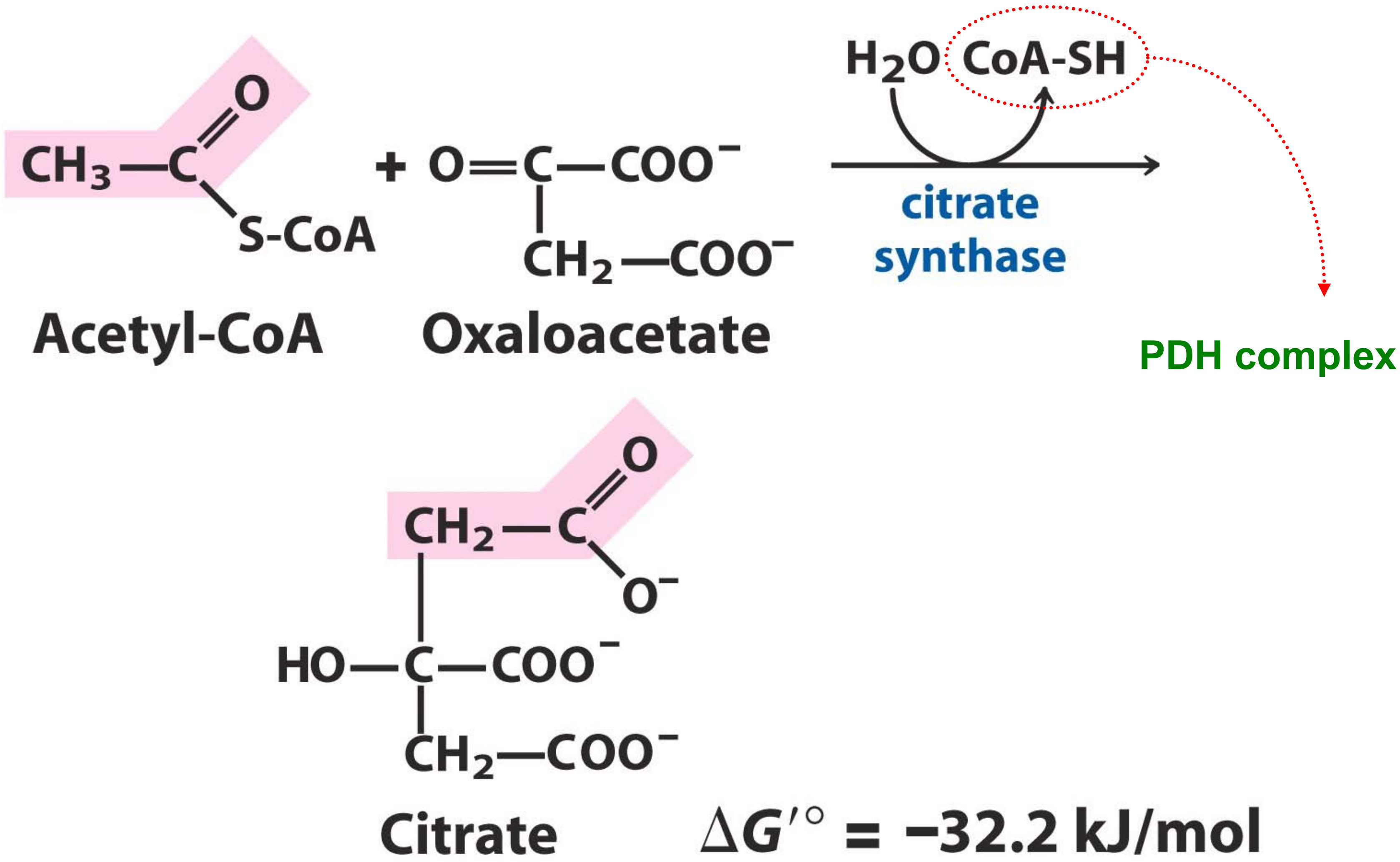
Synthetase : use ATP or another nucleoside triphosphate as a source of energy for synthetic reaction. (***Ligase***)

Kinase or **Phosphorylase**

Kinase : transfer a phosphoryl group from a nucleoside triphosphate such as ATP to an acceptor molecule.

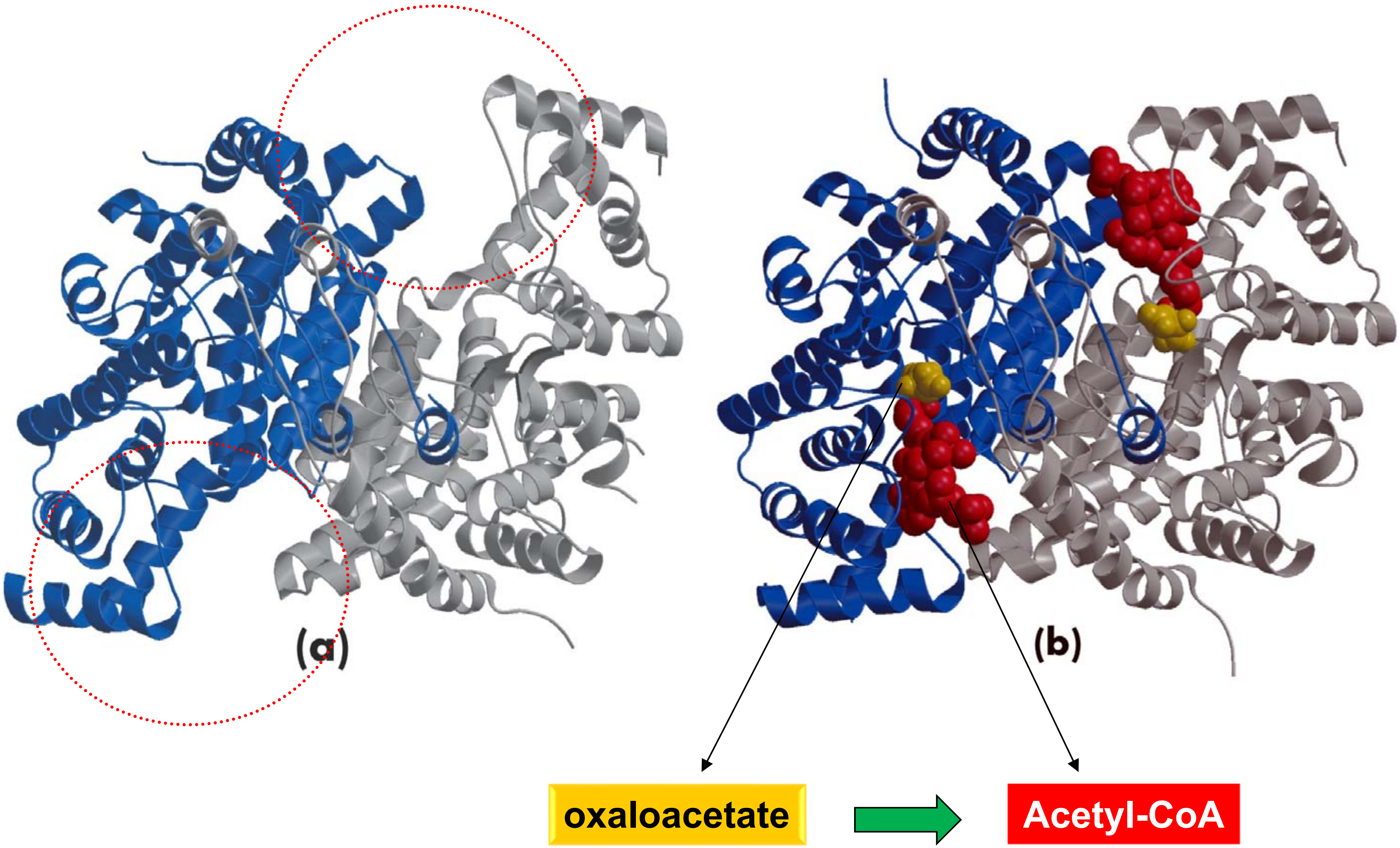
Phosphorylase : *phospholysis* is a displacement reaction in which phosphate is the attacking species and becomes covalently attached at the point of bond breakage. Such reactions are catalyzed by phosphorylase.

① *Formation of Citrate*



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

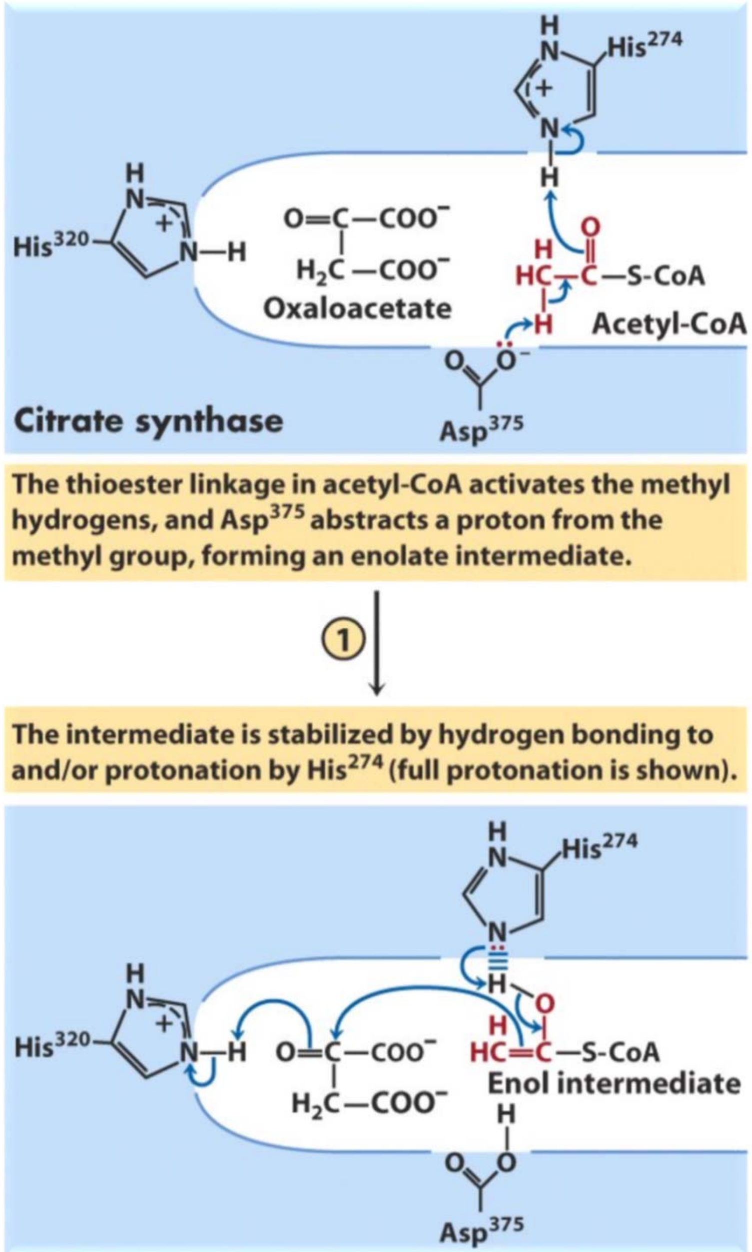
Structure of citrate synthase (*homodimeric*)



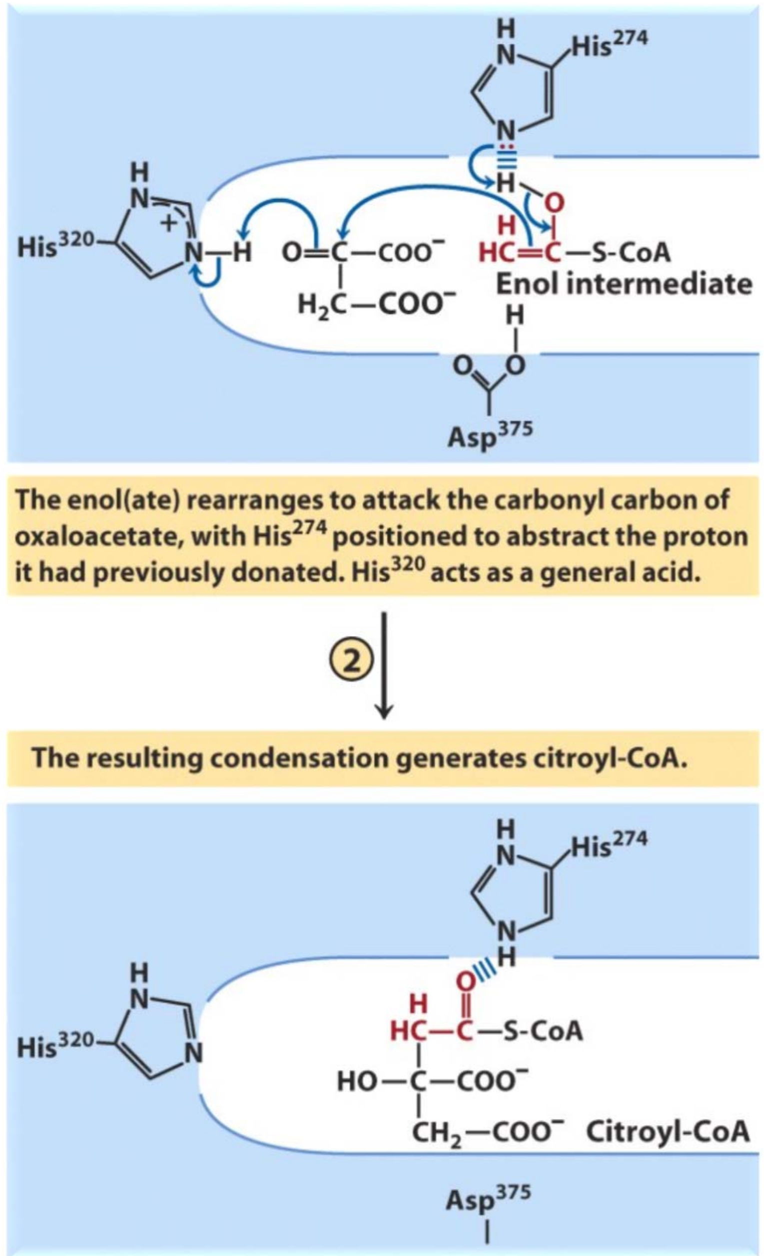
1st substrate

2nd substrate

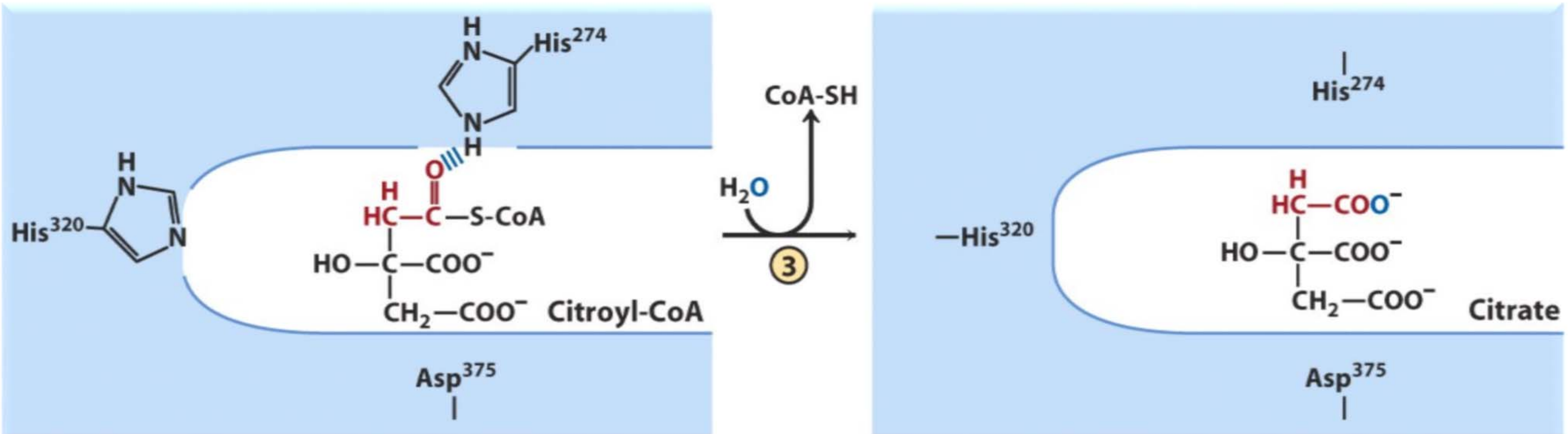
Citrate synthase (1)



