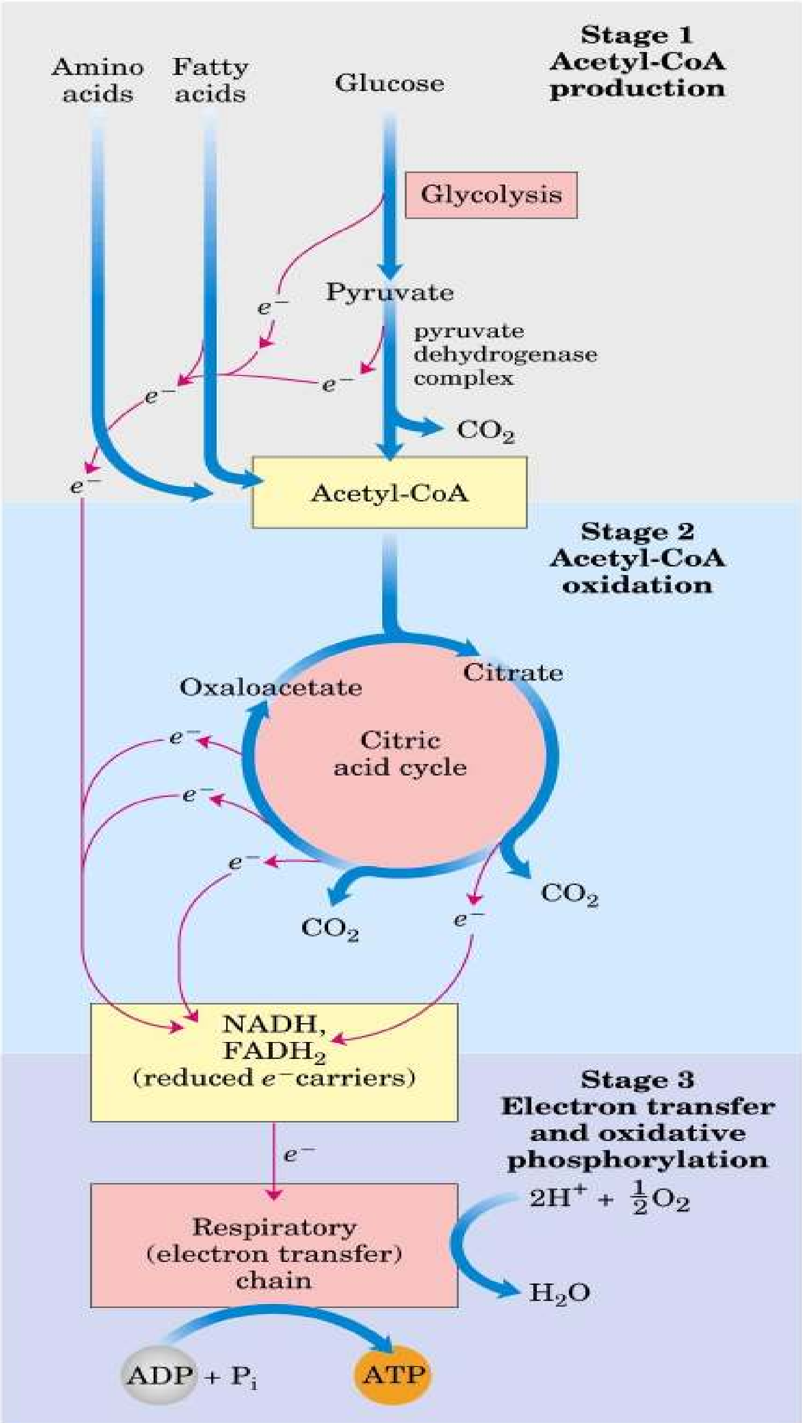
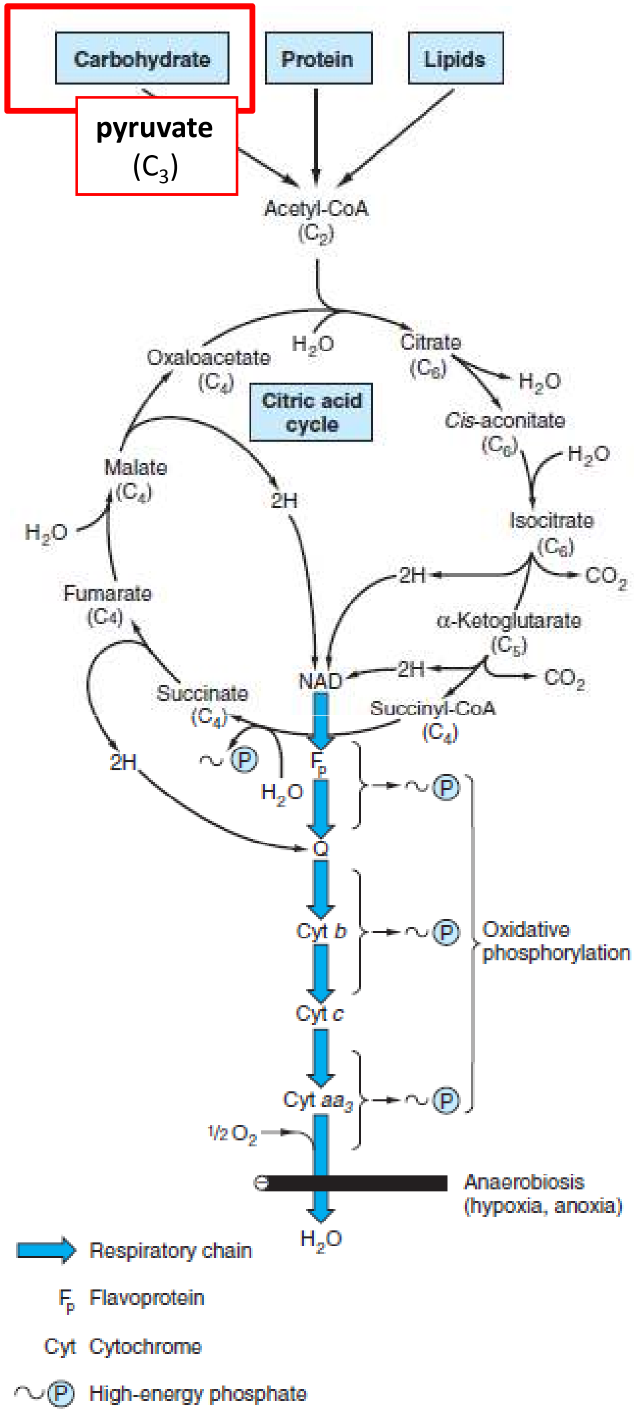
CITRIC ACID CYCLE
As Central Metabolic Pathway



- **Aerobic metabolism**
- **Energy metabolism**
  → Energy conservation/ATP synthesis by complete oxidation of fuel (bio)molecules to CO_2, and reduction of O_2 to H_2O

THREE STAGES OF CELLULAR RESPIRATION

1. **Acetyl-CoA production** - oxidation of fatty acids, glucose, and some amino acids.
2. **Oxidation of acetyl-CoA** in the citric acid cycle, CO_2 production, the energy released is conserved in the reduced electron carriers NADH and $FADH_2$.
3. Re-oxidation of coenzymes in respiratory chain, **electron transfer** to O_2, production of ATP.

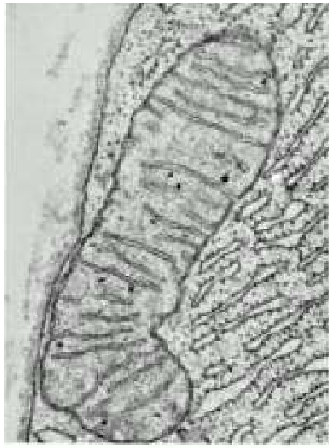


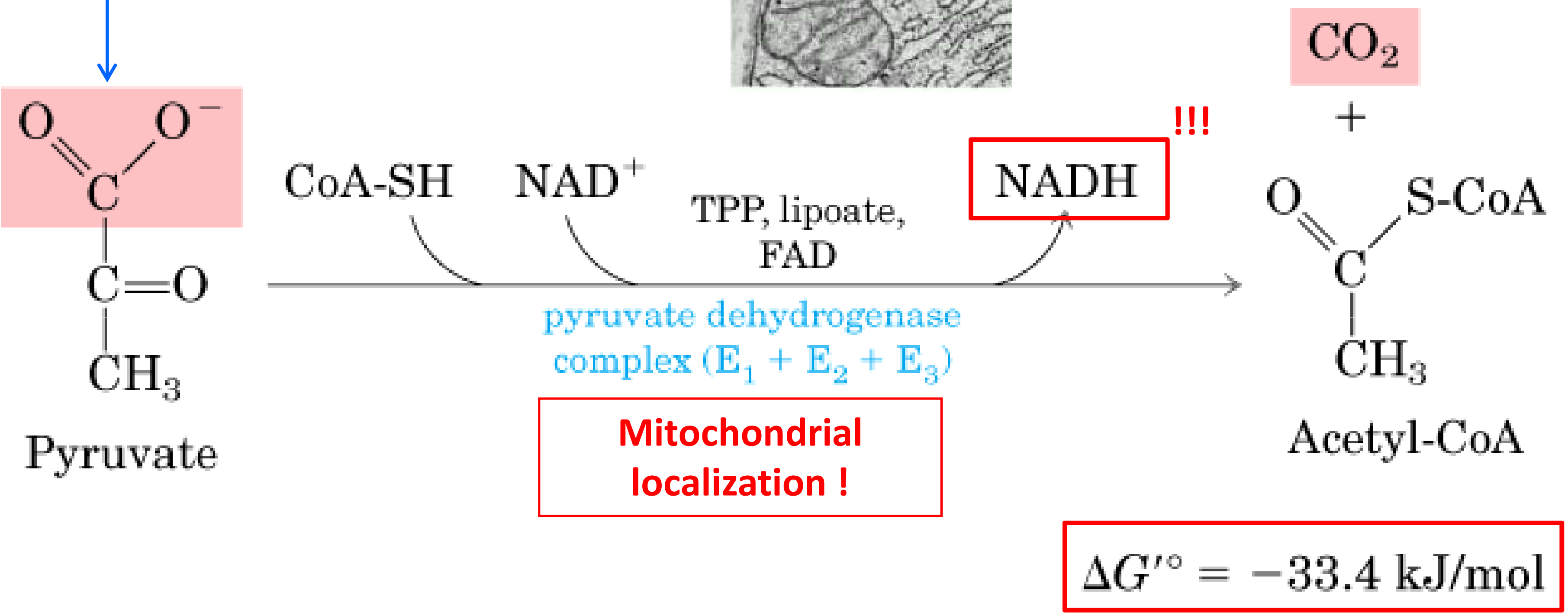
Oxidative decarboxylation of pyruvate and formation of acetyl-CoA and CO_2 catalyzed by the activity of pyruvate dehydrogenase (PDH) complex

↓

The link between glycolysis and the citric acid cycle!

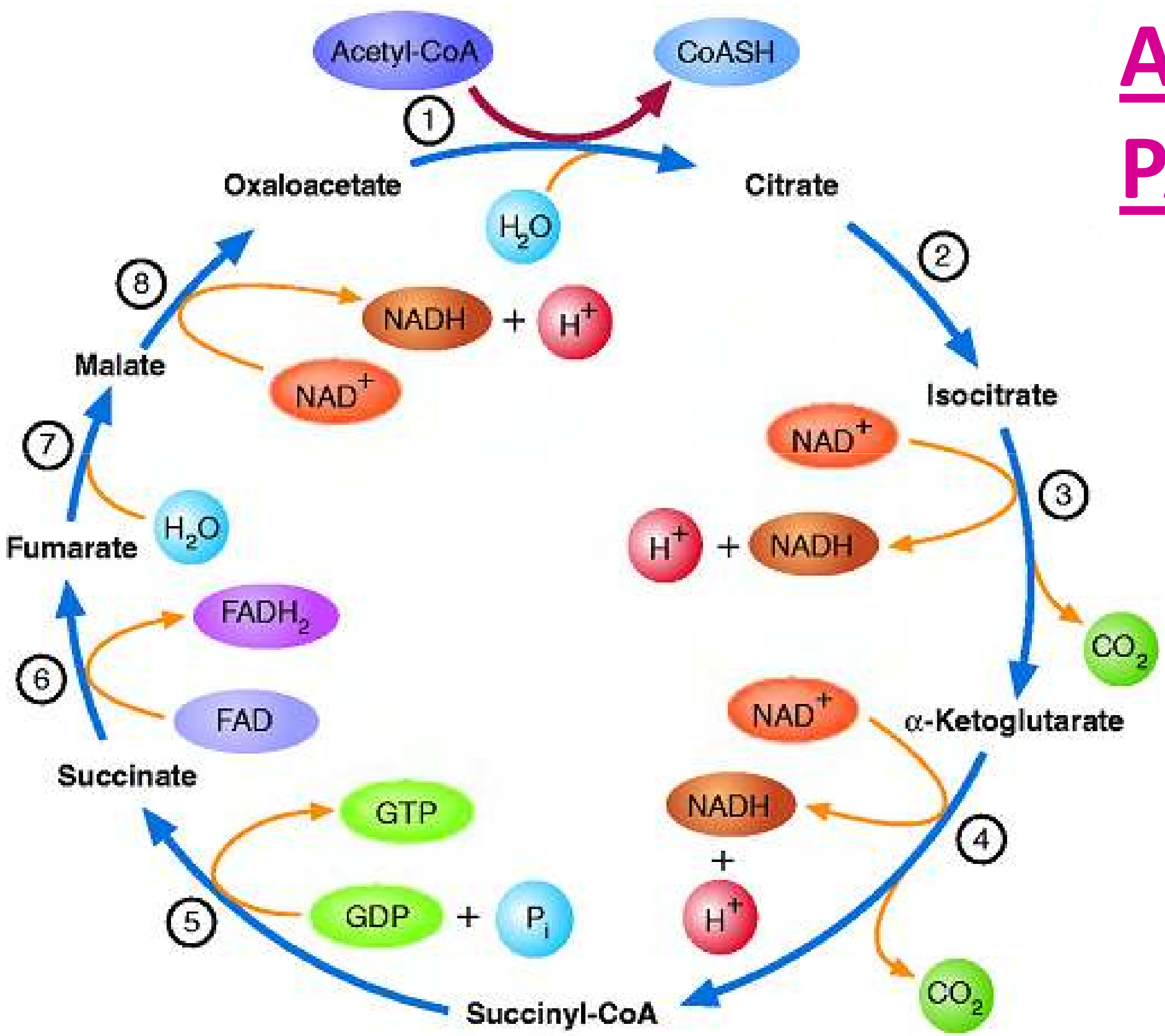
Special protein carrier transports **pyruvate** from cytosol to mitochondrial matrix in symport with H^+.