Citrate synthase (2)



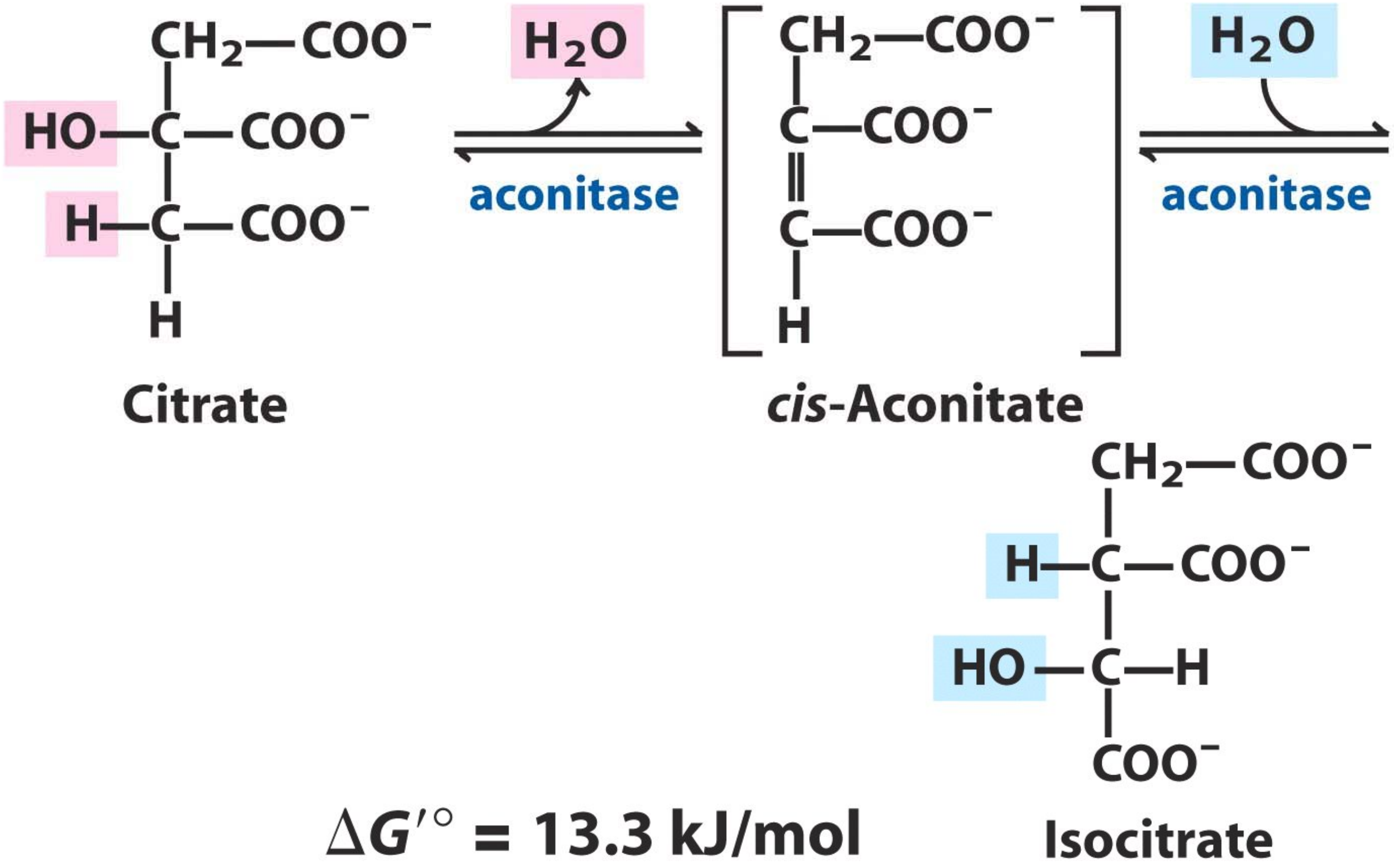
Citrate synthase (3)



The thioester is subsequently hydrolyzed, regenerating CoA-SH and producing citrate.

② *Formation of Isocitrate via cis-Aconitate*

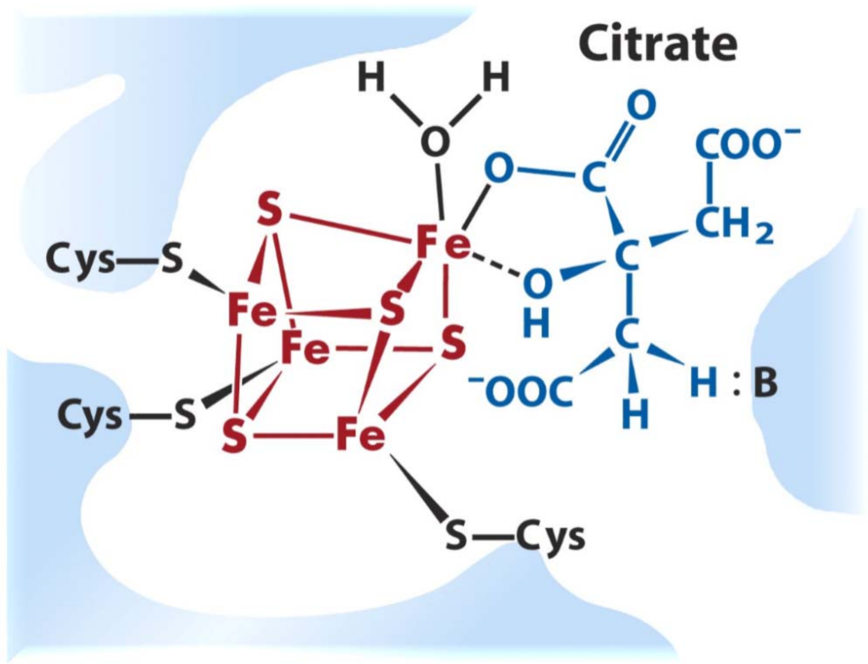
Aconitate hydratase



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

** at pH 7.4, 25 'C, less than 10% isocitrate*

Iron-sulfur center in aconitase



(*cytosolic*) Aconitase: (1) Aconitase activity

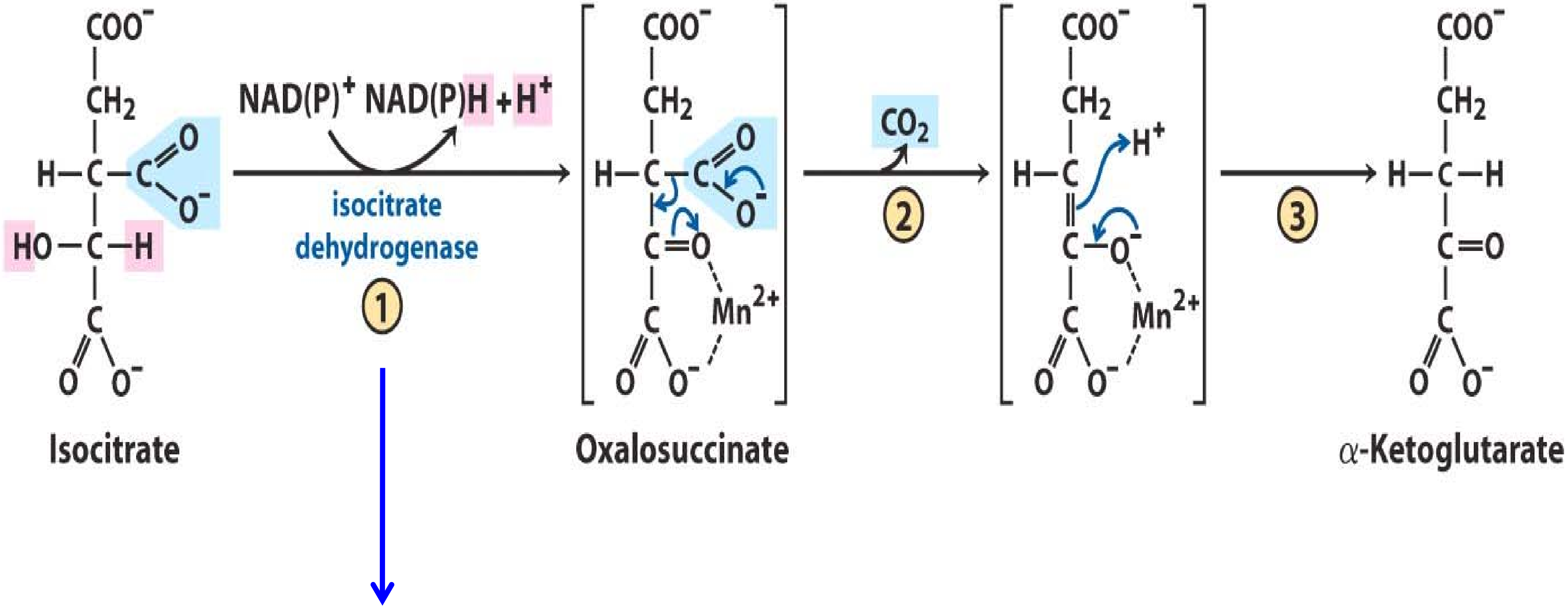
(2) Iron homeostasis ("*moonlighting*")

transferrin

transferrin receptor

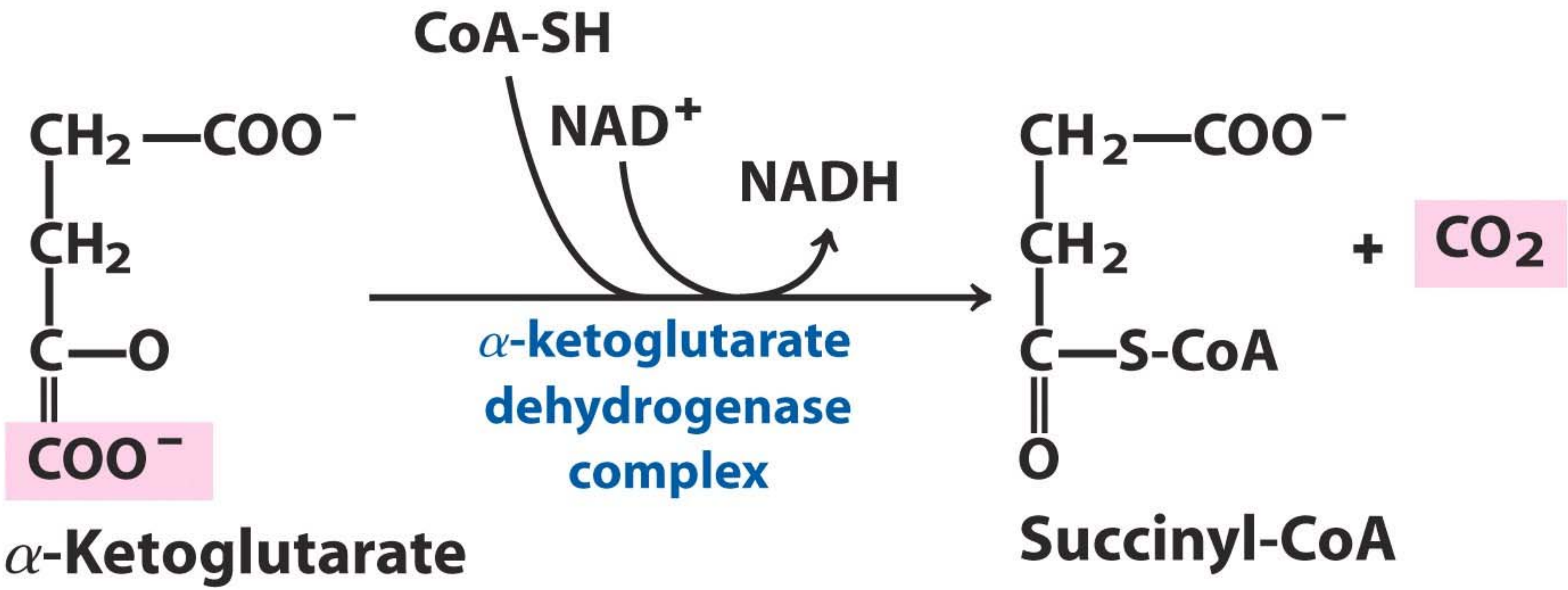
ferritin

③ *Oxidation of Isocitrate to α-ketoglutarate and CO_2*



2 different forms: NAD^+-dependent (in Mito. matrix)
$NADP^+$-dependent (in Mito. matrix & cytosol)

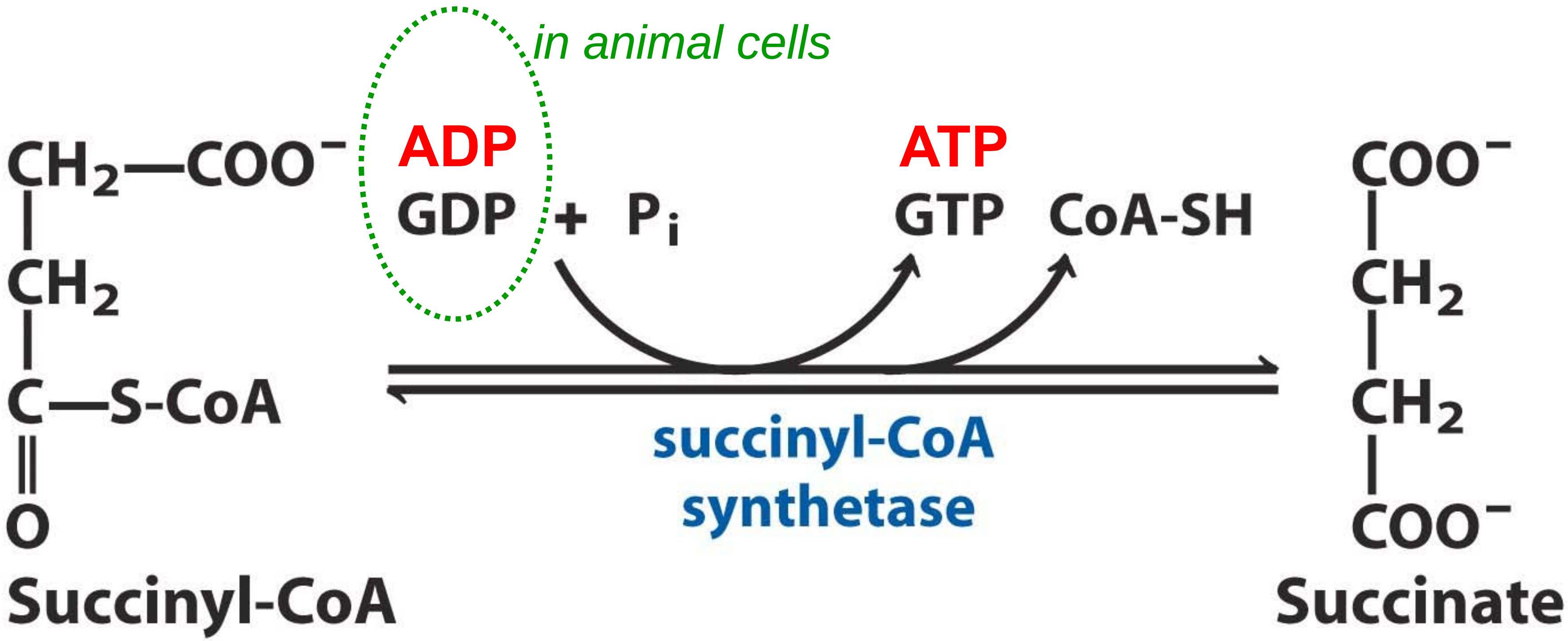
4 *Oxidation of α-ketoglutarate to Succinyl-CoA and CO_2*



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

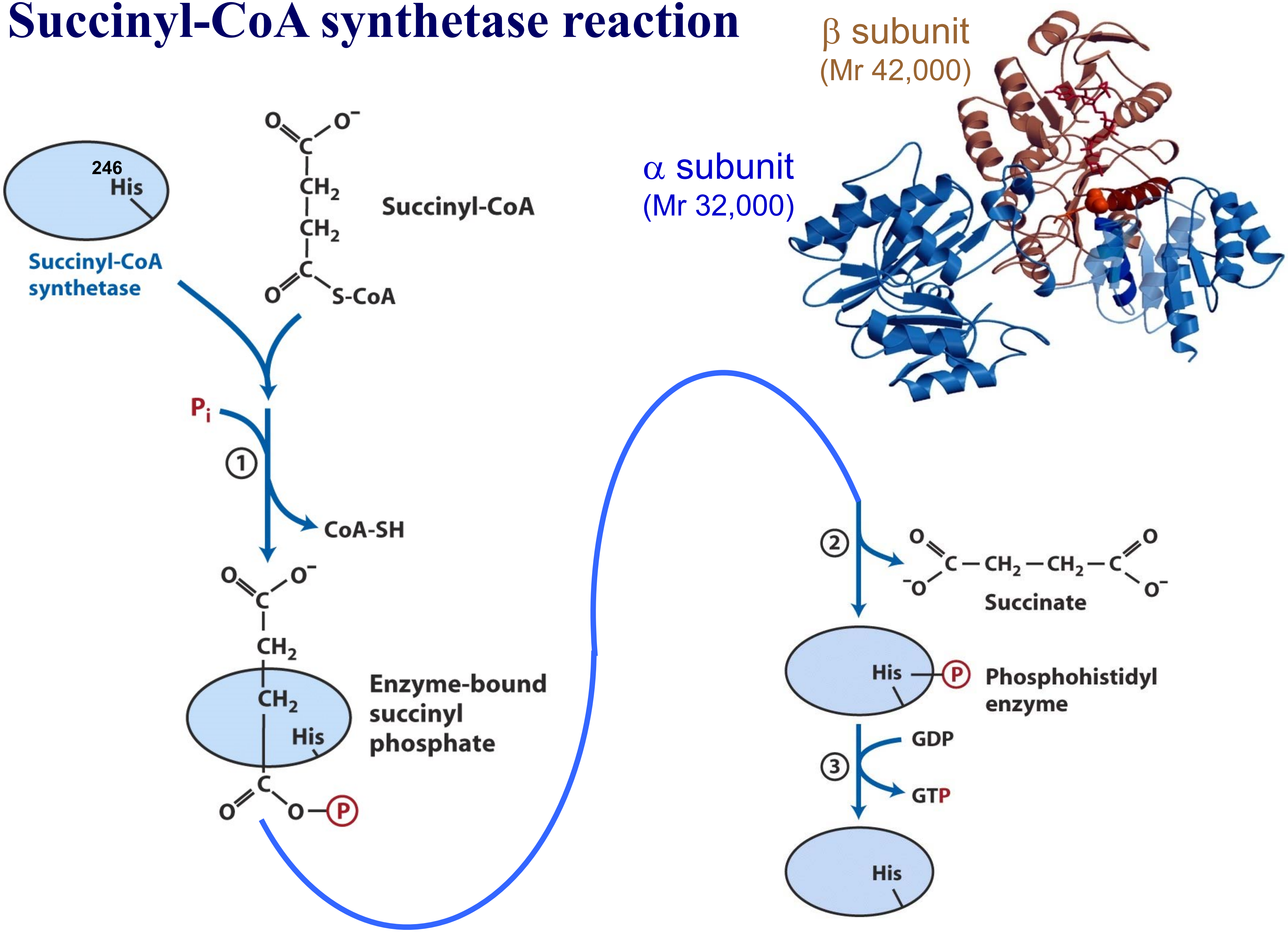
⑤ *Conversion of Succinyl-CoA to Succinate*