- irreversible reaction of **<u>oxidative decarboxylation</u>** → carboxyl group is removed as CO_2, acetyl group binds CoA
- NADH is reoxidized in respiratory chain

CITIC ACID CYCLE

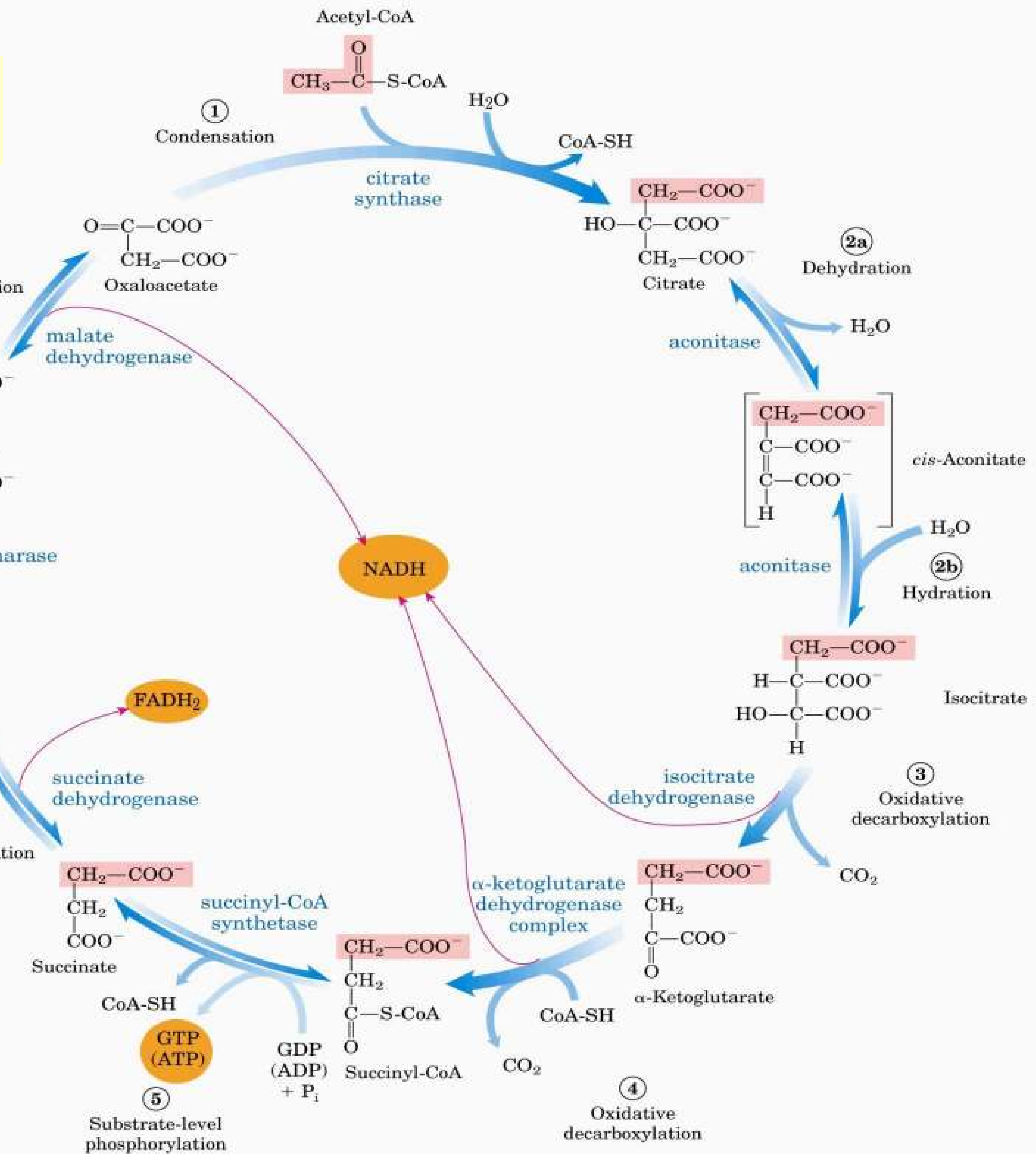


AMPHYBOLIC PATHWAY!

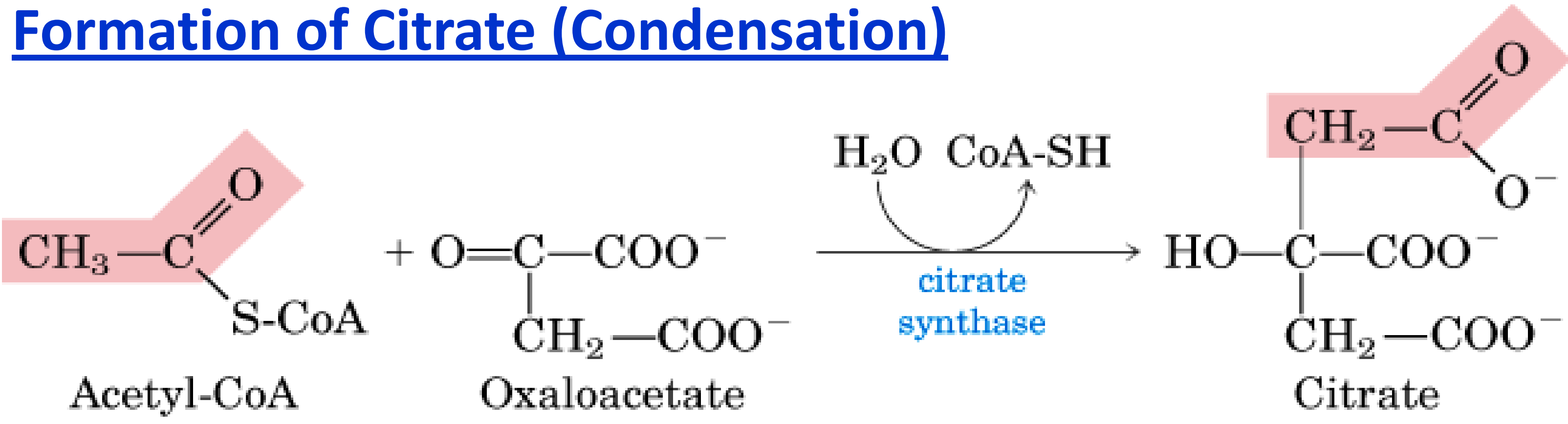
Citric acid cycle alone does not produce a lot of energy!

→ the energy of oxidation reactions is very efficiently conserved in the form of the reduced coenzymes NADH and $FADH_2$

Citric acid cycle: 8 reactions

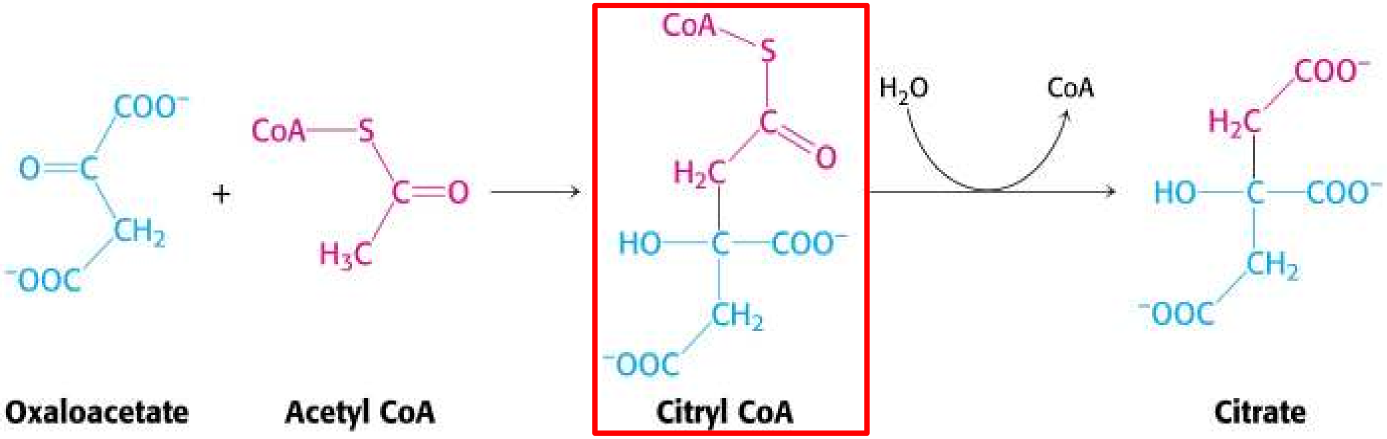


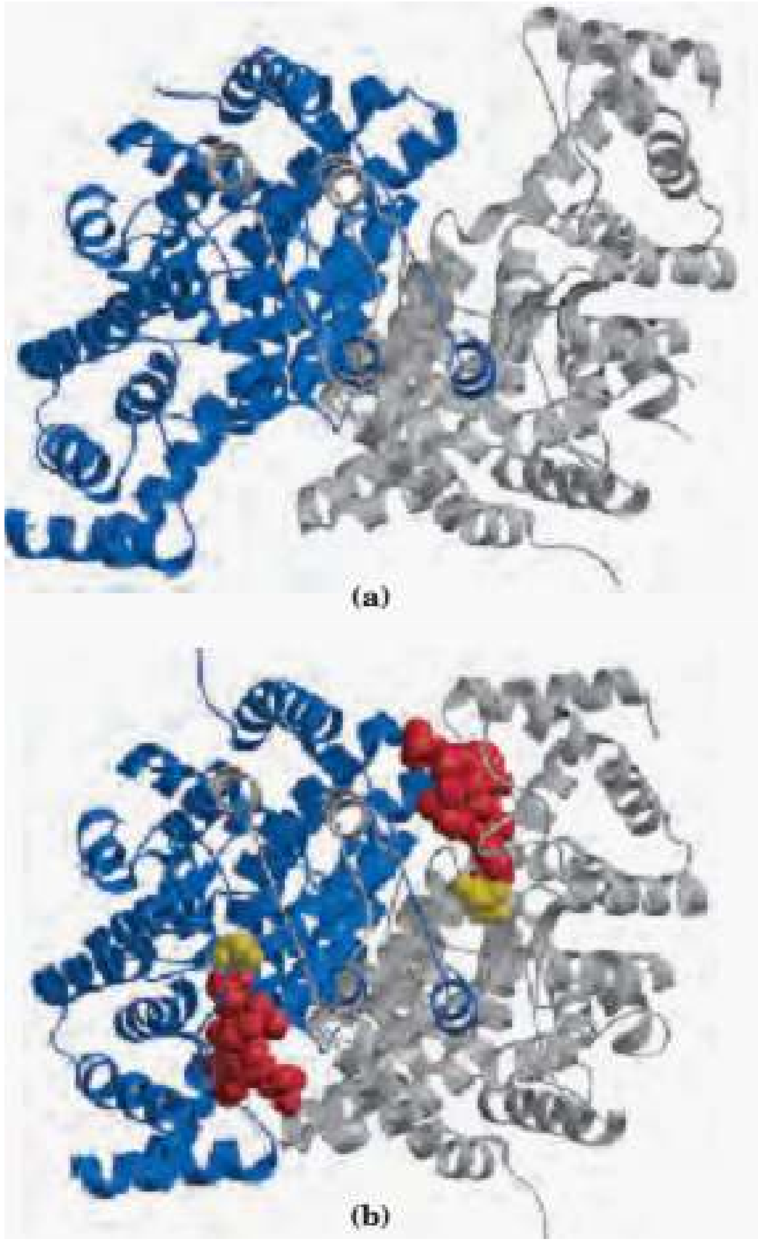
1. Formation of Citrate (Condensation)



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

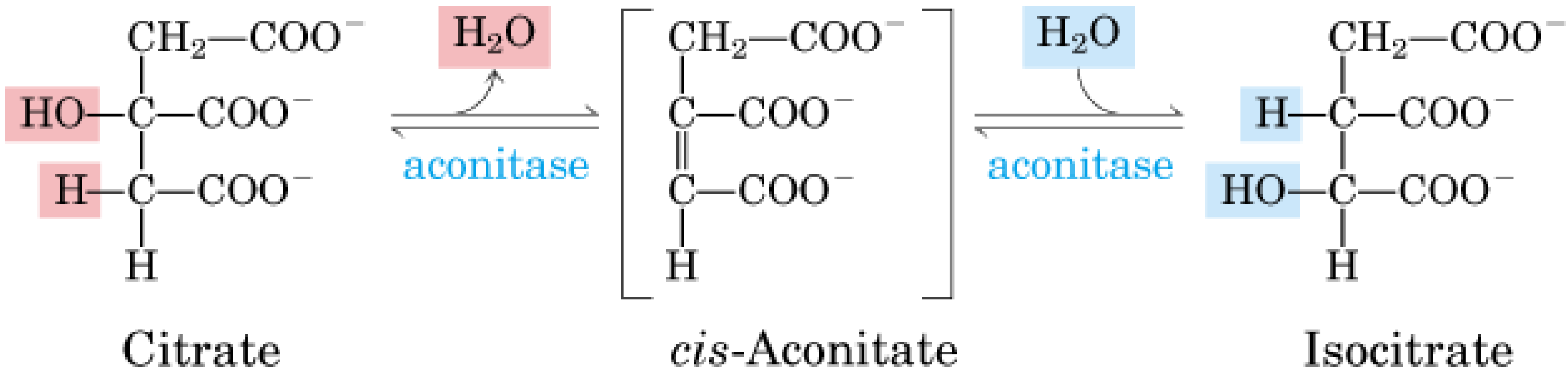
- **Aldol condensation** of acetyl-CoA and oxaloacetate
- **Irreversible** reaction catalyzed by **citrate synthase**
- **Hydrolysis** of a high-energy thioester intermediate **citryl-CoA** is very exergonic
  → it powers the synthesis of a new molecule from two precursors!
- Coenzyme A is recycled for needs of PDH activities





<u>Citrate synthase</u> exhibits sequential, ordered kinetics: **oxaloacetate** induces a major structural rearrangement leading to the creation of a binding site for **acetyl CoA**

2. Formation of Isocitrate (Dehydration and Hydration)



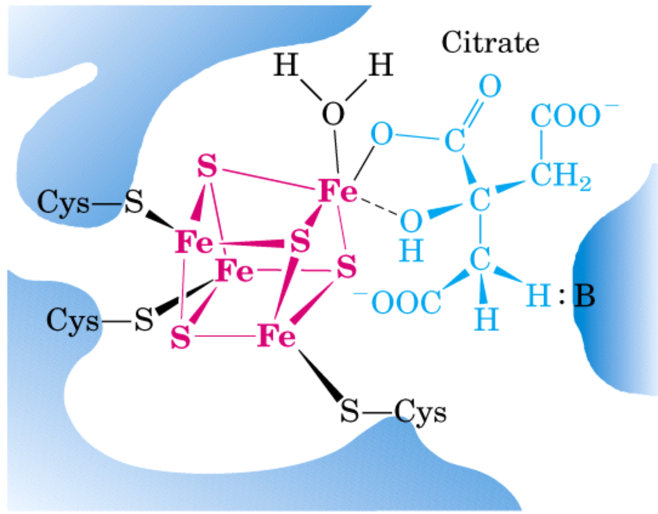
$\Delta G'^{\circ} = 13.3$ kJ/mol

- The tertiary hydroxyl group is not properly located in the citrate molecule for the oxidative decarboxylations that follow:

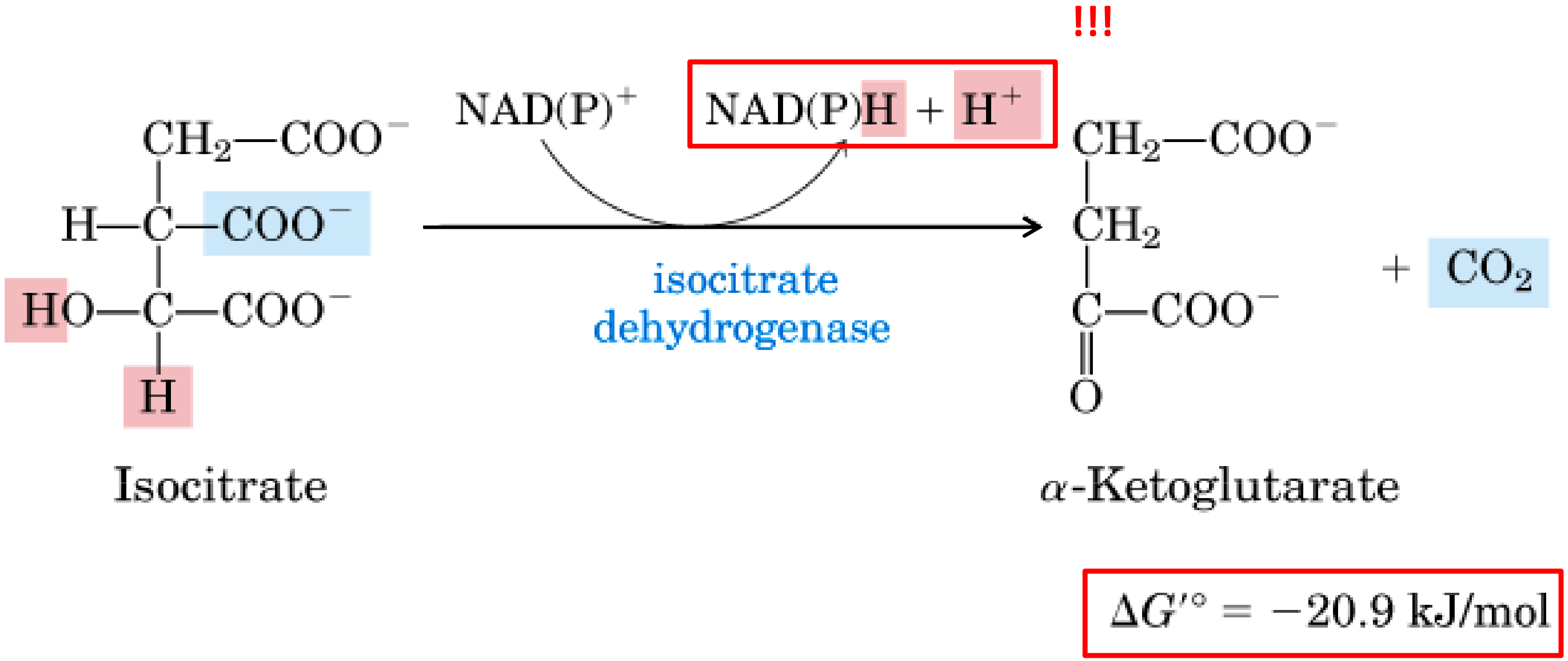
→ **isomerisation reaction** catalyzed by **aconitase**

- Intermediate is *cis*-aconitate

- Aconitase is a non-heme iron protein, contains an **iron-sulfur center**, which acts both in the binding of the substrate at the active site and in the catalytic addition or removal of H_2O

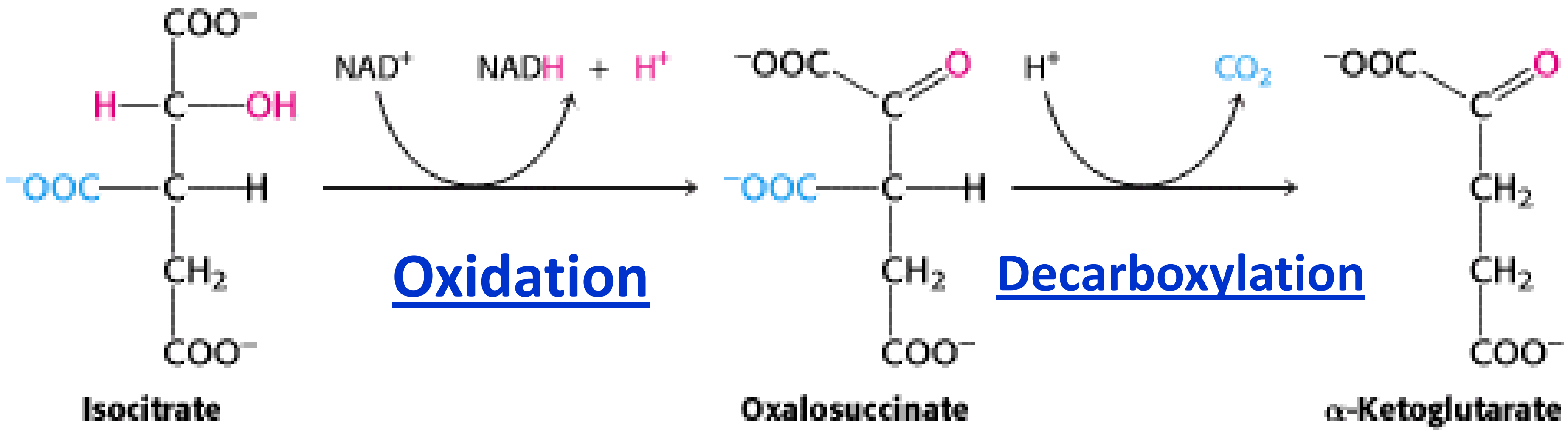


3. Oxidative Decarboxylation of Isocitrate

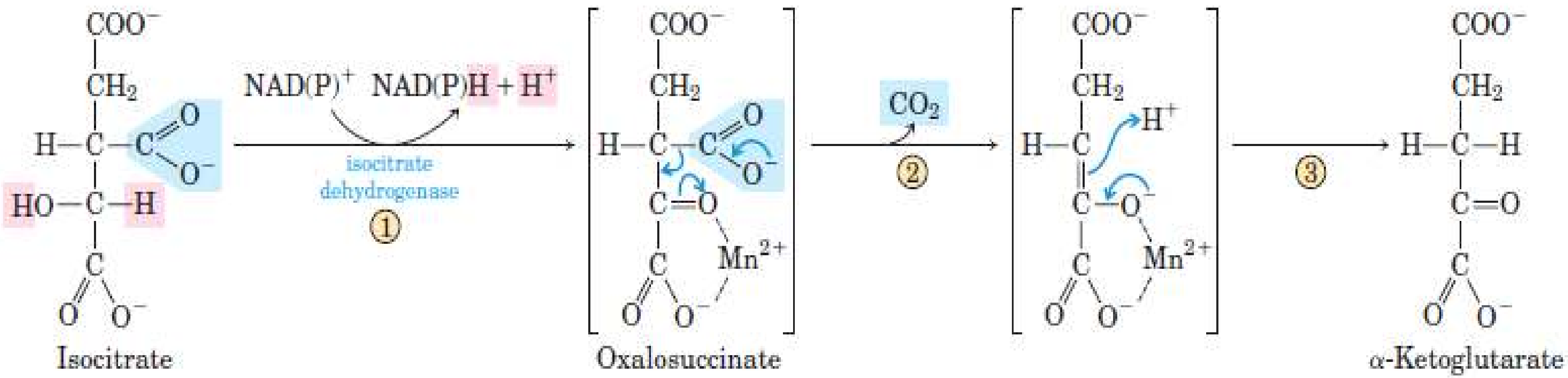


- Enzyme **isocitrate dehydrogenase** catalyzes **oxidative decarboxylation** of isocitrate and formation of α-ketoglutarate.
- **The rate of formation of α-ketoglutarate defines the overall rate of the cycle!** ***(Pace-maker reaction)***
- There are **two isoforms** using either NAD^+ or $NADP^+$ as electron acceptors.
- The intermediate in this reaction is oxalosuccinate →→→

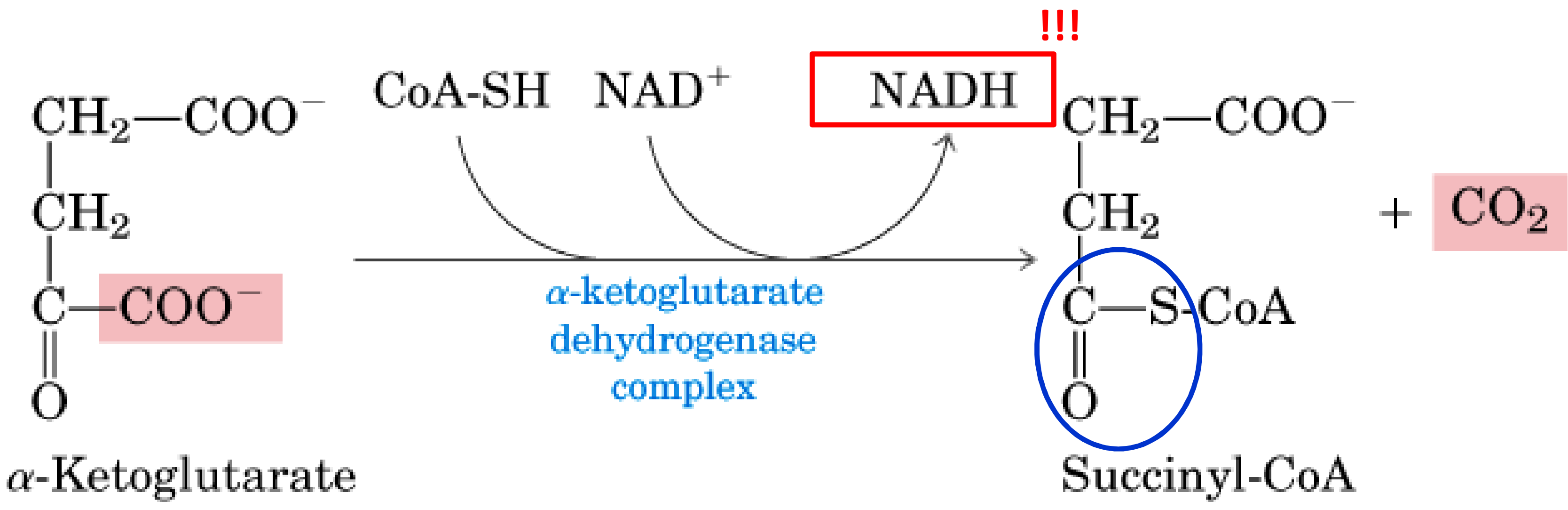
3. Oxidative Decarboxylation of Isocitrate



→ the intermediate **oxalosuccinate**, an unstable ketoacid



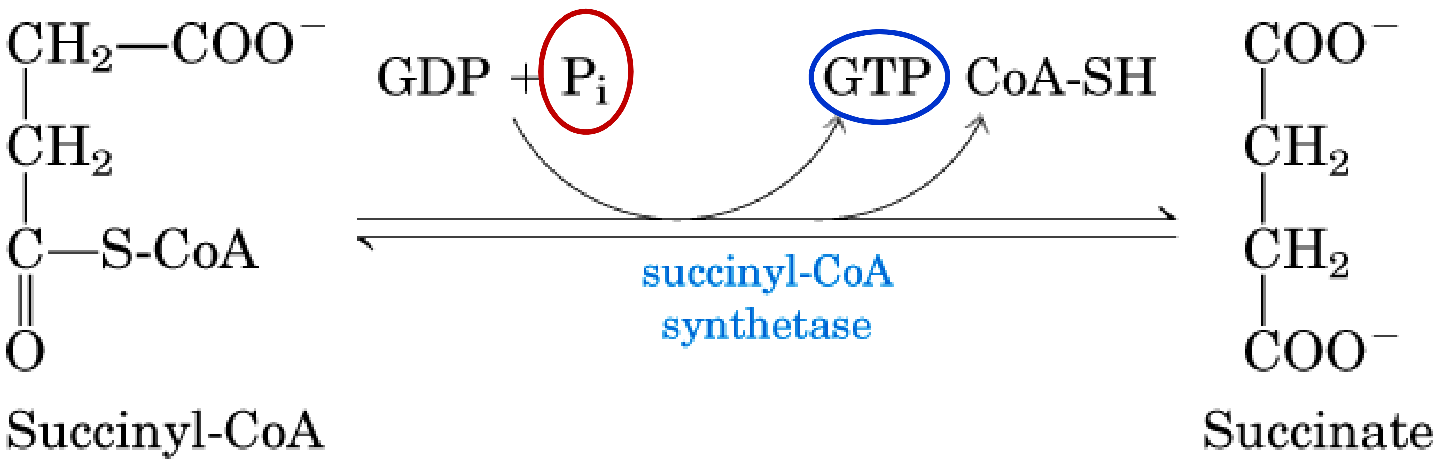
4. Oxidative Decarboxylation of α-Ketoglutarate



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

- The **energy of** α-ketoglutarate **oxidation is conserved** in succinyl-CoA **tioesther bond**!
- The oxidative decarboxylation of α-ketoglutarate closely resembles that of pyruvate, also an α-ketoacid (identical type of the reaction)
- The complex that catalyzes the **oxidative decarboxylation** of α-ketoglutarate → **α-ketoglutarate dehydrogenase is homologous** to the PDH complex:
    - it includes three enzymes, homologous to E1, E2, and E3 of the PDH complex, as well as enzyme-bound TPP, bound lipoate, FAD, NAD^+, and coenzyme A
- Reaction is **exergonic**, irreversible in physiol. conditions.