Succinic thiokinase

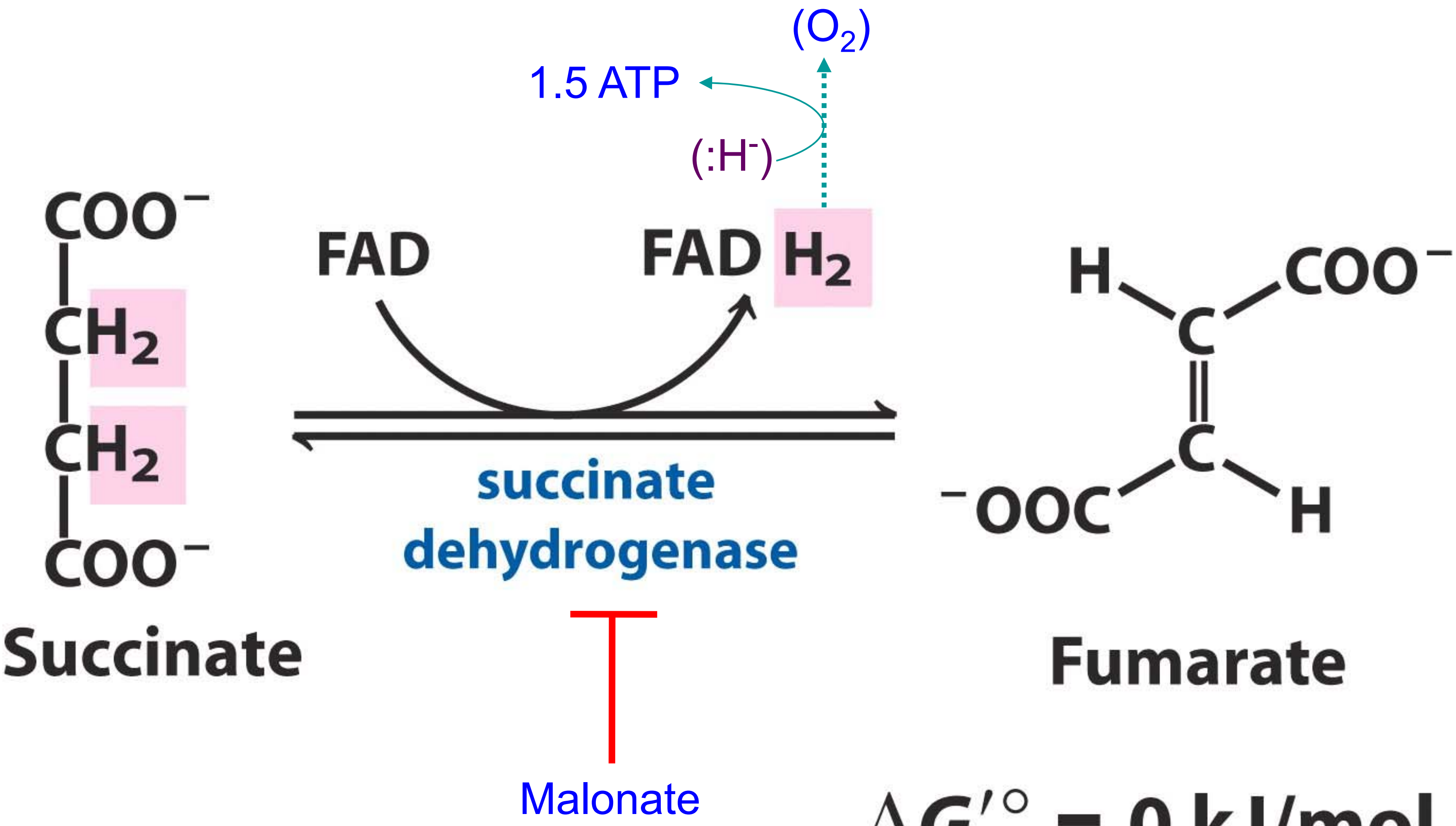


$\Delta G'^{\circ} = -2.9$ kJ/mol

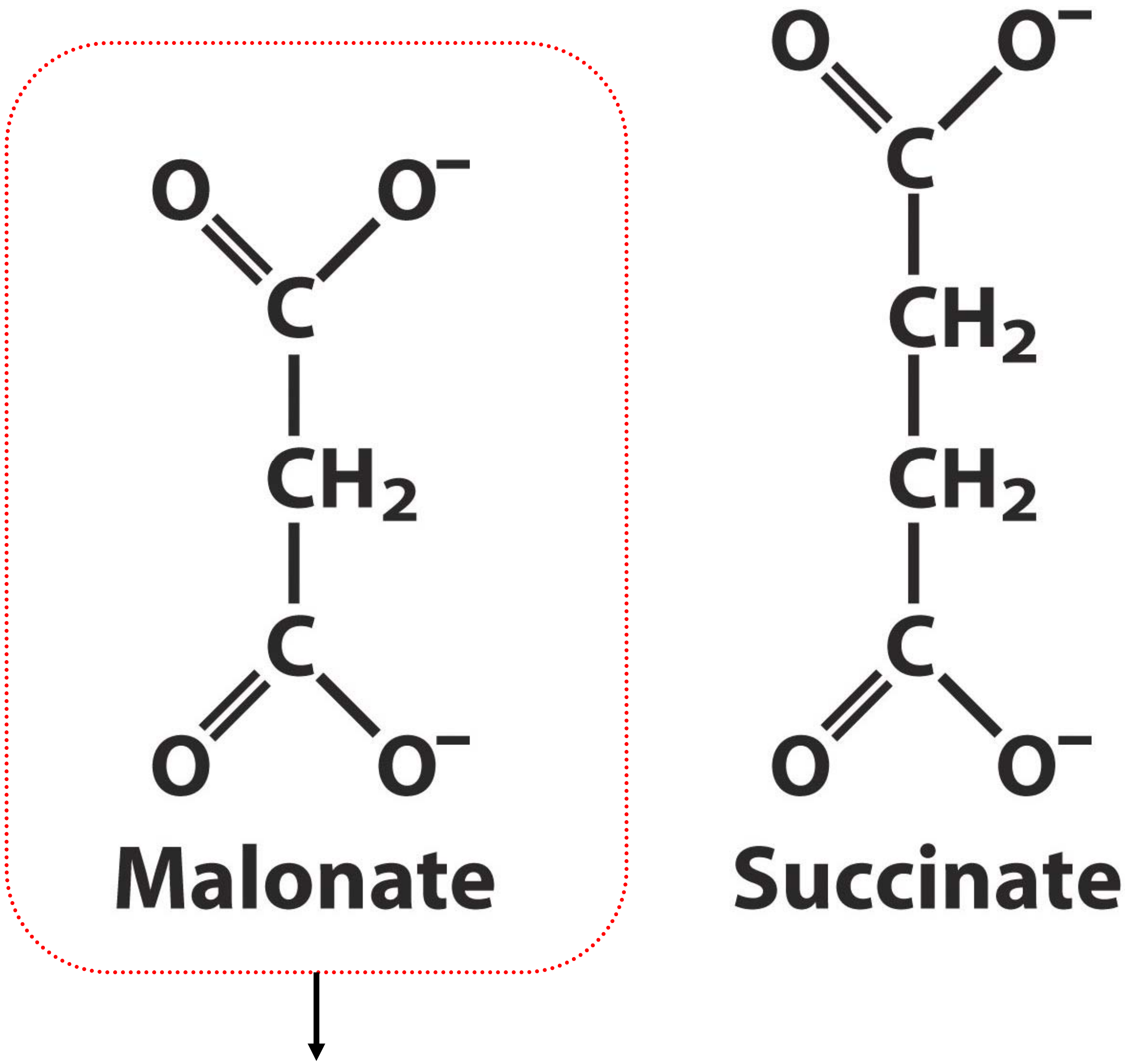
Succinyl-CoA synthetase reaction



⑥ *Oxidation of Succinate to Fumarate*



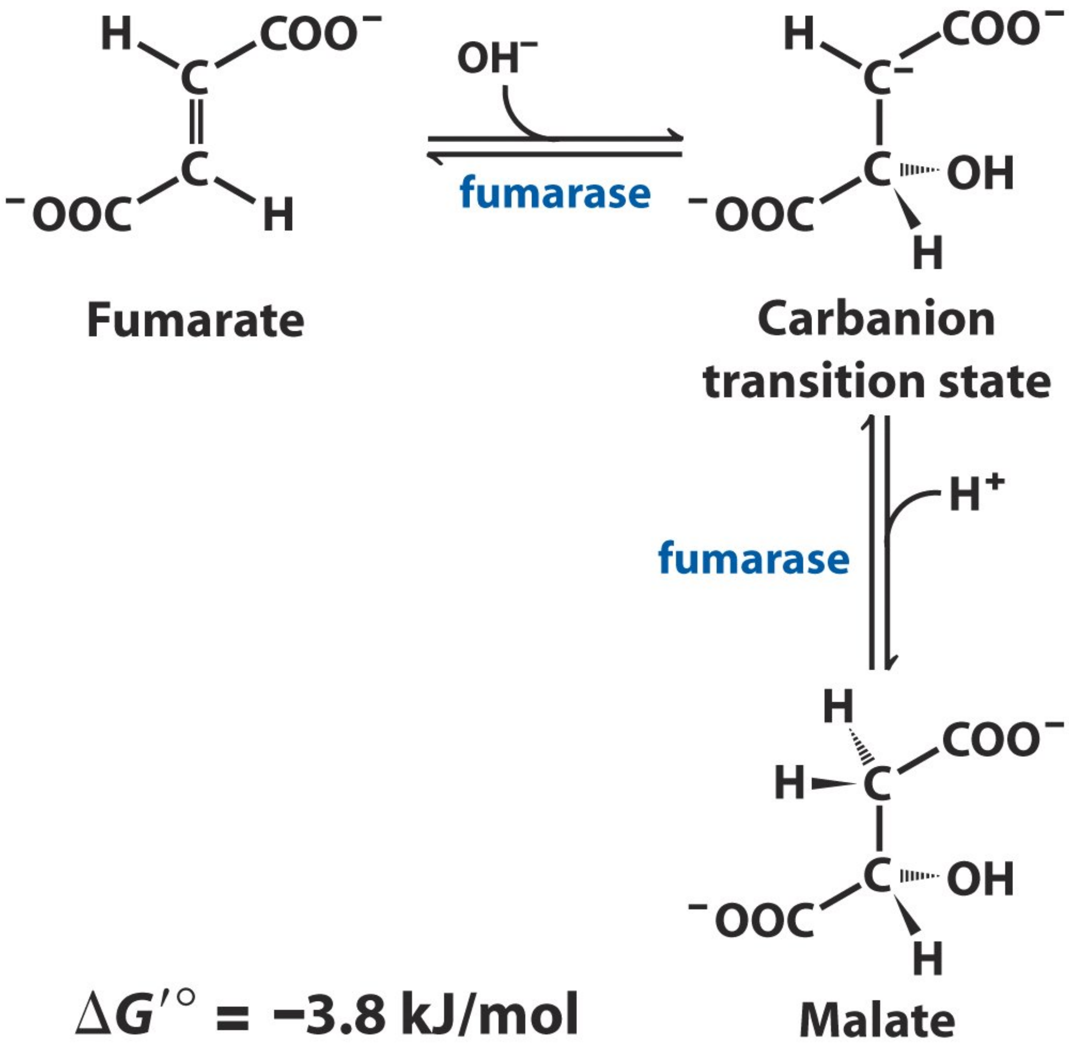
$$\Delta G'^{\circ} = 0 \text{ kJ/mol}$$



Strong competitive inhibitor of
succinate dehydrogenase (*TCA cycle blocker*)

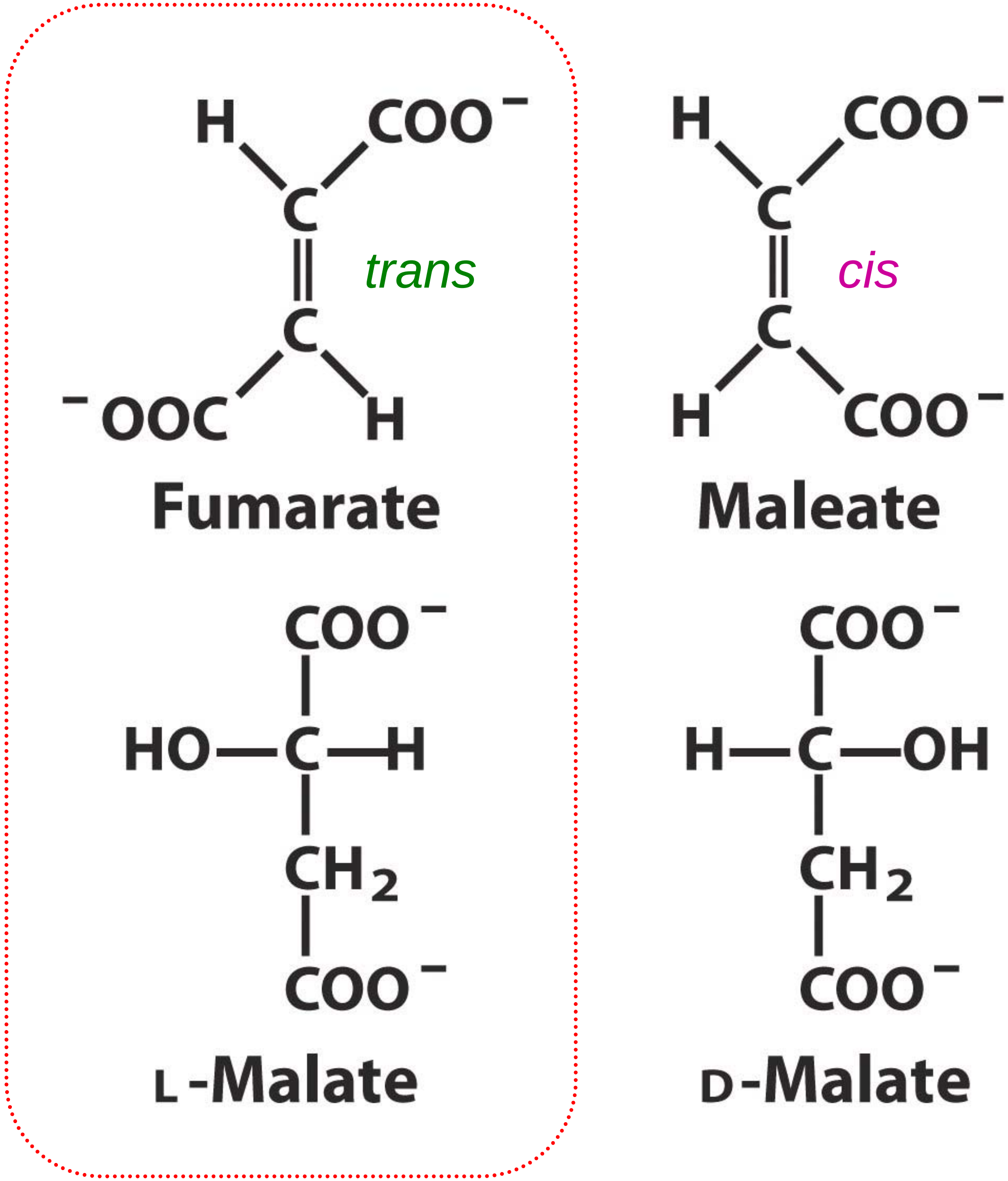
⑦ *Hydration of Fumarate to Malate*

Fumarate hydratase

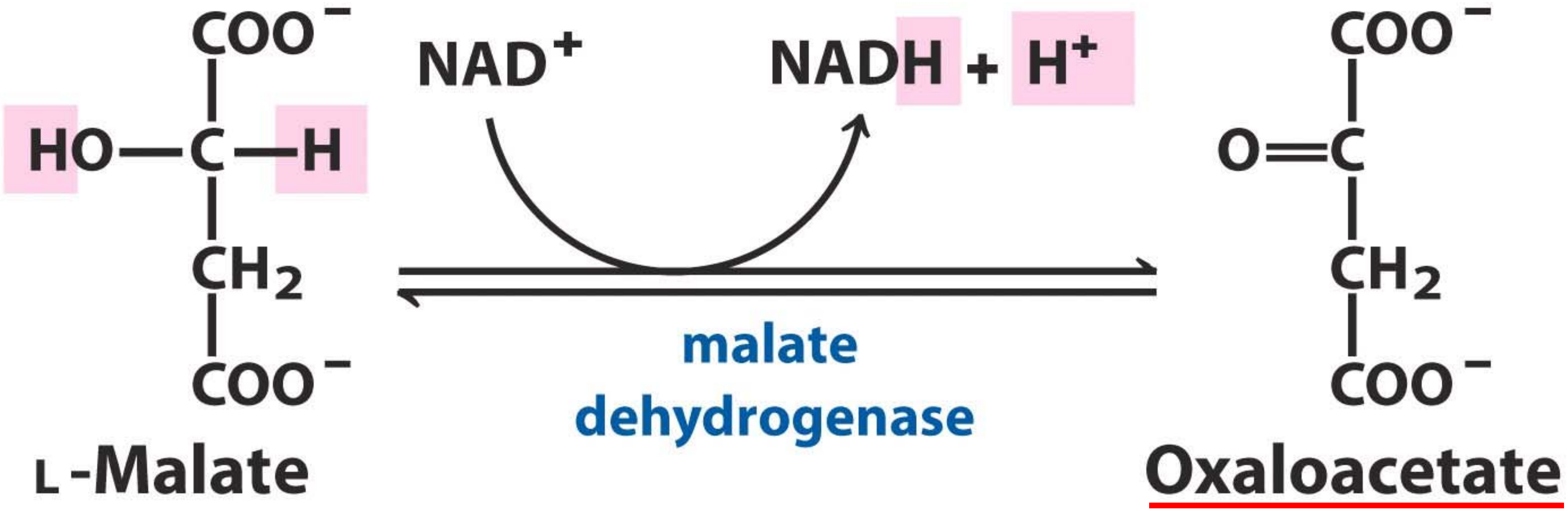


$\Delta G'^{\circ} = -3.8$ kJ/mol

Fumarase : highly stereospecific



⑧ Oxidation of Malate to Oxaloacetate



(< 10^{-6} M)