5. Substrate-Level Phosphorylation reaction (SuccinateFormation)



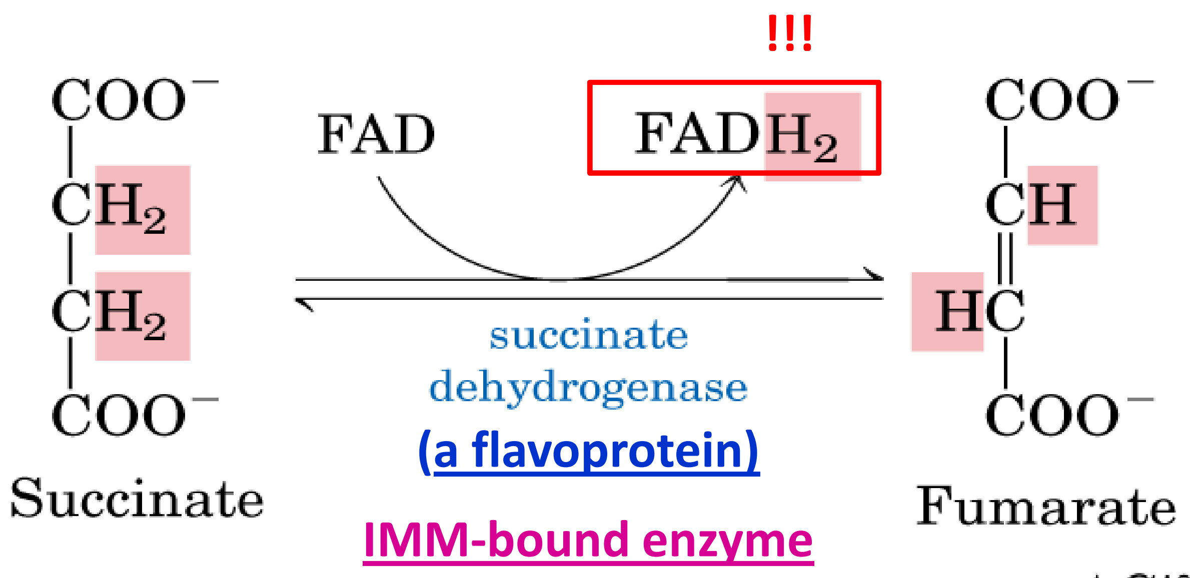
$\Delta G'^{\circ}_{hydrolysis} \approx -36$ kJ/mol

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

- Free energy of the **hydrolysis of thioester** succinyl-CoA is conserved in either GTP or ATP (used to drive the synthesis of a phosphoanhydride bond).
- Reaction is catalyzed by **succinyl-CoA synthetase** (succinic thiokinase):
  → 2 isoenzymes with different specificity for either ADP or GDP
- Reversible exchange GTP/ATP is catalyzed by **nucleoside diphosphate kinase**.

$$\mathrm{GTP} + \mathrm{ADP} \rightleftharpoons \mathrm{GDP} + \mathrm{ATP} \qquad \Delta G'^{\circ} = 0 \text{ kJ/mol}$$

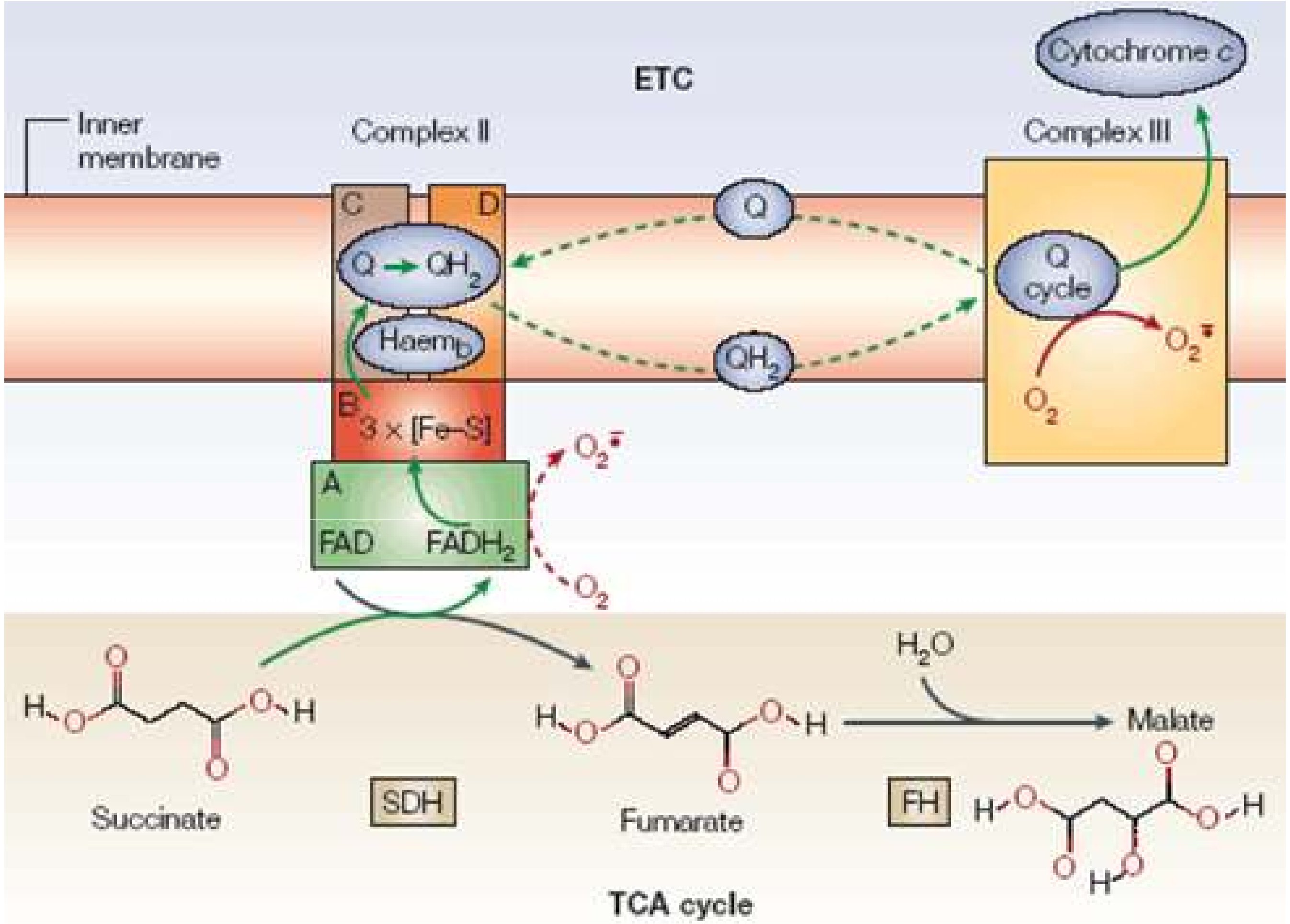
6. Succinate Dehydrogenation (oxidation)



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

- Succinate is oxidized to **fumarate** by the flavoprotein succinate dehydrogenase
- **FAD** is the **e^- acceptor** (hydrogen acceptor) in this reaction, as the cofactor covalently bound to the enzyme
- **The only enzyme of the citric acid cycle that is tightly bound to the inner mitochondrial membrane (IMM)!**
    - $FADH_2$ does not dissociate from the enzyme (in contrast to NADH produced in other oxidation-reduction reactions);
    - rather, two electrons are transferred from $FADH_2$ **directly** to iron-sulfur clusters of the enzyme

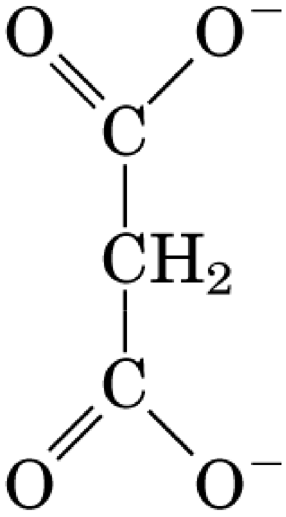
Succinate dehydrogenase or Complex II (succinate-ubiquinone oxidoreductase) of the electron-transport chain



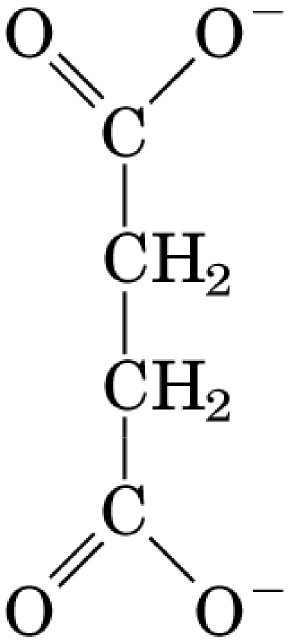
- bound to the inner mitochondrial membrane
- contains 3 different iron-sulfur clusters and <u>covalently bound FAD</u>

- electrons are directly transported from $FADH_2$ to coenzyme Q of the respiratory chain
  – *succinate dehydrogenase (Complex II) is directly associated with the electron-transport chain,* ***the link between the citric acid cycle and ATP formation***

Illustration source: Nature Rev Cancer, 2005, Nature Publishing Group, www.medscape.com



Malonate

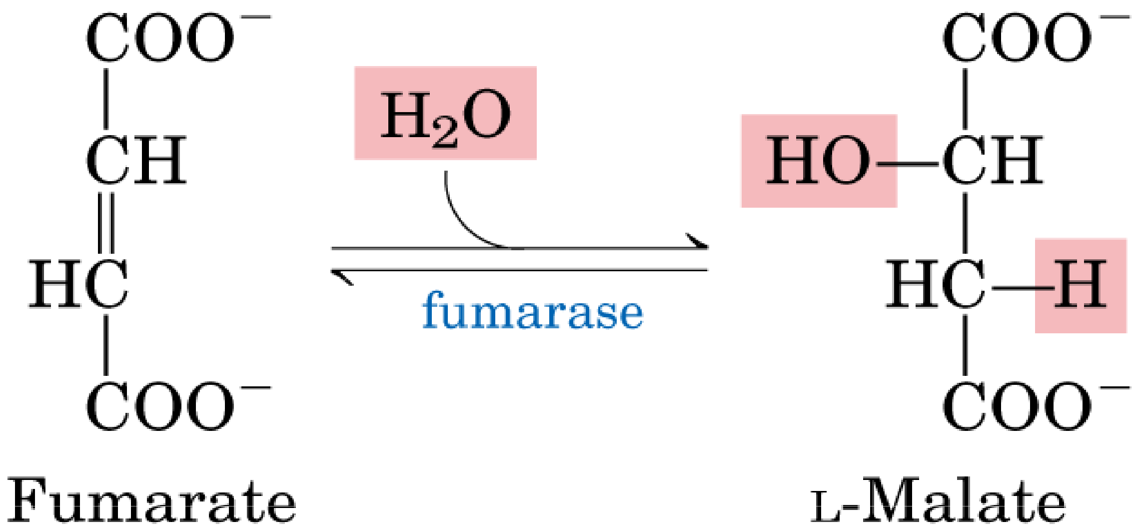


Succinate

Malonate, an analog of succinate not normally present in cells, is a strong **competitive inhibitor** of **succinate dehydrogenase**.

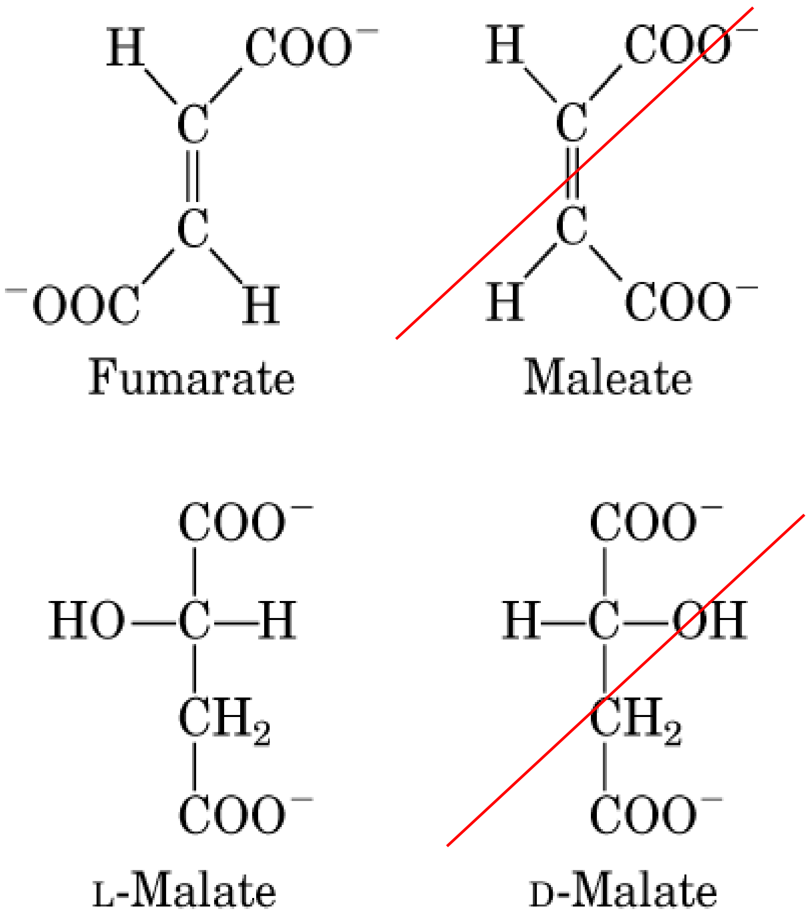
Its addition to mitochondria blocks the activity of the citric acid cycle.

7. Hydration of Fumarate to Malate



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

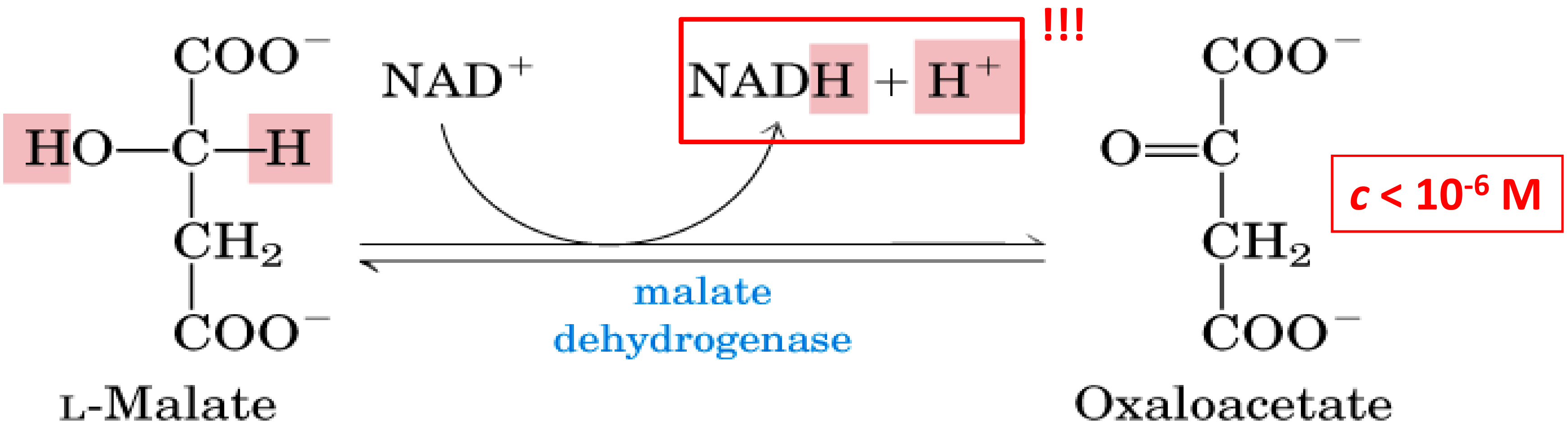
- The reversible hydration of fumarate to **L-malate** is catalyzed by fumarase *(formally **fumarate hydratase**).*
- This enzyme is highly stereospecific; **it catalyzes hydration of the *trans* double bond of fumarate** (exclusively).



Fumarase is highly stereospecific for substrates **fumarate** and **L-malate.**

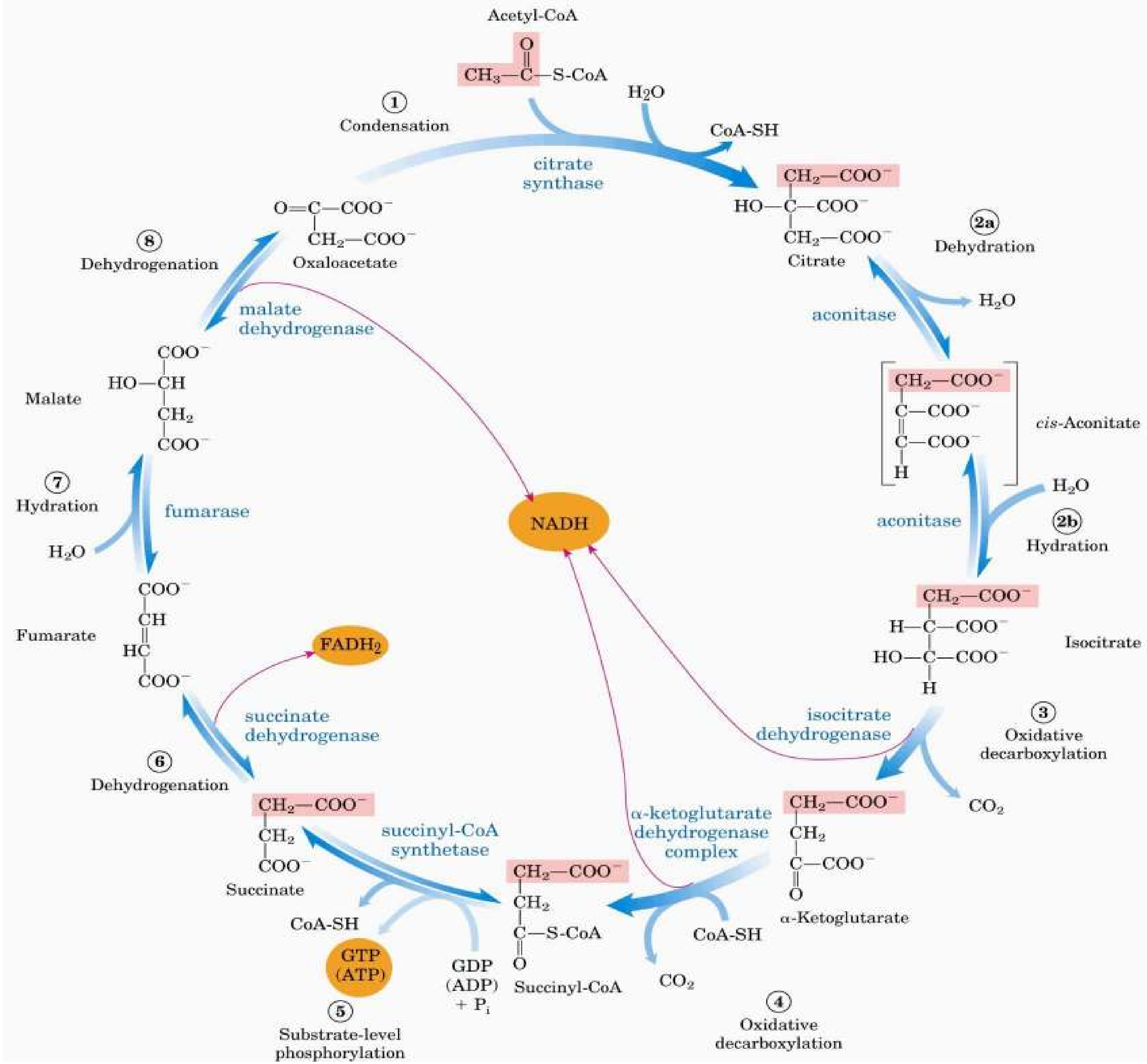
It catalyzes hydration of the *trans* double bond of fumarate, and **not** the *cis* double bond of maleate, the *cis* isomer of fumarate.

8. Oxidation of Malate to Oxaloacetate



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

- NAD-linked **L-malate dehydrogenase** catalyzes **the oxidation** of L-malate to oxaloacetate
- under standard thermodinamic contitions, the equilibrium of this reaction lies far to the left, but:
  - oxaloacetate is continually removed by the **highly exergonic citrate synthase reaction** → this keeps the concentration of oxaloacetate in the cell extremely low ($<10^{-6}$ M), pulling the malate dehydrogenase reaction toward the formation of oxaloacetate


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

The Net reaction of Citric Acid Cycle:

Acetyl-CoA + 3 NAD^+ + FAD + GDP + P_i + 2 H_2O → CoA-SH + 3 NADH + $FADH_2$ + GTP + 2 CO_2 + $3H^+$

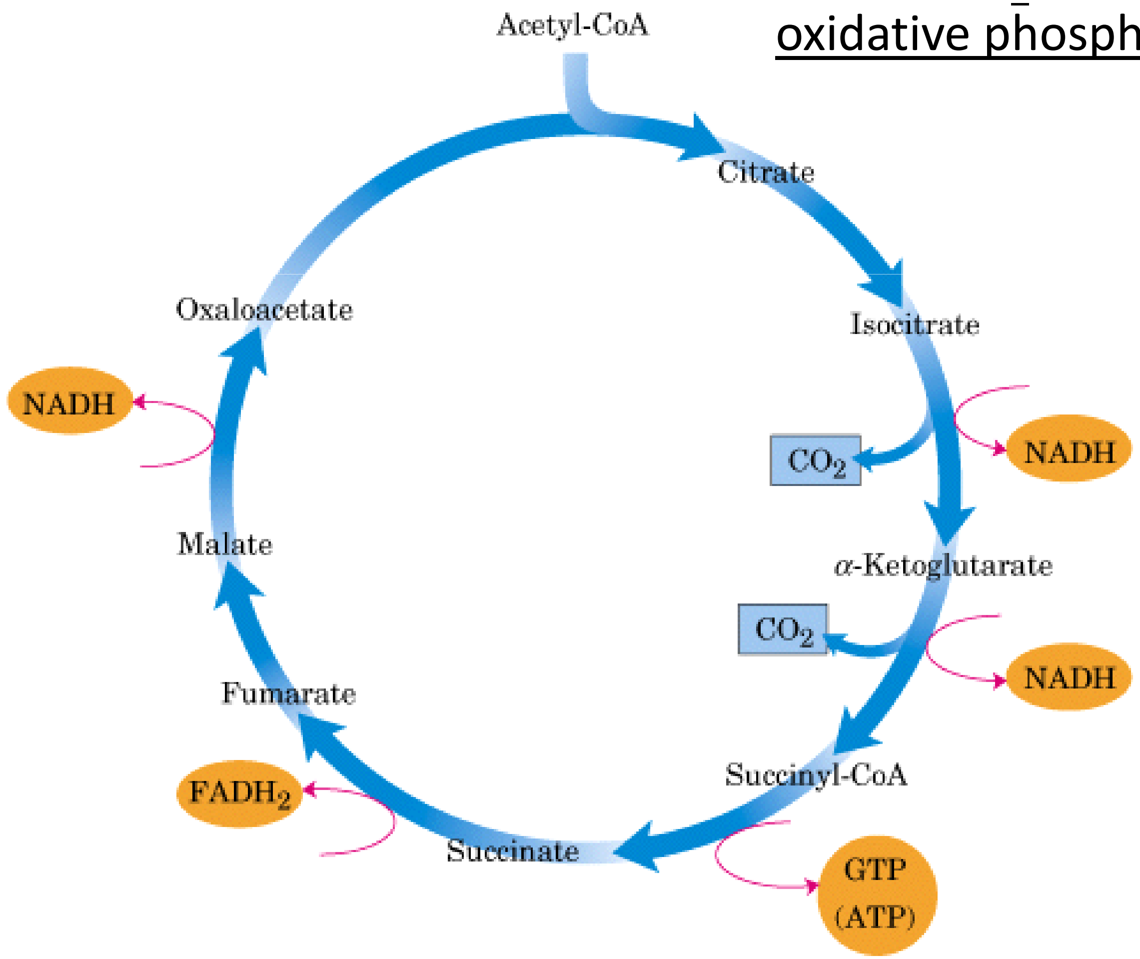
TABLE 17.2 Citric acid cycle

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.

Oxidation energy is very efficiently conserved in citric acid cycle!

- **Transfer** of **two electrons** from NADH to O_2 in ETC drives the formation of 2.5 ATP in oxidative phosphorylation, and transfer of **two electrons** from $FADH_2$ to O_2 (in ETC) yields 1.5 ATP in oxidative phosphorylation



1 NADH ($2e^-$) → 2.5 ATP

1 $FADH_2$ ($2e^-$) → 1.5 ATP

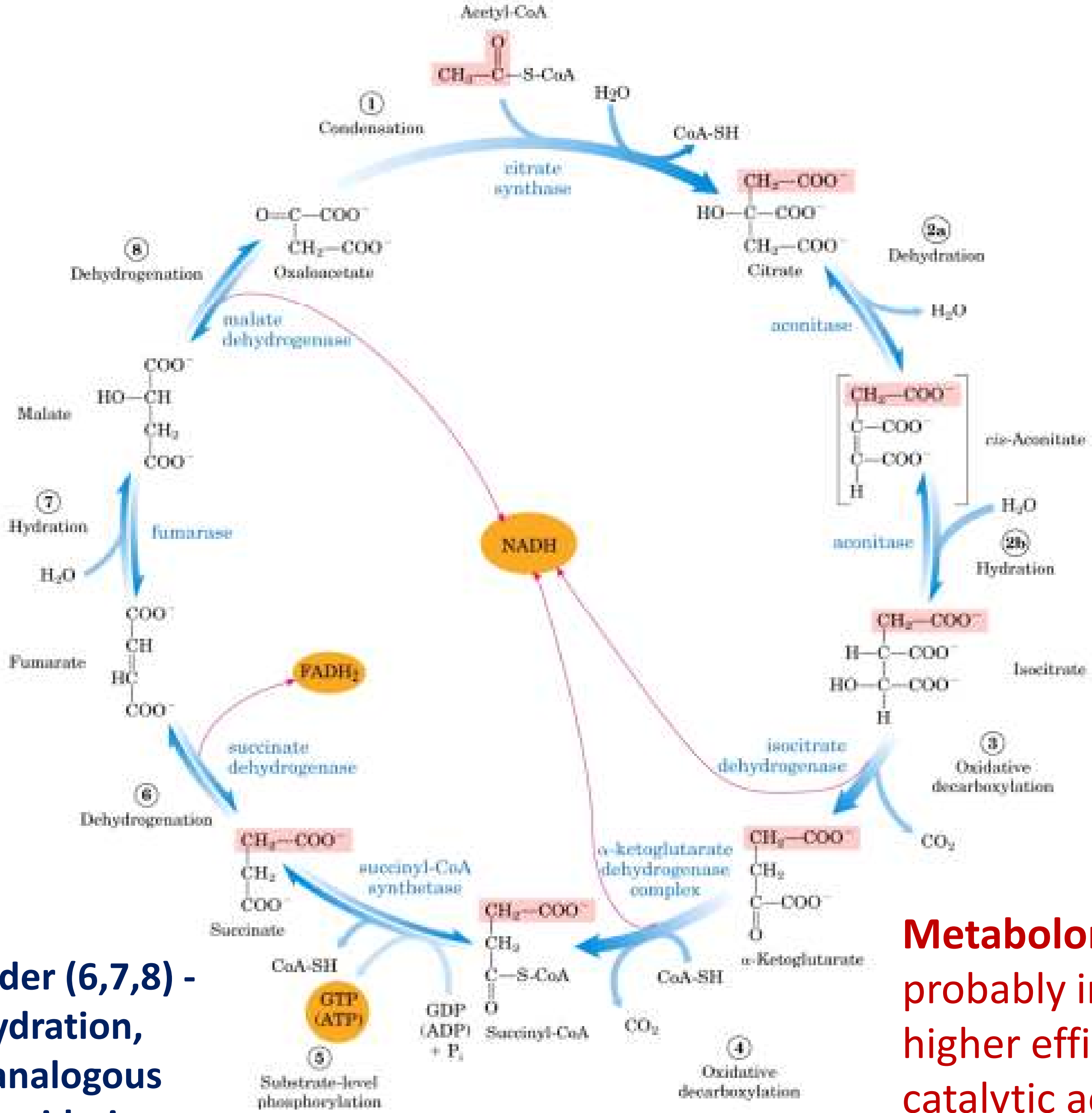
How many ATP molecules are produced by complete oxidation of 1 molecule of acetyl-CoA?