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

Products of one turn of the citric acid cycle

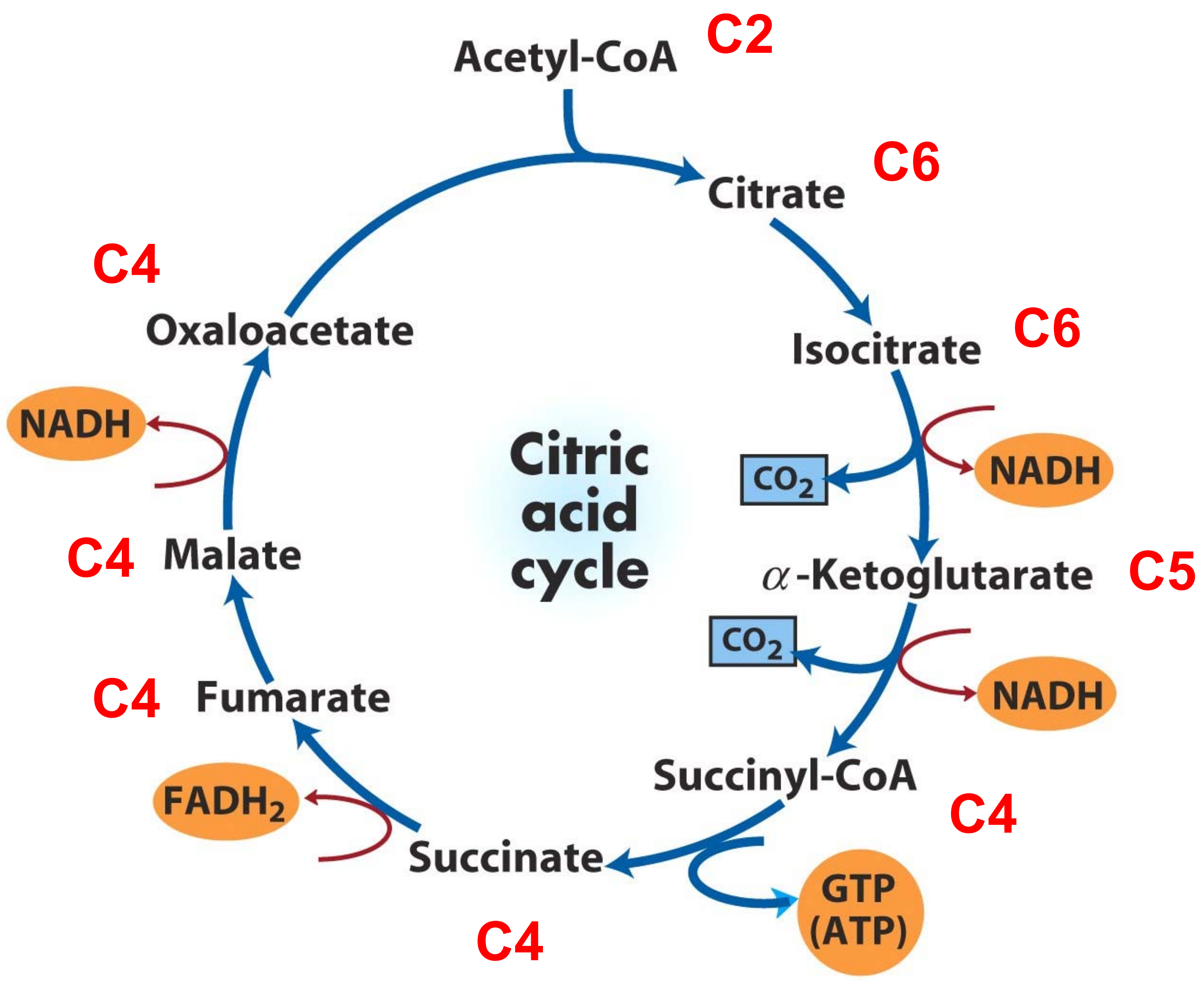


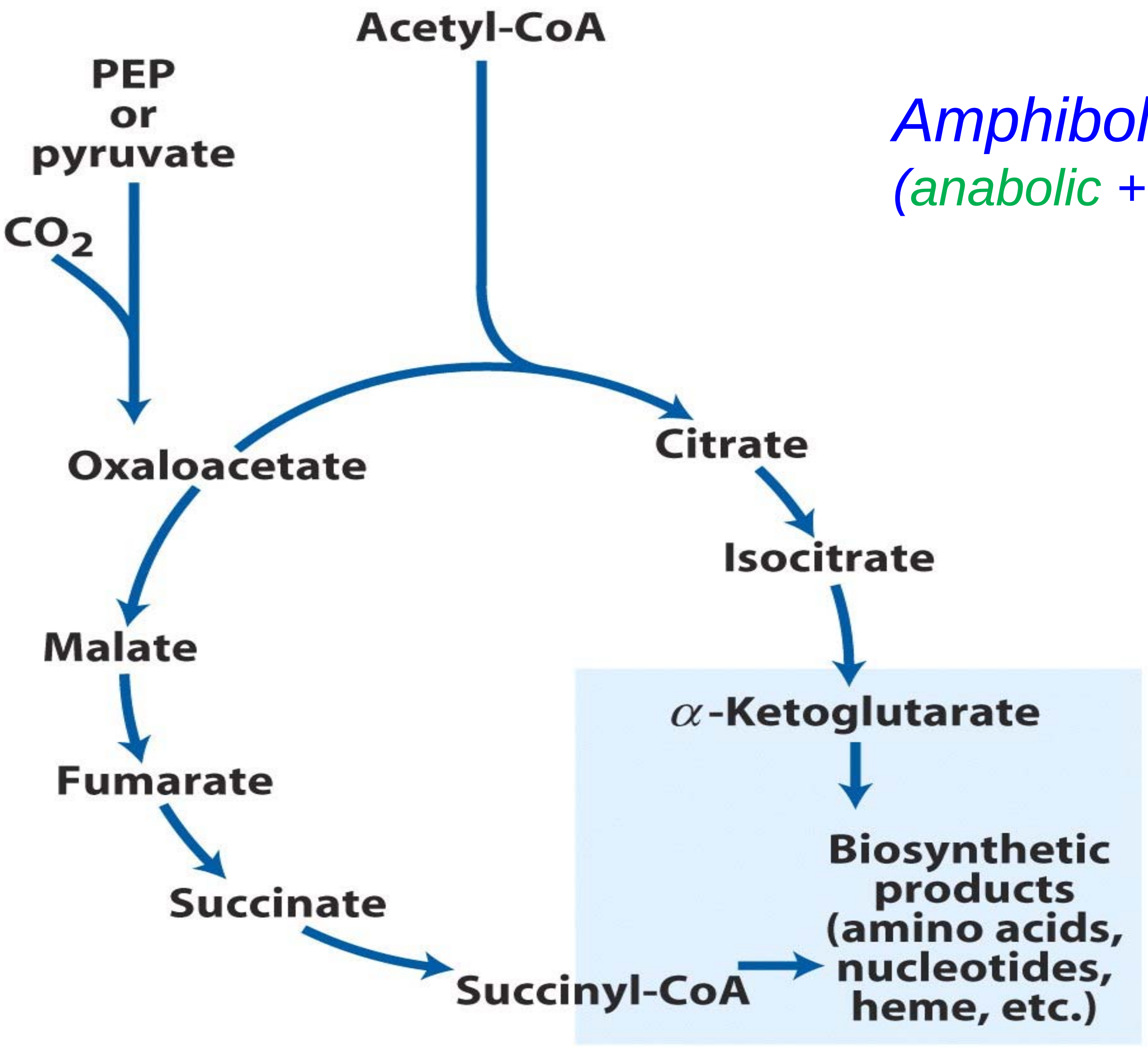
TABLE 16–1 Stoichiometry of Coenzyme Reduction and ATP Formation in the Aerobic Oxidation of Glucose via Glycolysis, the Pyruvate Dehydrogenase Complex Reaction, the Citric Acid Cycle, and Oxidative Phosphorylation

Reaction	*Number of ATP or reduced coenzyme directly formed*	*Number of ATP ultimately formed**
Glucose ⟶ glucose 6-phosphate	−1 ATP	−1
Fructose 6-phosphate ⟶ fructose 1,6-bisphosphate	−1 ATP	−1
2 Glyceraldehyde 3-phosphate ⟶ 2 1,3-bisphosphoglycerate	2 NADH	3 or 5†
2 1,3-Bisphosphoglycerate ⟶ 2 3-phosphoglycerate	2 ATP	2
2 Phosphoenolpyruvate ⟶ 2 pyruvate	2 ATP	2
2 Pyruvate ⟶ 2 acetyl-CoA	2 NADH	5
2 Isocitrate ⟶ 2 α-ketoglutarate	2 NADH	5
2 α-Ketoglutarate ⟶ 2 succinyl-CoA	2 NADH	5
2 Succinyl-CoA ⟶ 2 succinate	2 ATP (or 2 GTP)	2
2 Succinate ⟶ 2 fumarate	2 $FADH_2$	3
2 Malate ⟶ 2 oxaloacetate	2 NADH	5
Total		30–32

*This is calculated as 2.5 ATP per NADH and 1.5 ATP per $FADH_2$. A negative value indicates consumption.

† This number is either 3 or 5, depending on the mechanism used to shuttle NADH equivalents from the cytosol to the mitochondrial matrix; see Figures 19–27 and 19–28.

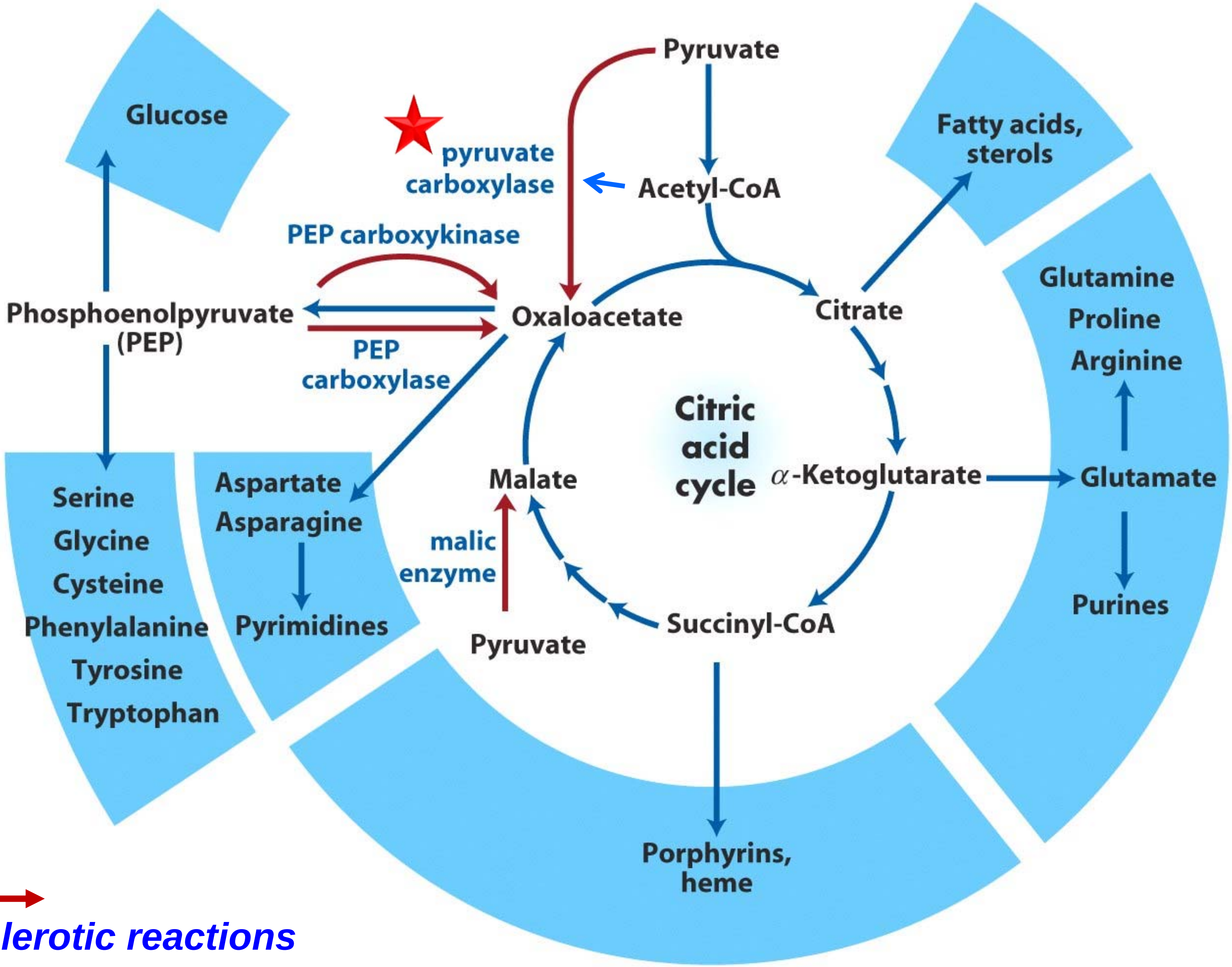
Biosynthetic precursors produced by an incomplete citric acid cycle in anaerobic bacteria



Amphibolic pathway

(anabolic + catabolic)

Role of the citric acid cycle in anabolism




anaplerotic reactions

TABLE 16–2 Anaplerotic Reactions

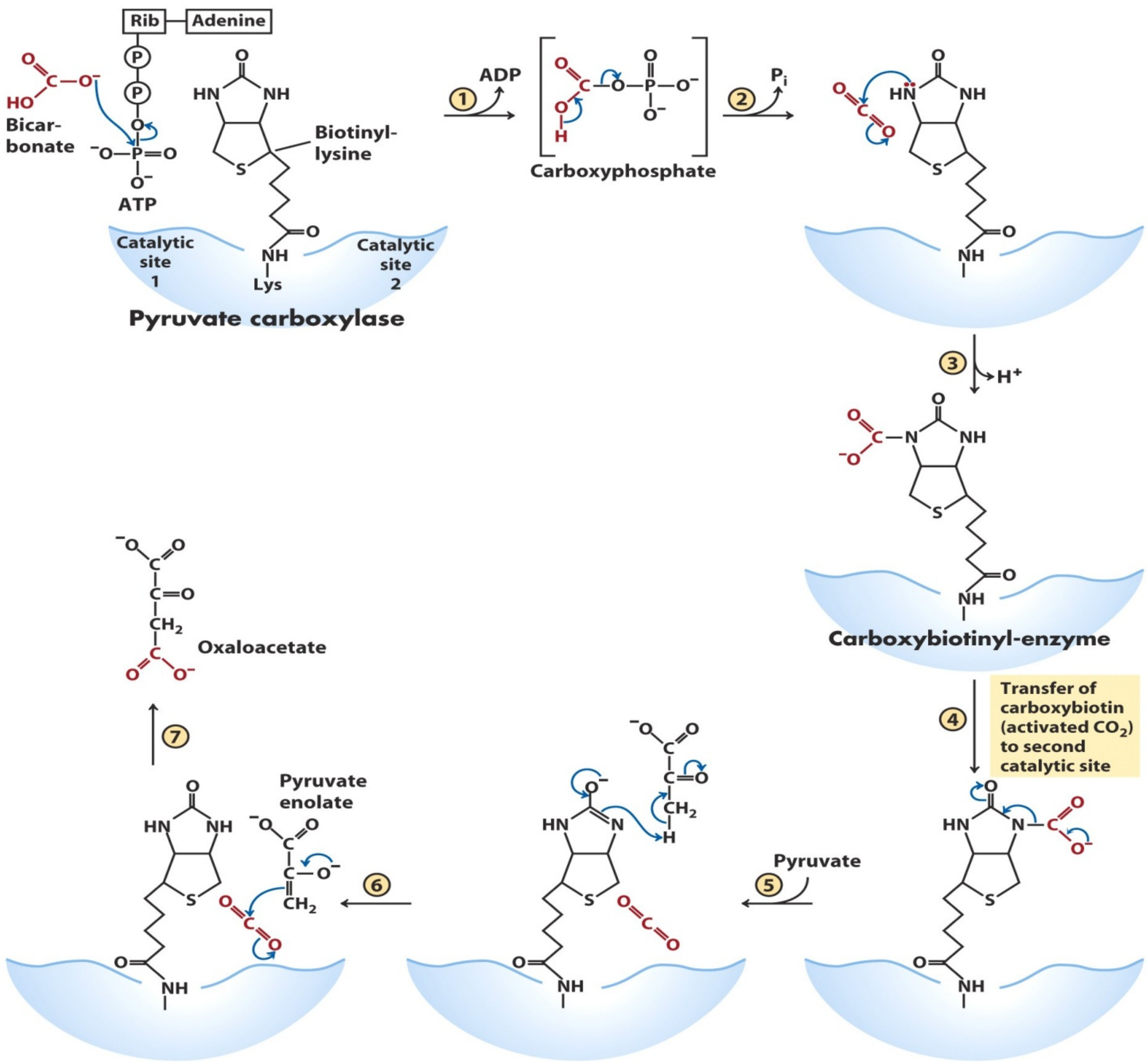
Reaction	*Tissue(s)/organism(s)*
Pyruvate + HCO_3^- + ATP $\xrightleftharpoons{\text{pyruvate carboxylase}}$ oxaloacetate + ADP + P_i	Liver, kidney
Phosphoenolpyruvate + CO_2 + GDP $\xrightleftharpoons{\text{PEP carboxykinase}}$ oxaloacetate + GTP	Heart, skeletal muscle
Phosphoenolpyruvate + HCO_3^- $\xrightleftharpoons{\text{PEP carboxylase}}$ 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