10 ATP

TABLE 19–5 ATP Yield from Complete Oxidation of Glucose

Process	*Direct product*	*Final ATP*
Glycolysis	2 NADH (cytosolic)	3 or 5*
	2 ATP	2
Pyruvate oxidation (two per glucose)	2 NADH (mitochondrial matrix)	5
Acetyl-CoA oxidation in citric acid cycle	6 NADH (mitochondrial matrix)	15
(two per glucose)	2 $FADH_2$	3
	2 ATP or 2 GTP	2
Total yield per glucose		30 or 32

*** The number depends on which shuttle system transfers reducing equivalents into the mitochondrion.**

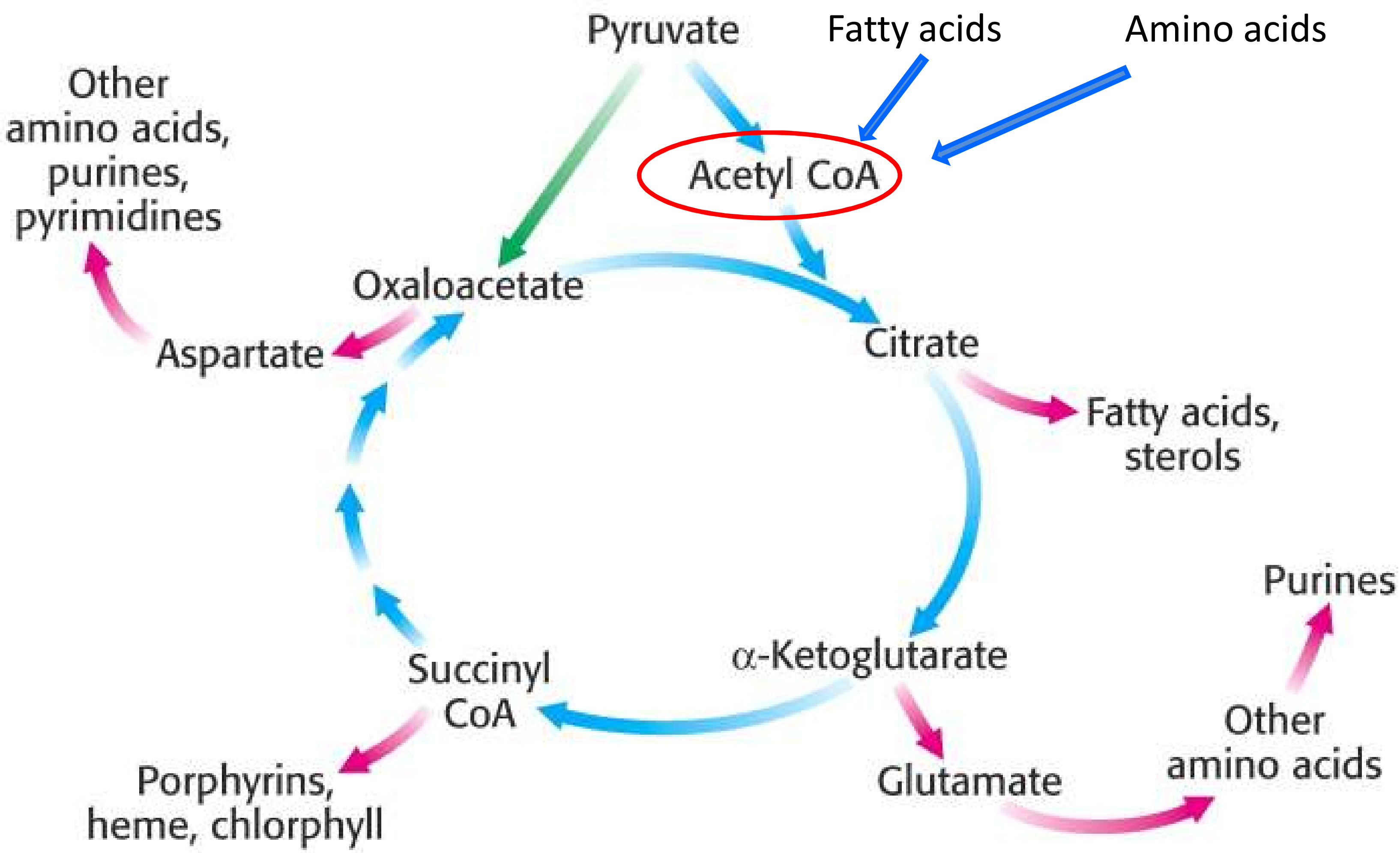


Reactions order (6,7,8) - oxidation, hydration, oxidation – analogous to fatty acid oxidation reactions!

Metabolon: Enzymes are probably interconnected - higher efficiency of catalytic activity (substrate channeling)

CITRIC ACID CYCLE IS AMPHYBOLIC -
roles in catabolism and anabolism of biomolecules

Citric acid cycle intermediates are precursors (intermediates) for biosynthesis of important biomolecules!



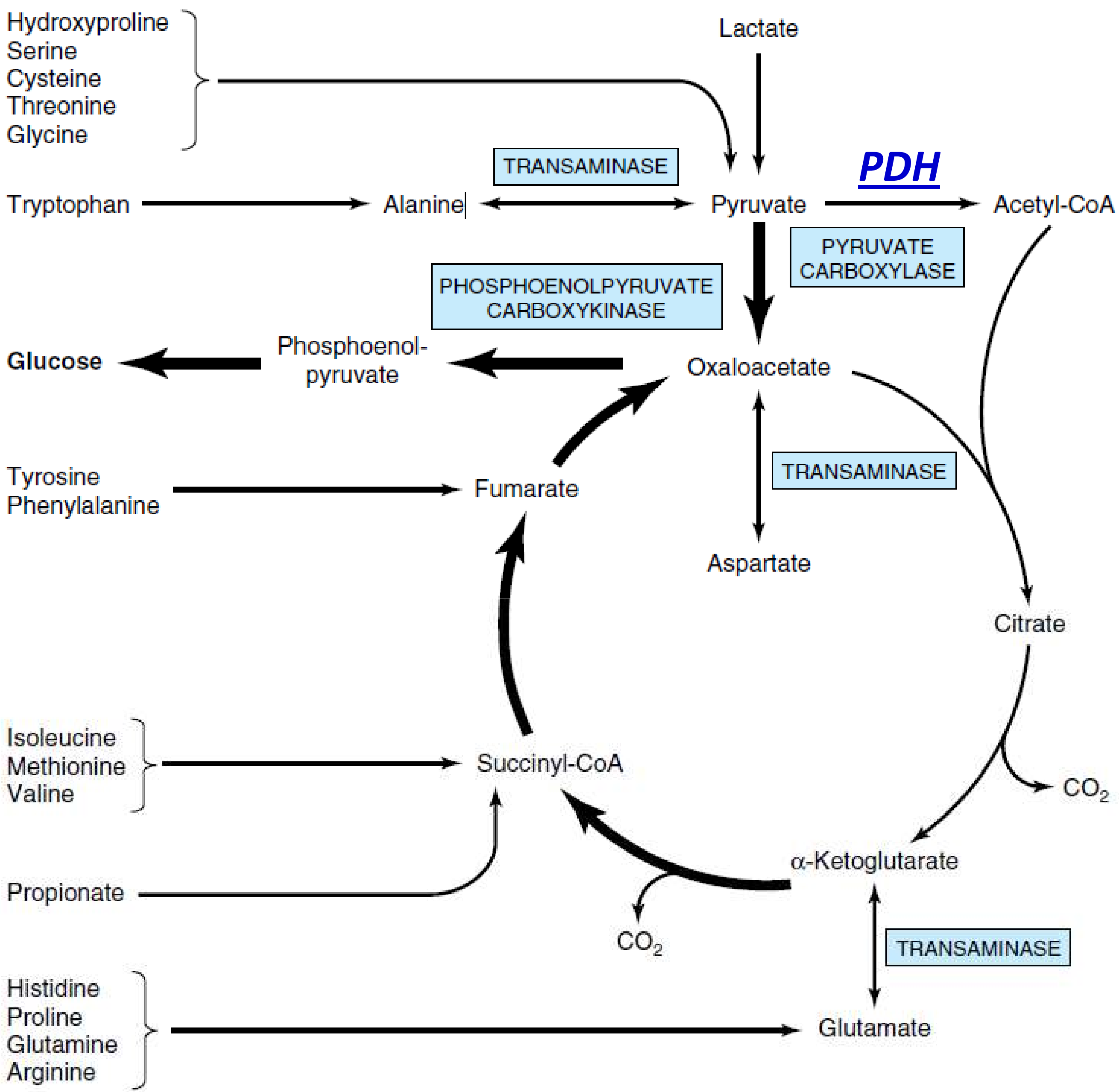


Figure 16–4. Involvement of the citric acid cycle in transamination and gluconeogenesis. The bold arrows indicate the main pathway of gluconeogenesis.

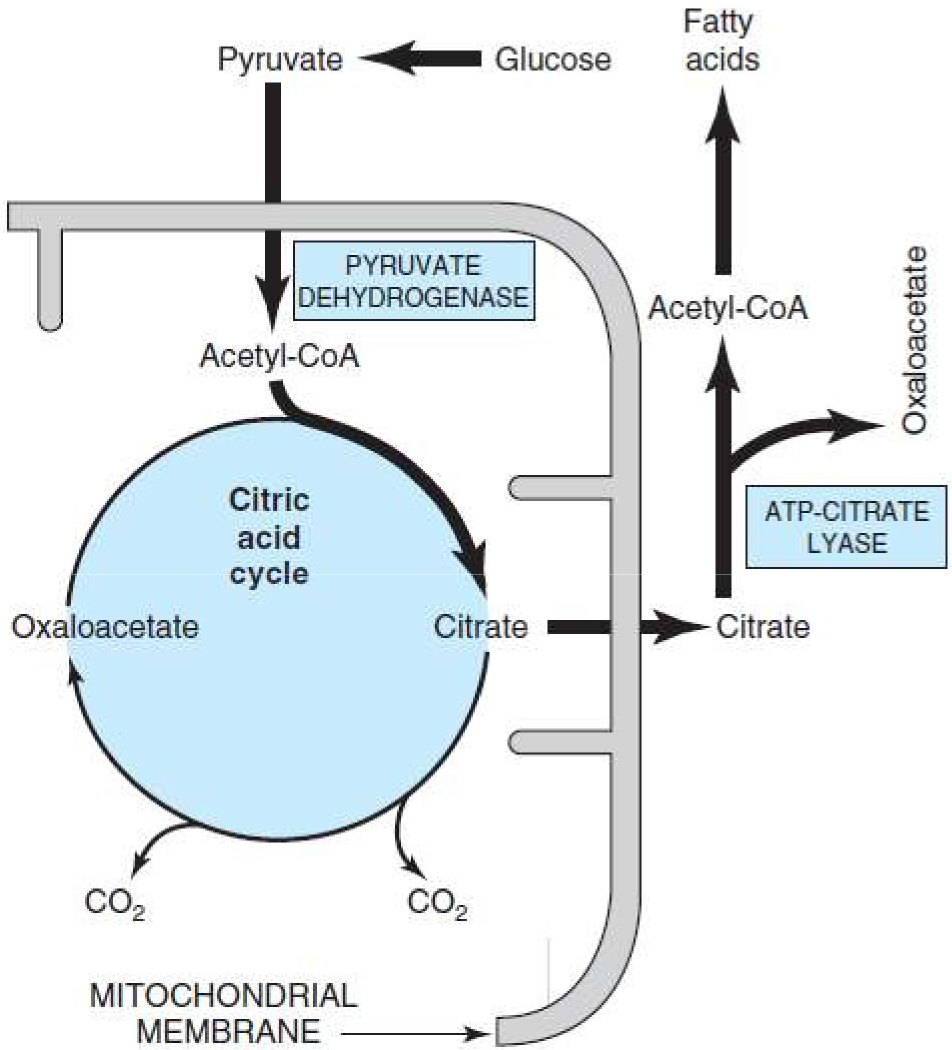


Figure 16–5. Participation of the citric acid cycle in fatty acid synthesis from glucose. See also Figure 21–5.

Concentrations of the citric acid cycle intermediates remain constant!

Anaplerotic* reactions replenish citric acid cycle intermediates!

- intermediates must be replenished if any are drawn off for biosyntheses

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

**anaplerotic - to "fill up"* (*Greek* ἀνά = 'up' and πληρόω = 'to make full, to complete')

Anaplerotic reactions

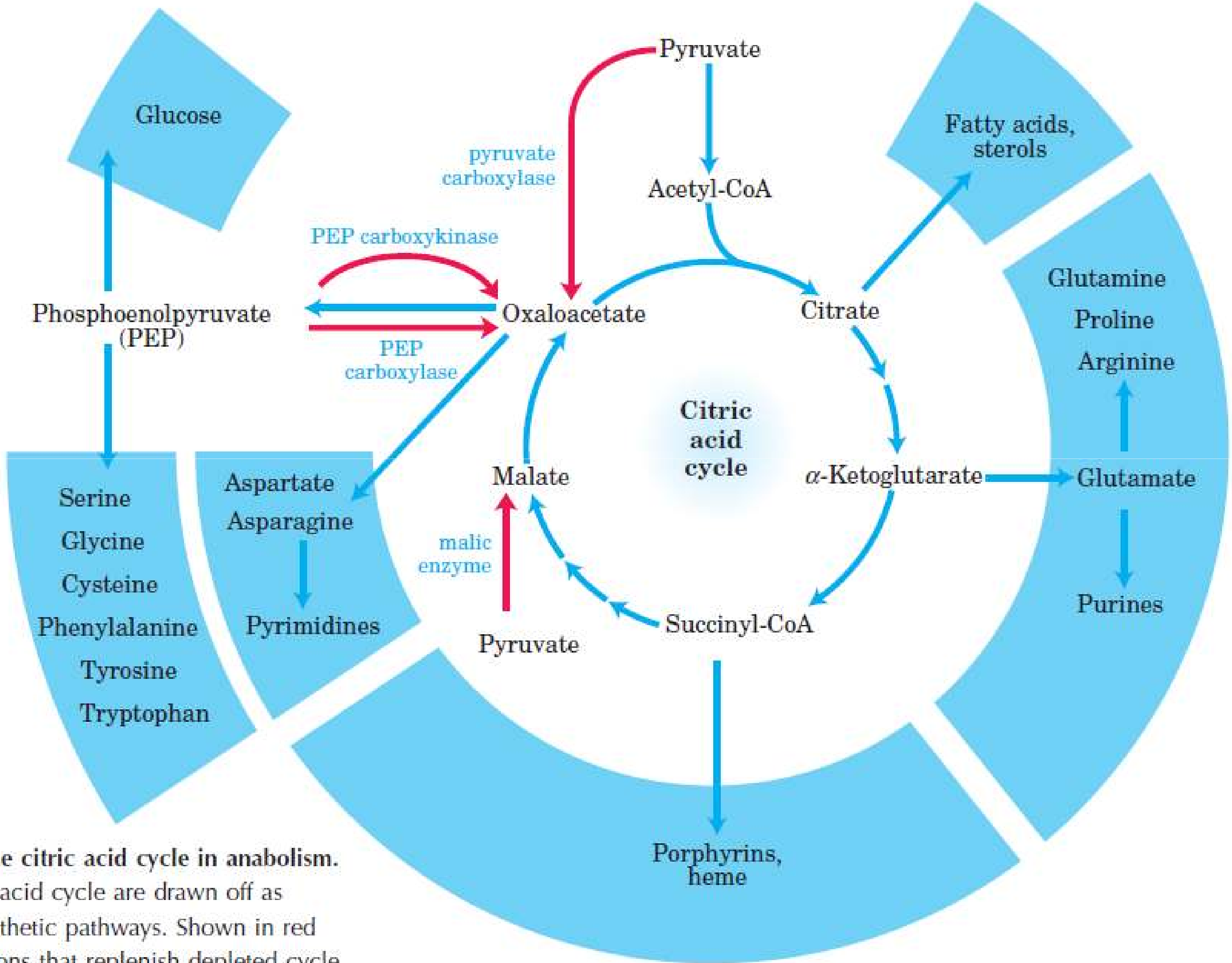
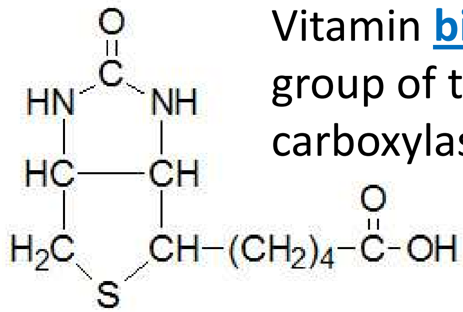


FIGURE 16–15 Role of the citric acid cycle in anabolism. Intermediates of the citric acid cycle are drawn off as precursors in many biosynthetic pathways. Shown in red are four anaplerotic reactions that replenish depleted cycle intermediates (see Table 16–2).

The most important anaplerotic reaction in mammalian liver and kidneys is reversible **pyruvate carboxylation** catalyzed by **pyruvate carboxylase**.

$$\text{Pyruvate} + HCO_3^- + \text{ATP} \xrightleftharpoons{\text{pyruvate carboxylase}} \text{oxaloacetate} + \text{ADP} + P_i$$

- **pyruvate carboxylase is a regulatory enzyme – acetyl-CoA is its alosteric activator!**
- the enzyme is virtually inactive in the absence of acetyl-CoA
- **acetyl-CoA** signifies the need for more oxaloacetate:

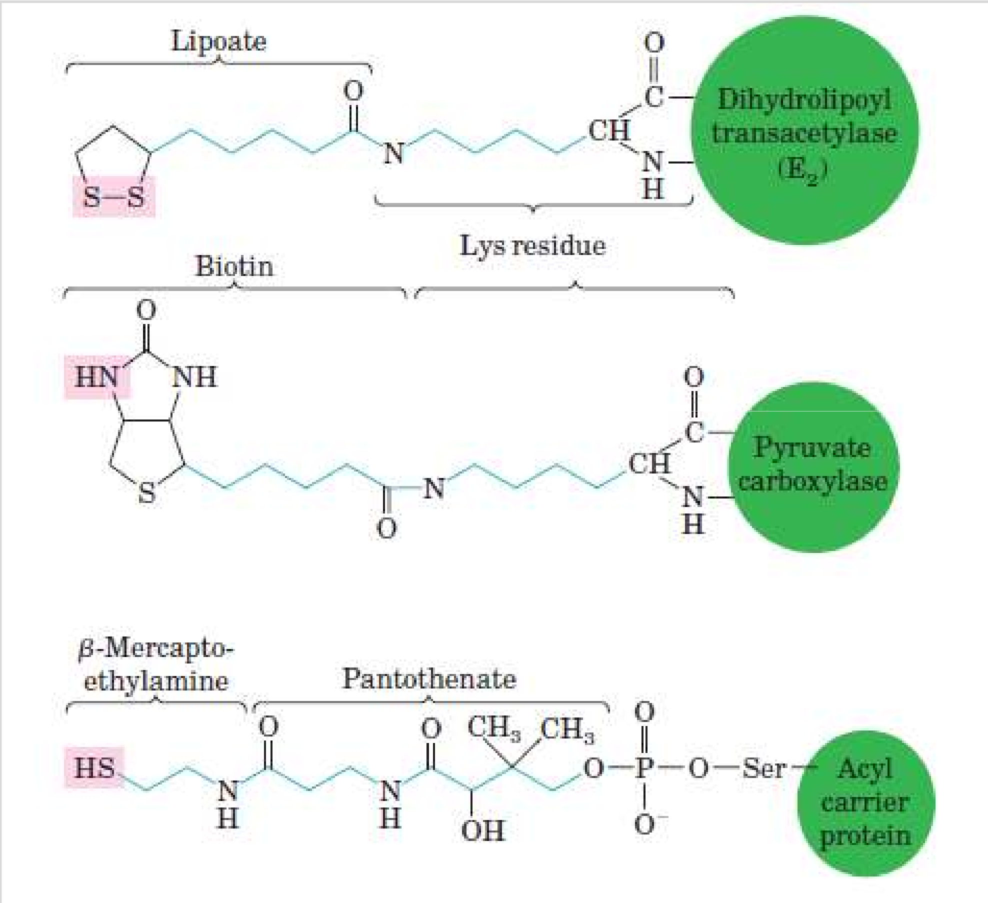


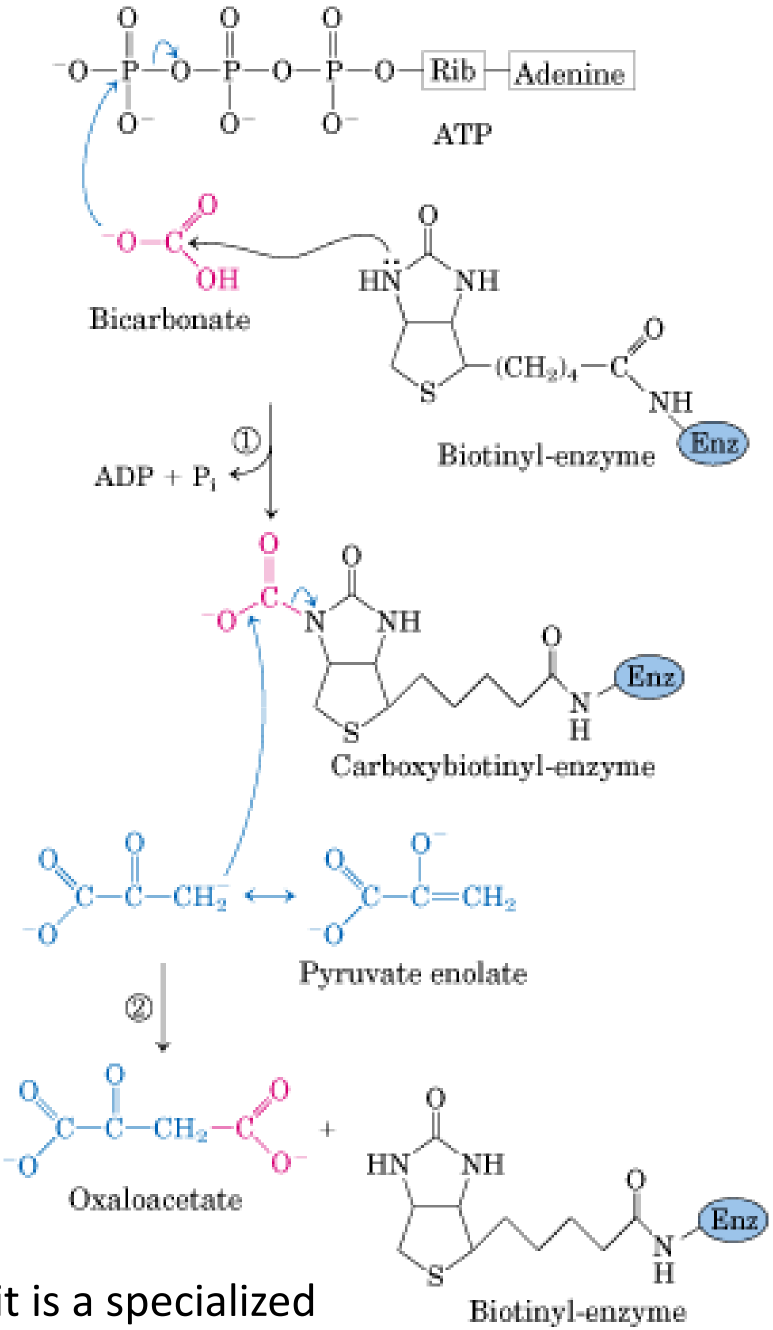
Vitamin **biotin** is prosthetic group of the pyruvate carboxylase.

Why patients with rare pyruvate carboxylase defficiency have high concentration of lactate in urine (lactic aciduria)?

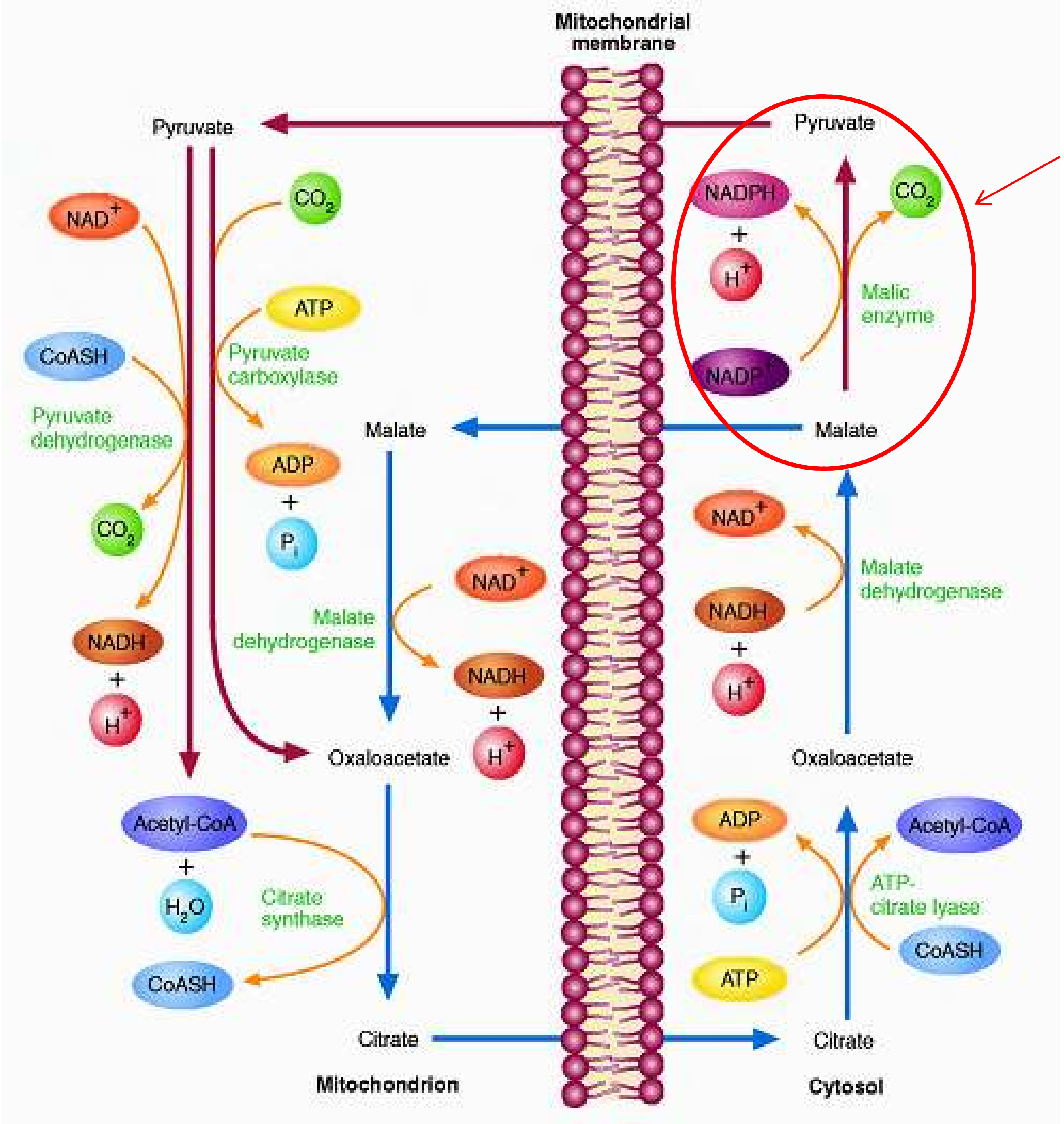
Pyruvate carboxylase **cofactors**

lipoate, **biotin** and **mercaptoethylamin- pantothenate** act as intermediate carriers from one active site to another in the enzyme complex.