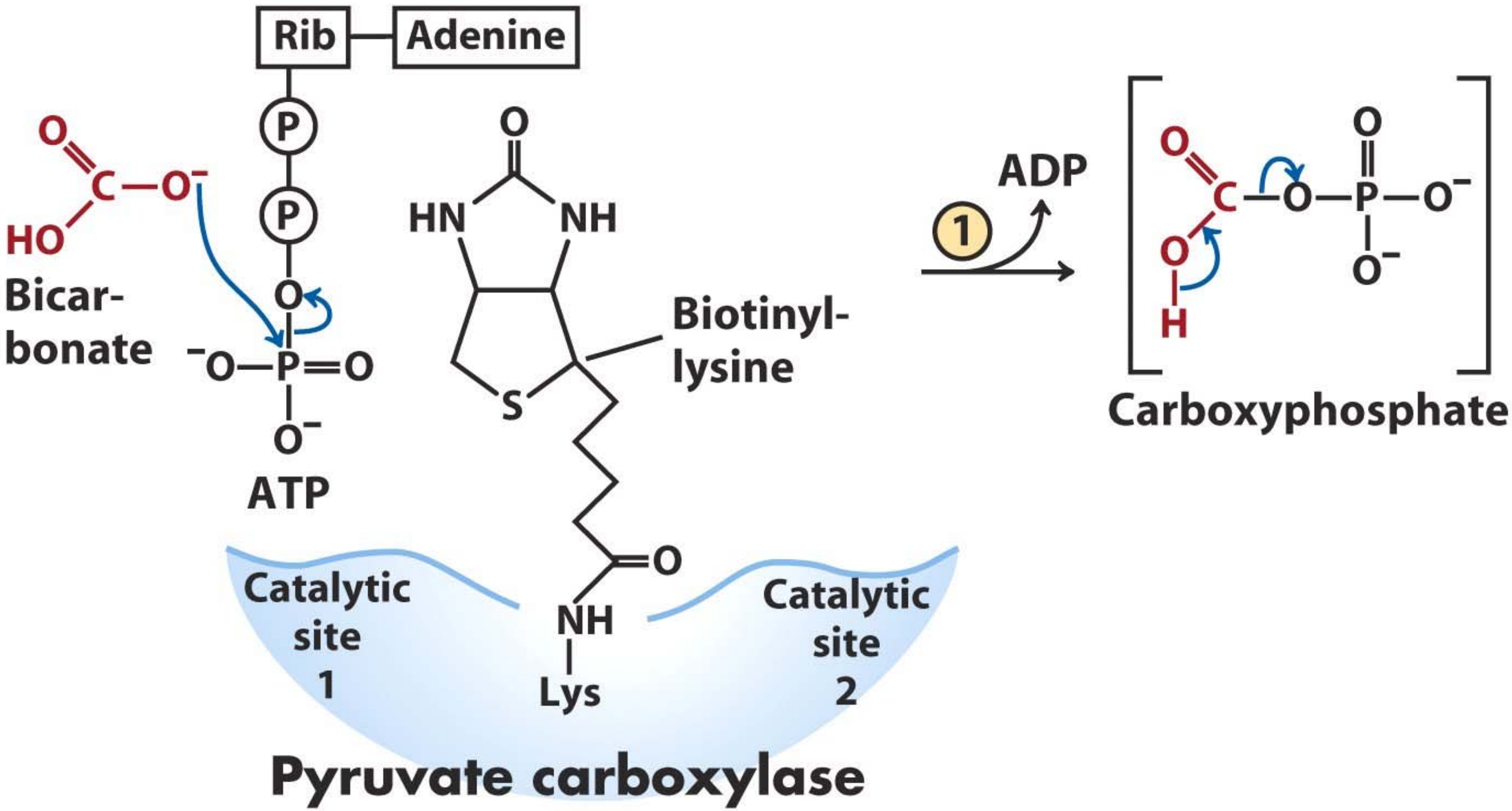
*** As intermediates of the citric acid cycle are removed to serve as biosynthetic precursors, they replenished by anaplerotic reactions***

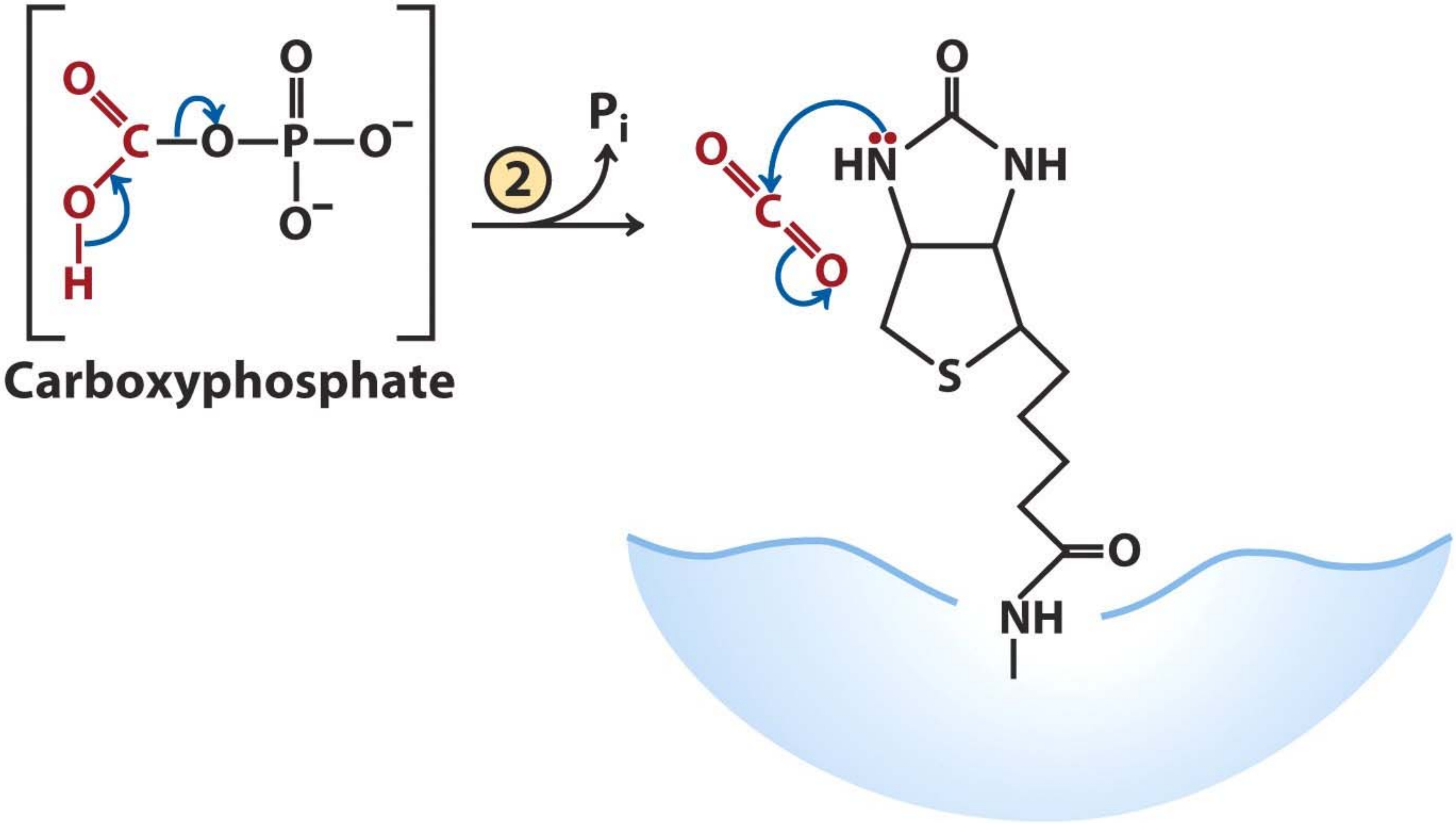
↓

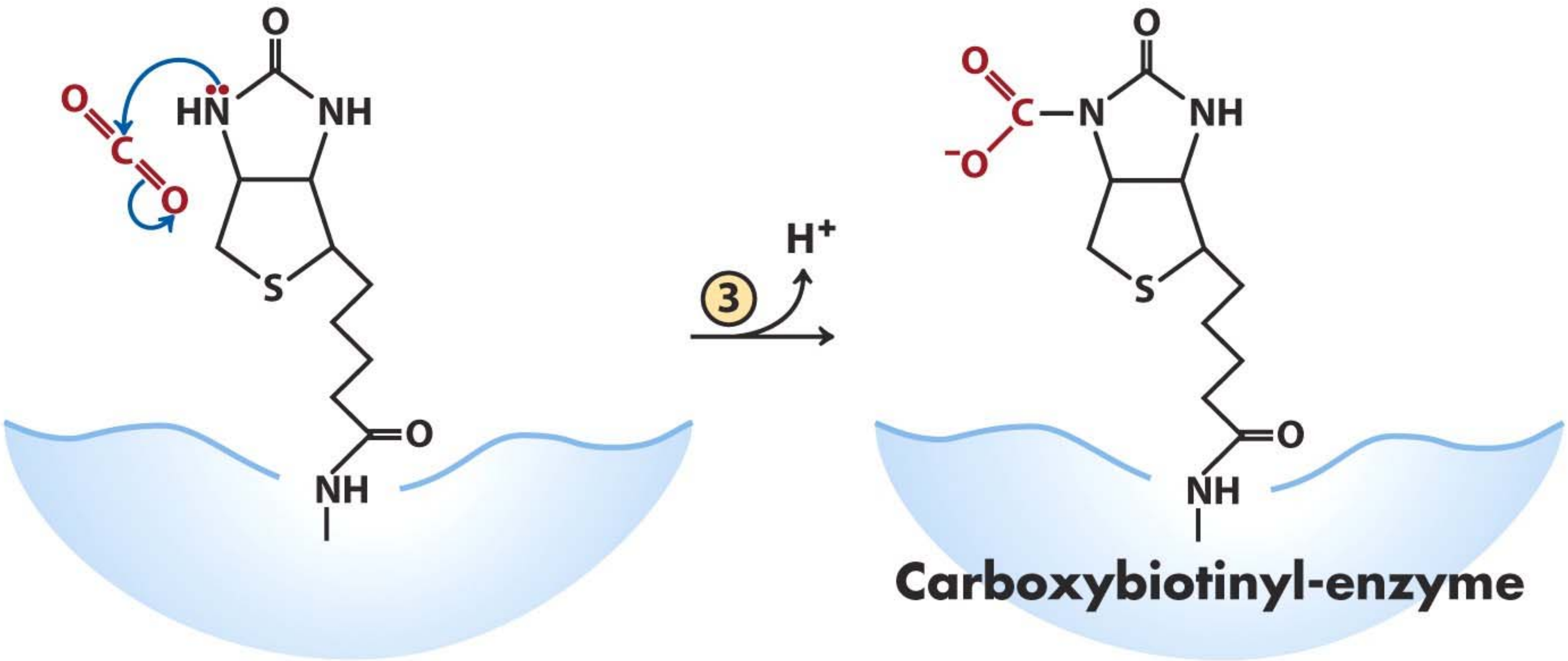
The concentration of the citric acid cycle intermediates remain almost constant

Pyruvate carboxylase reaction

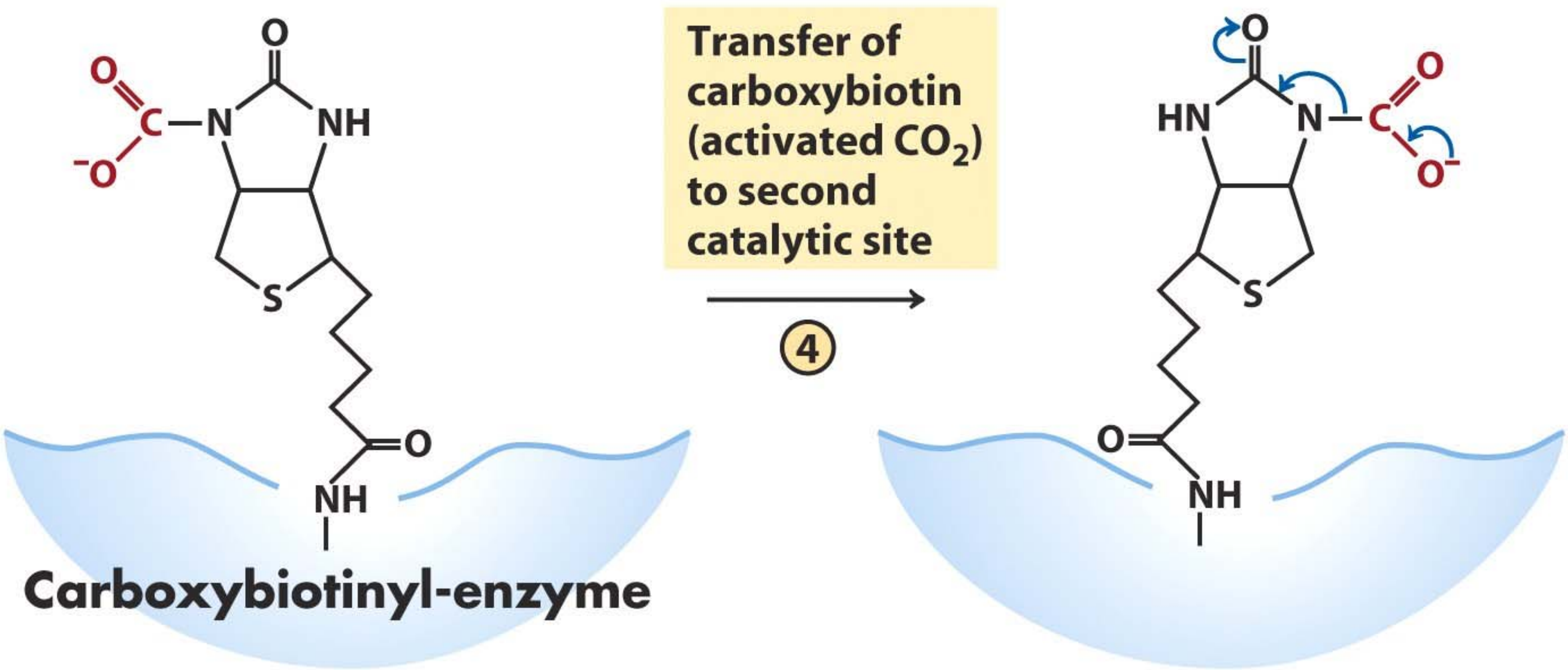


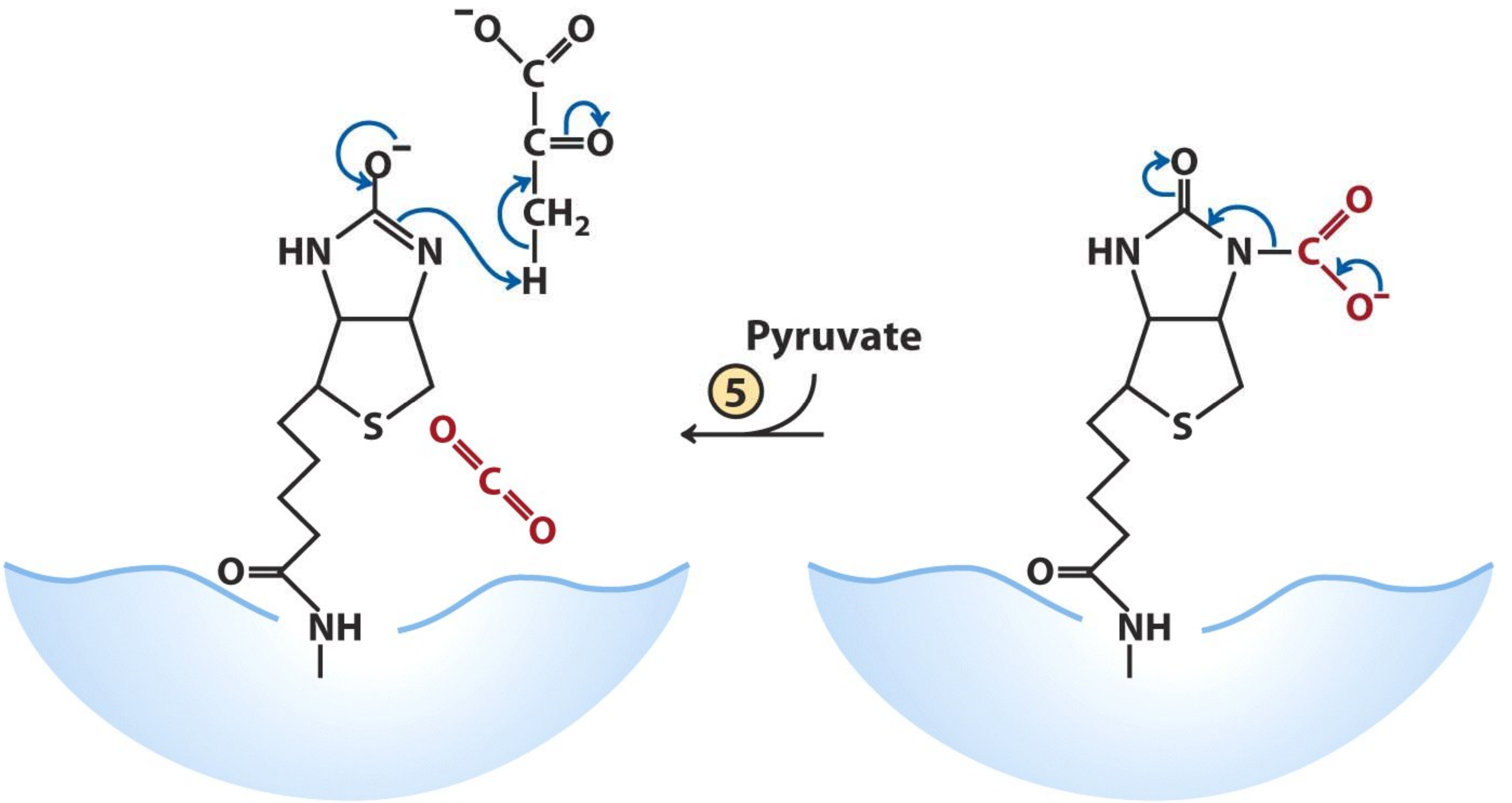

Rib
Adenine
Bicar-
bonate
ATP
Biotinyl-
lysine
ADP
1
Carboxyphosphate
Catalytic
site
1
Lys
Catalytic
site
2
Pyruvate carboxylase


O
C
O
H
O
P
O⁻
O⁻
Carboxyphosphate
2
Pi
O
C
O
O
HN
NH
S
O
NH

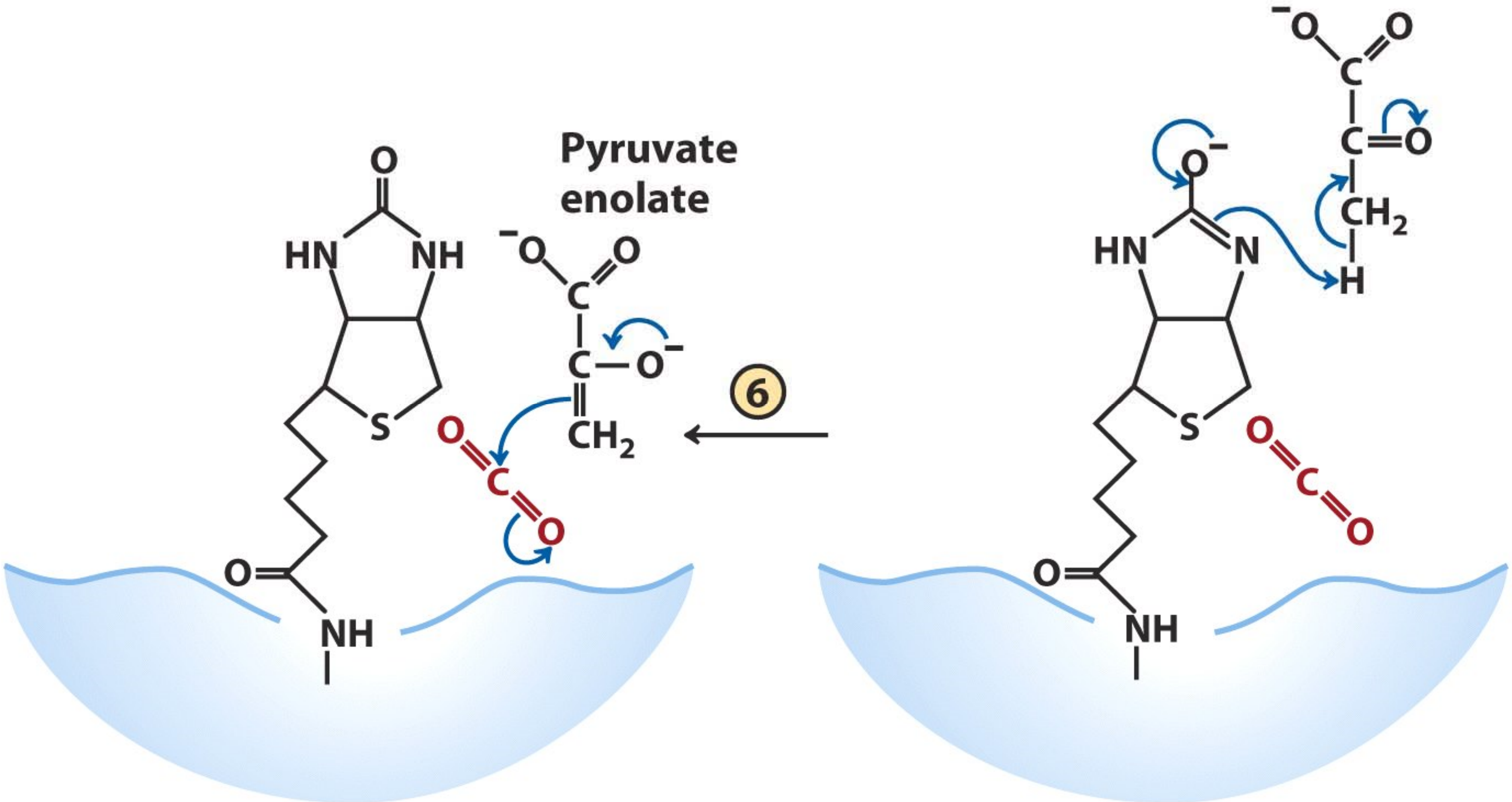


O
HN
NH
O
C
O
S
NH
O
3
H+
O
O
C
N
NH
-O
S
NH
O
Carboxybiotinyl-enzyme

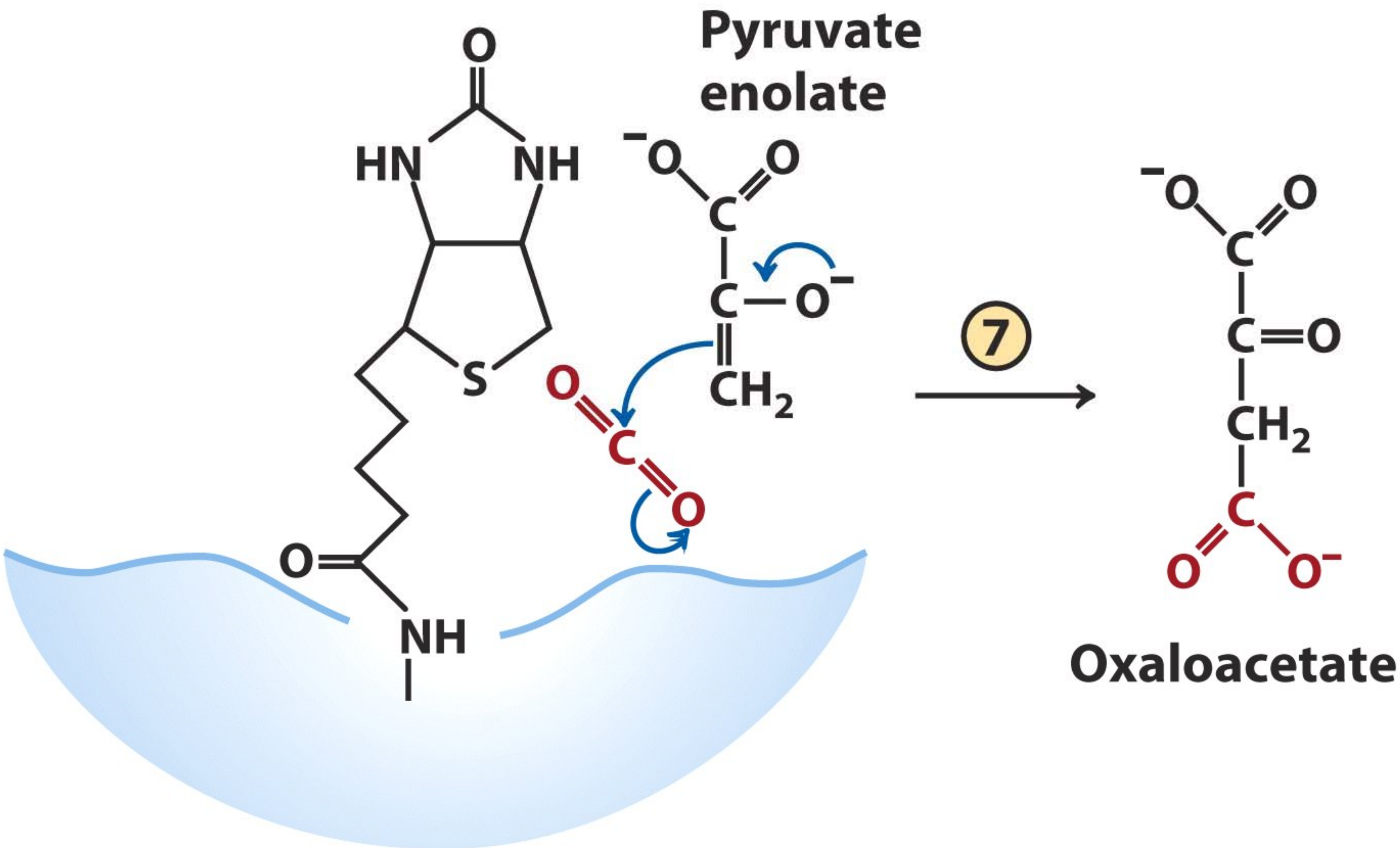

Transfer of carboxybiotin (activated CO_2) to second catalytic site
4
Carboxybiotinyl-enzyme



Pyruvate
5

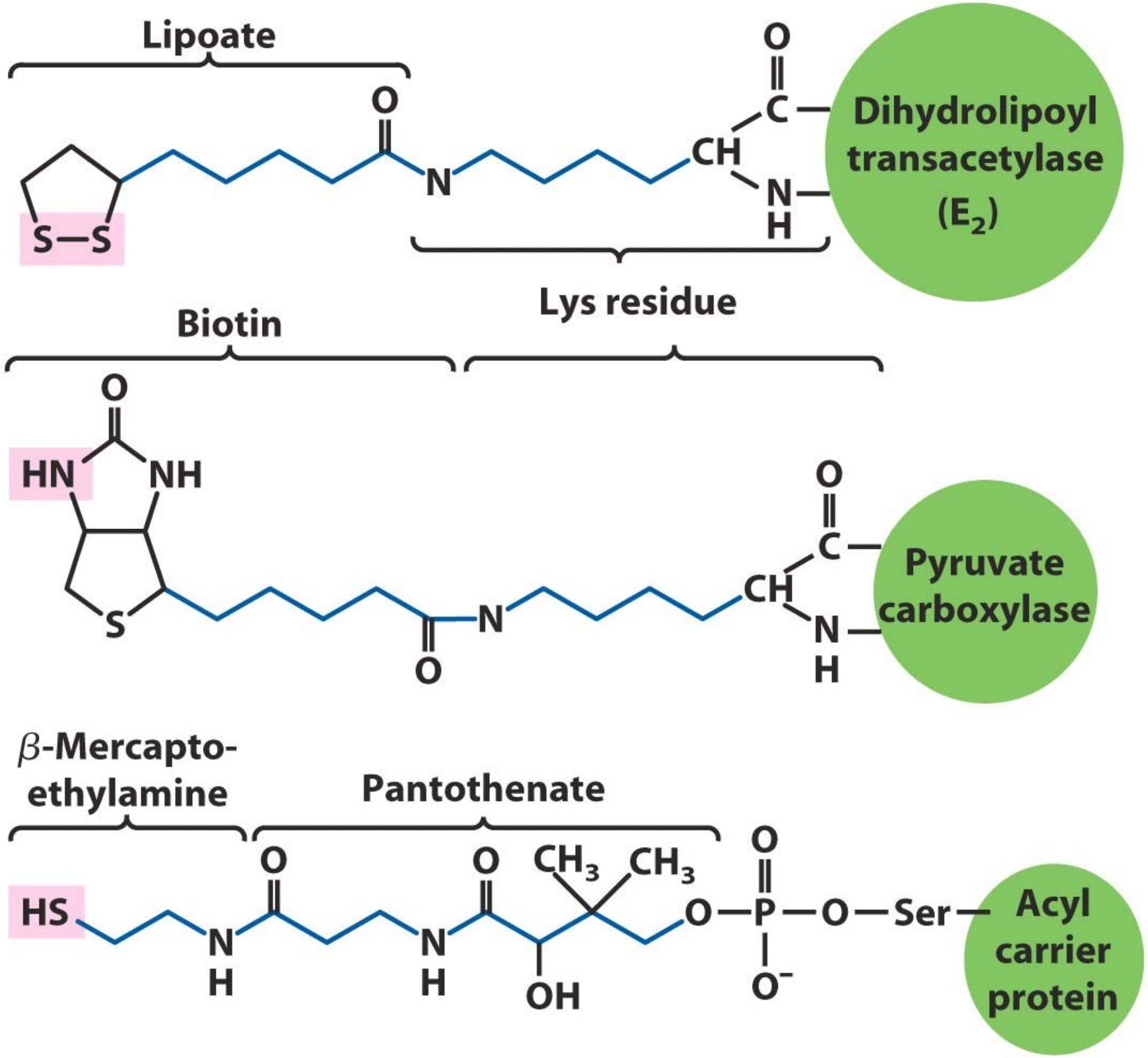


Pyruvate
enolate
6

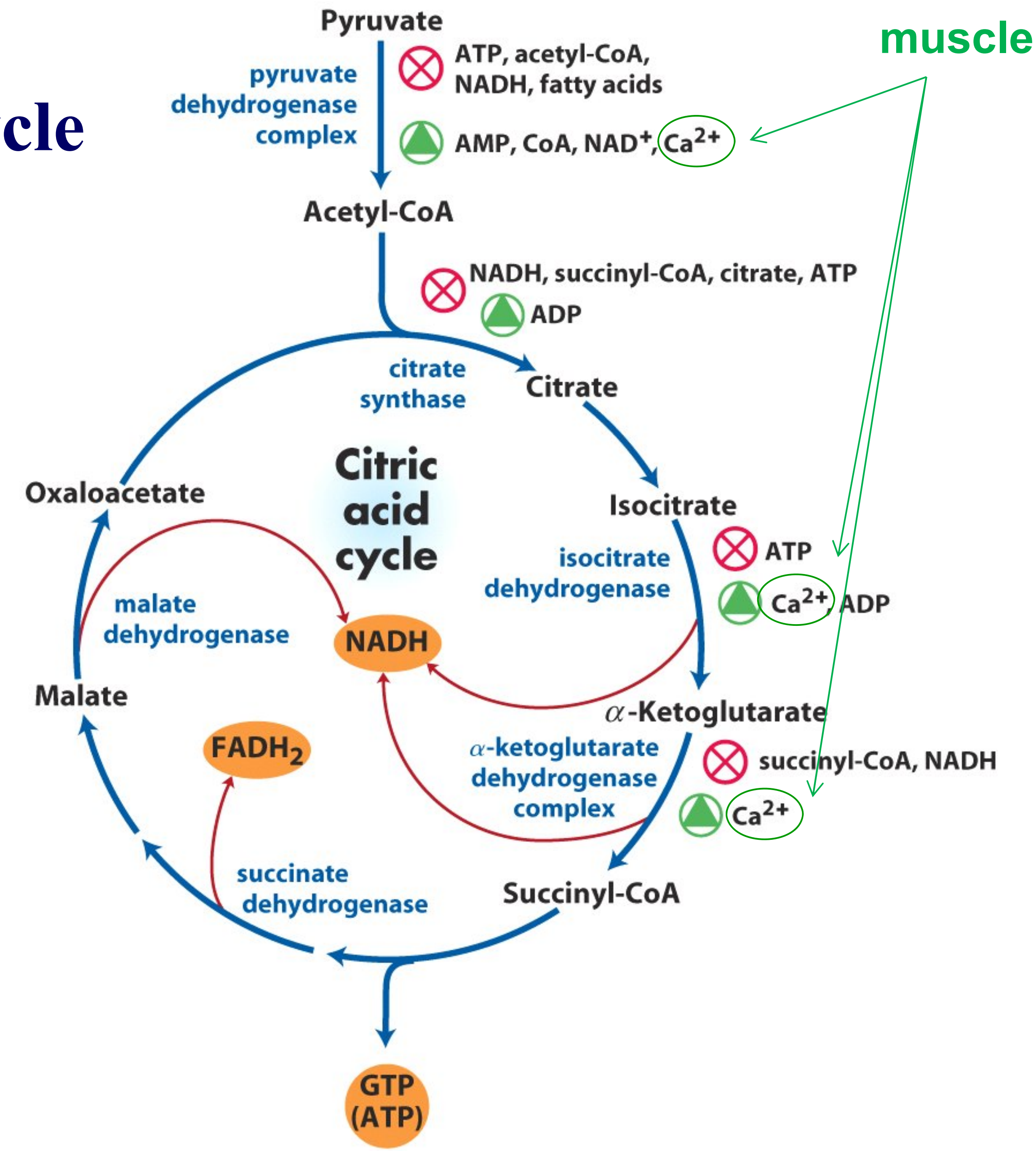

Pyruvate
enolate
7
Oxaloacetate

Biological tethers

Avidin (Mr 70,000)
rich in raw eggs



Regulation of the citric acid cycle



The Glyoxylate Cycle

Vertebrates:

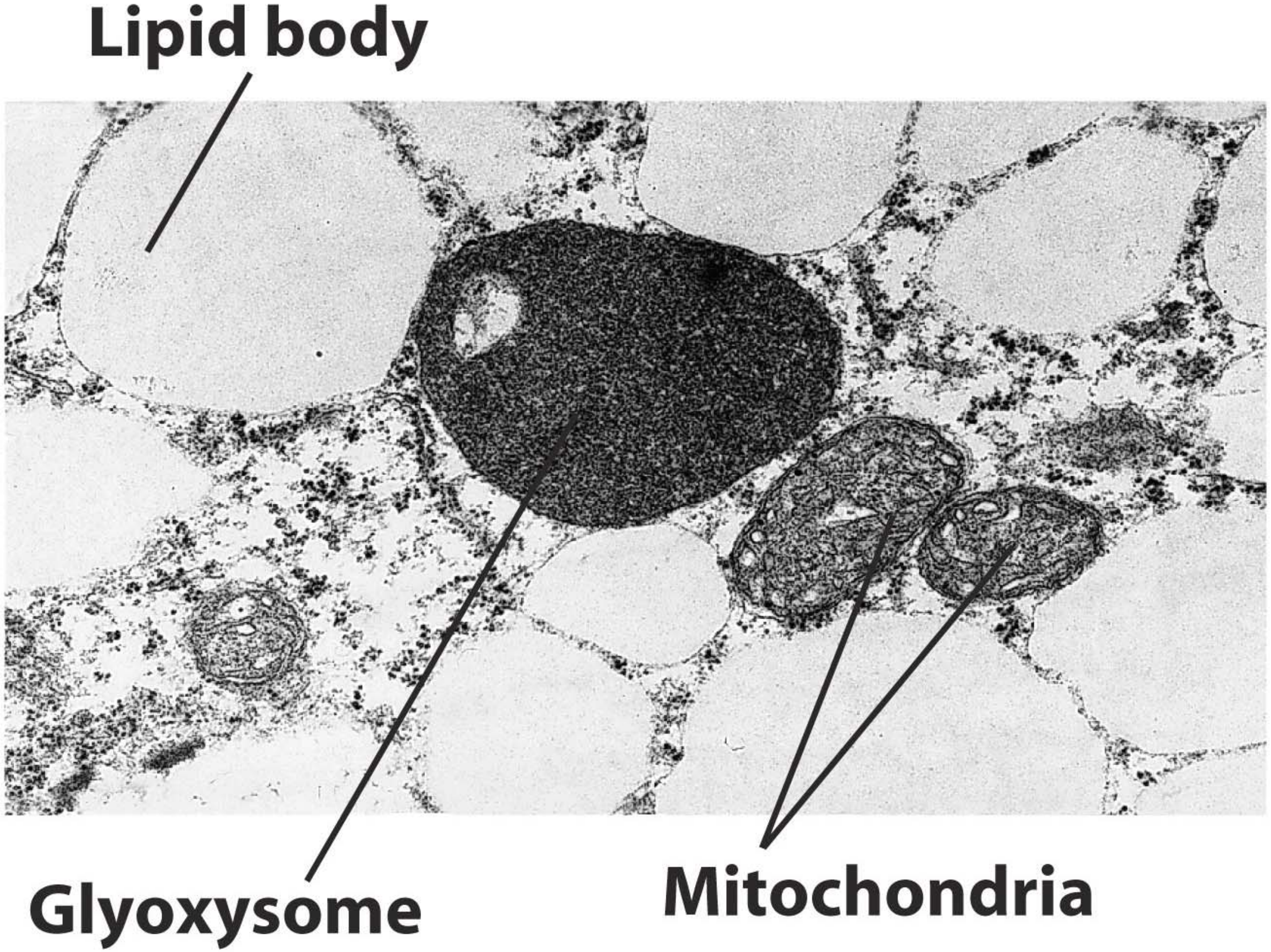
cannot convert fatty acids, or the acetate to carbohydrates

Plants, certain invertebrates, some microorganism:

acetate can serve both as an energy-rich fuel and as a source of PEP for cabohydrate synthesis

↓

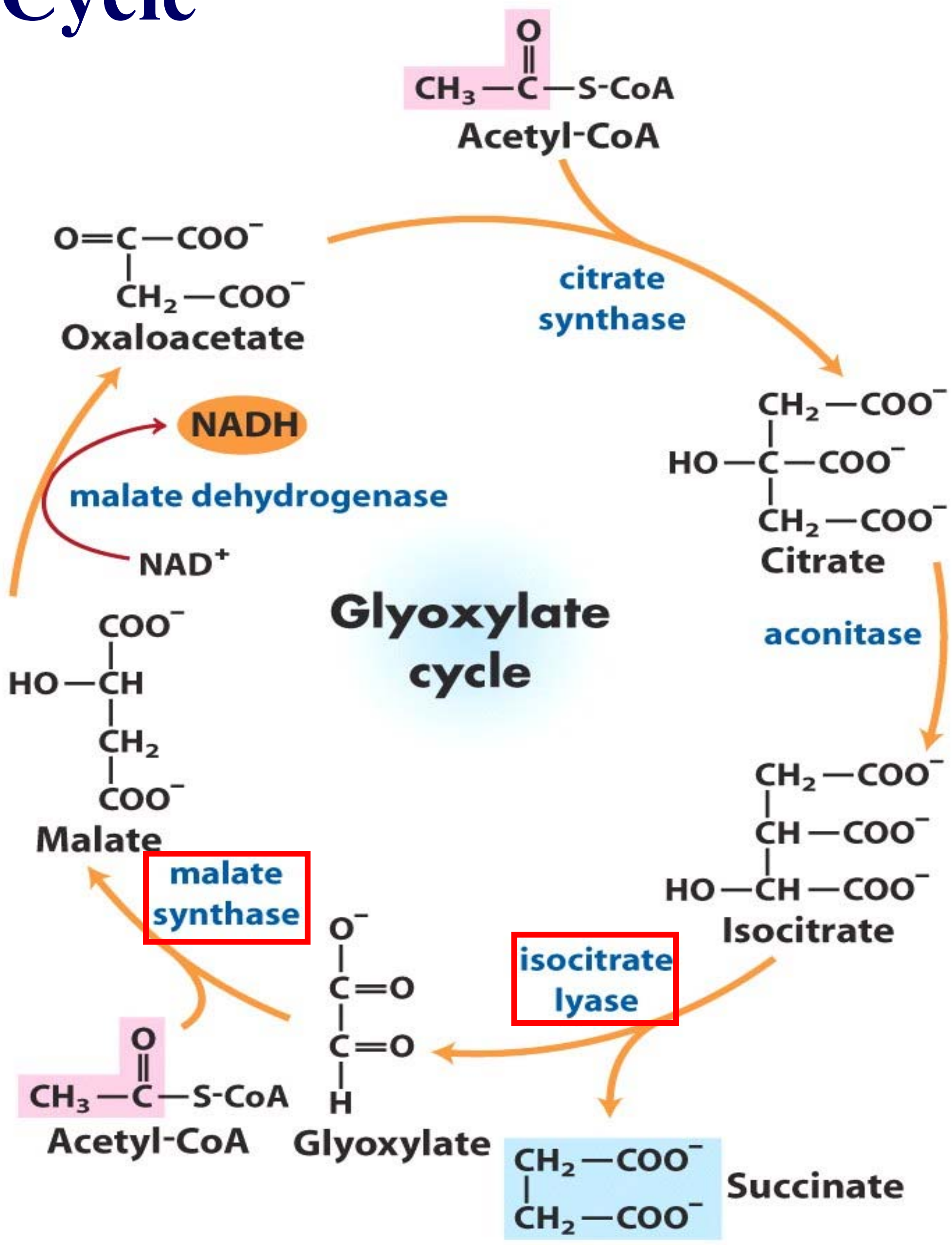
Glyoxylate Cycle



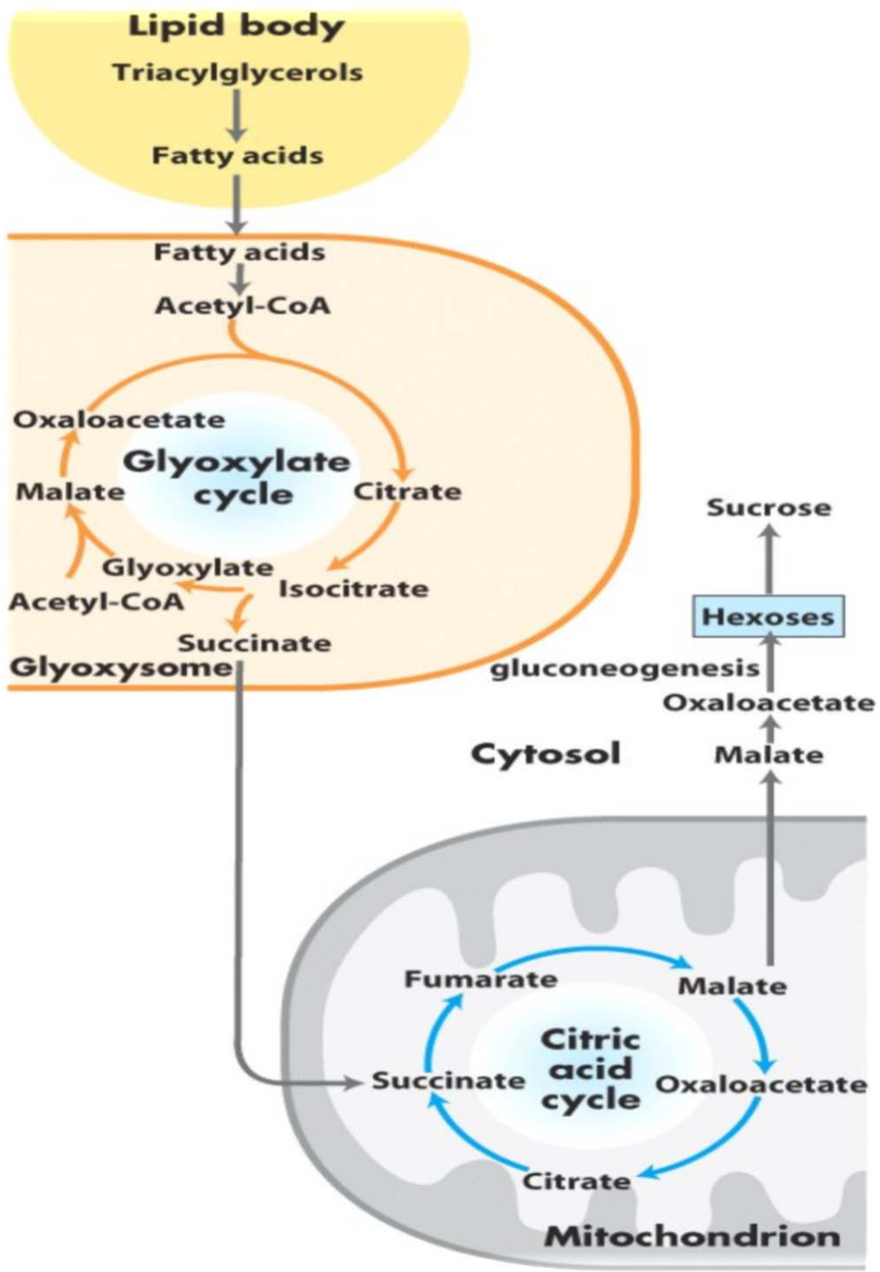
enzymes of the glyoxylate cycle
are sequestered in membrane-bounded organells

EM of a germinating cucumber seed

The Glyoxylate Cycle



The glyoxylate and citric acid cycles



Coordinated regulation of glyoxylate and citric acid cycles