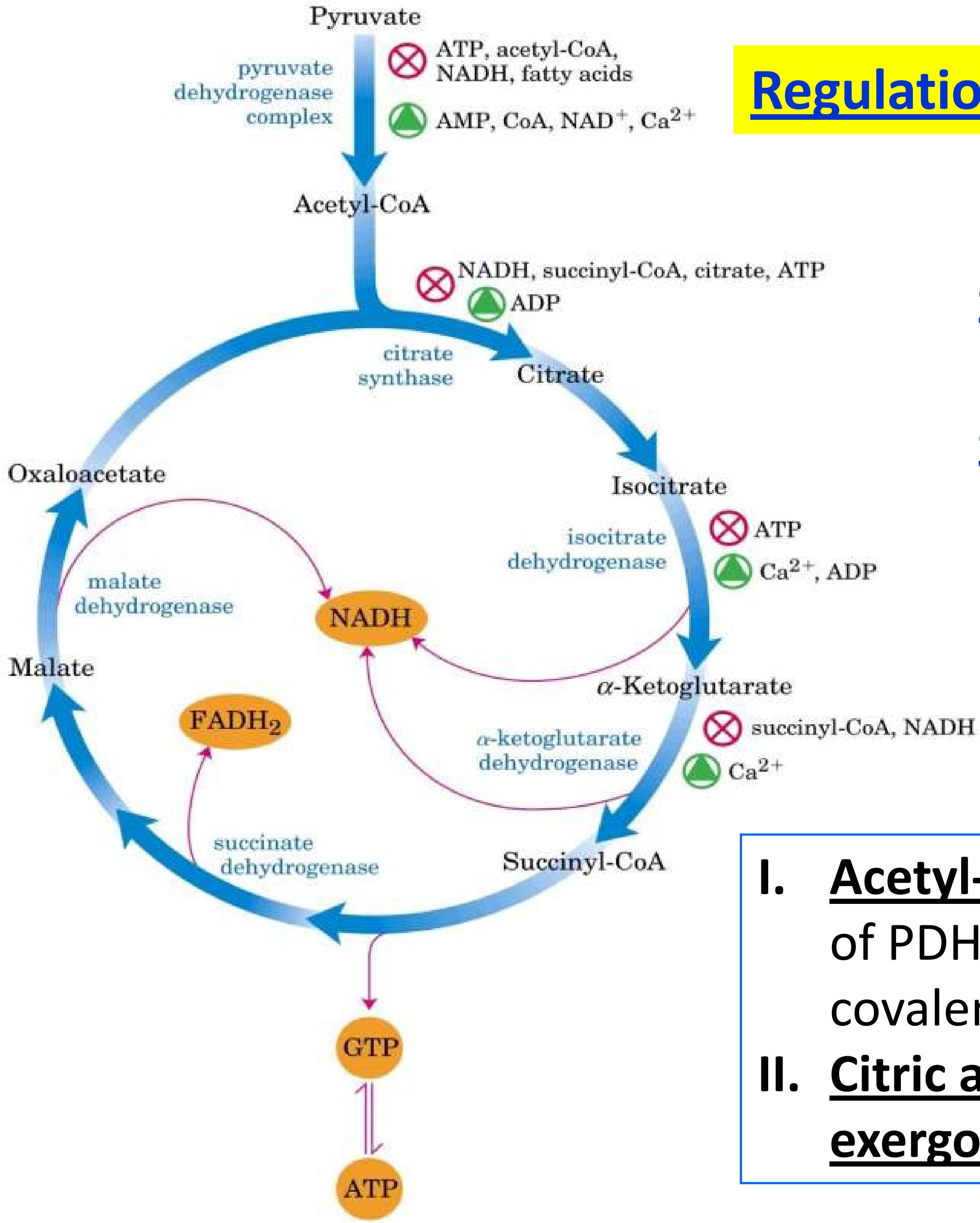
Biotin plays a key role in many carboxylation reactions - it is a specialized **carrier of one-carbon groups** in their most oxidized form: CO_2



Malic enzyme - **bound to NADP$^+$**

- catalyzes anaplerotic reactions!
- second important role - cell supply with NADPH for fatty acid biosynthesis

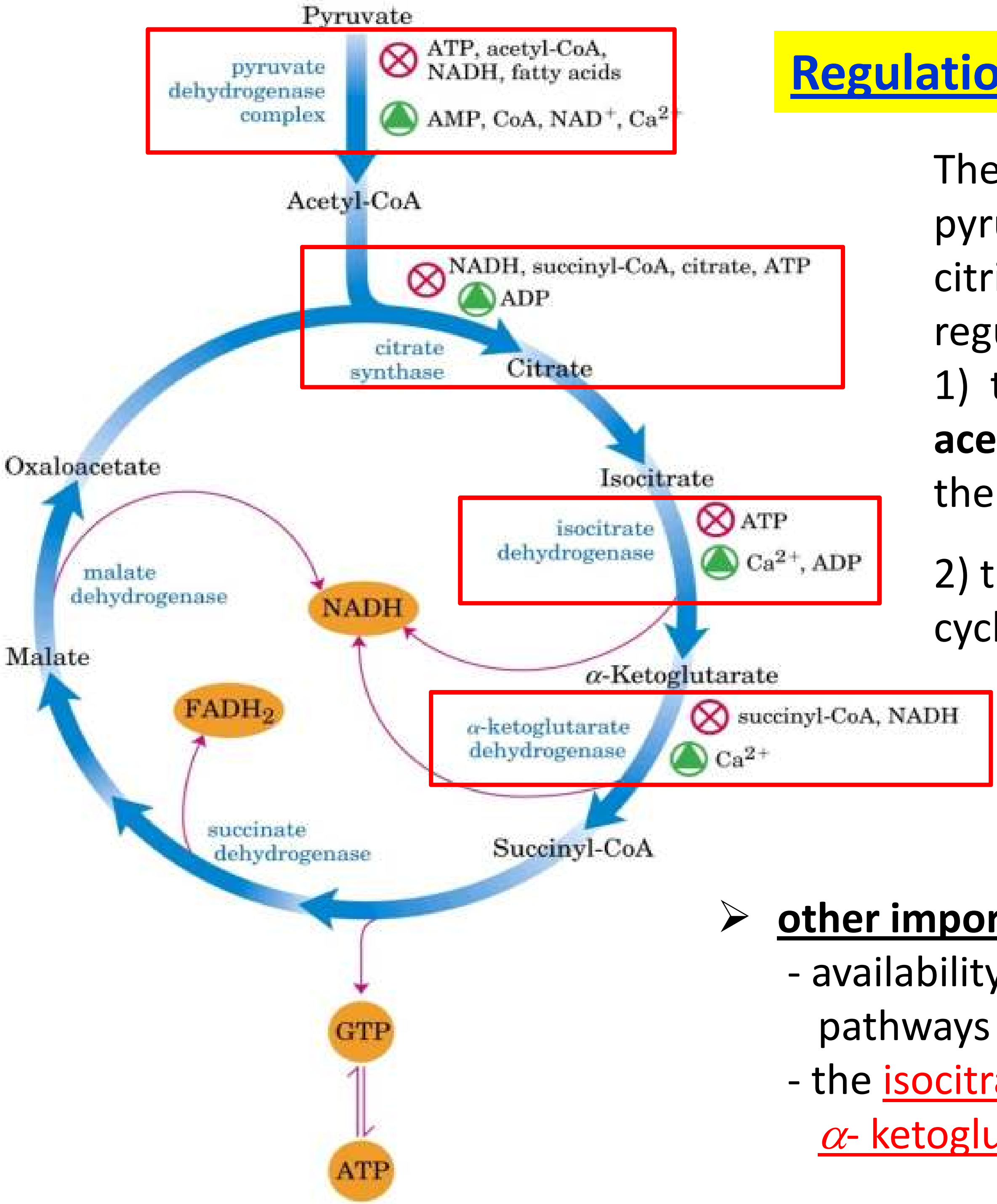
Regulation of the Citric Acid Cycle



1) **Substrate availability**
2) **Inhibition by accumulating products**
3) **Allosteric feedback inhibition of the enzymes that catalyze early steps in the cycle**

I. **Acetyl-CoA formation**: Regulation of PDH complex by allosteric and covalent mechanisms

II. **Citric acid cycle**: Regulation of **exergonic** reactions



<u>Regulation of the Citric Acid Cycle</u>

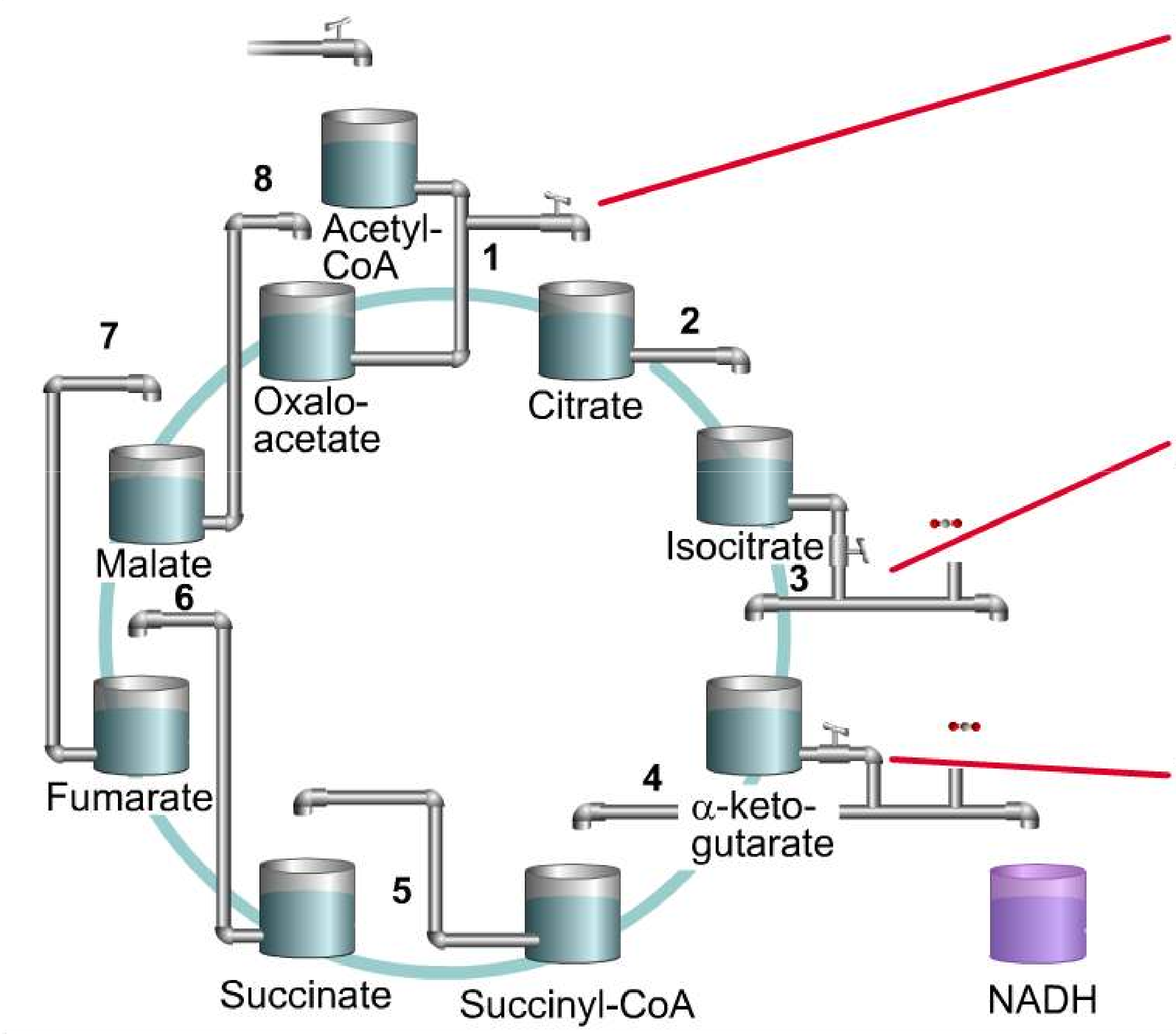
The flow of carbon atoms from pyruvate (into and through the citric acid cycle) is under tight regulation at two levels:

1) the **conversion of pyruvate to acetyl-CoA**, the starting material for the cycle

2) the **entry of acetyl-CoA** into the cycle - the <u>citrate synthase reaction</u>

- **<u>other important regulation points</u>:**
  - availability of intermediates from other pathways (fatty acids, amino acids)
  - the <u>isocitrate dehydrogenase</u> and <u>α- ketoglutarate dehydrogenase reactions</u>

Inhibition



INHIBITION OF STEP 1: citrate synthase
- Substrate Availability (acetyl-CoA, oxaloacetate)
- Product Inhibition (citrate)
- Feedback Inhibition (NADH from pool generated by entire cycle, succinyl-CoA)

INHIBITION OF STEP 3: isocitrate dehydrogenase
- Product Inhibition (NADH)
- **Feedback Inhibition** (NADH from pool generated by entire cycle)

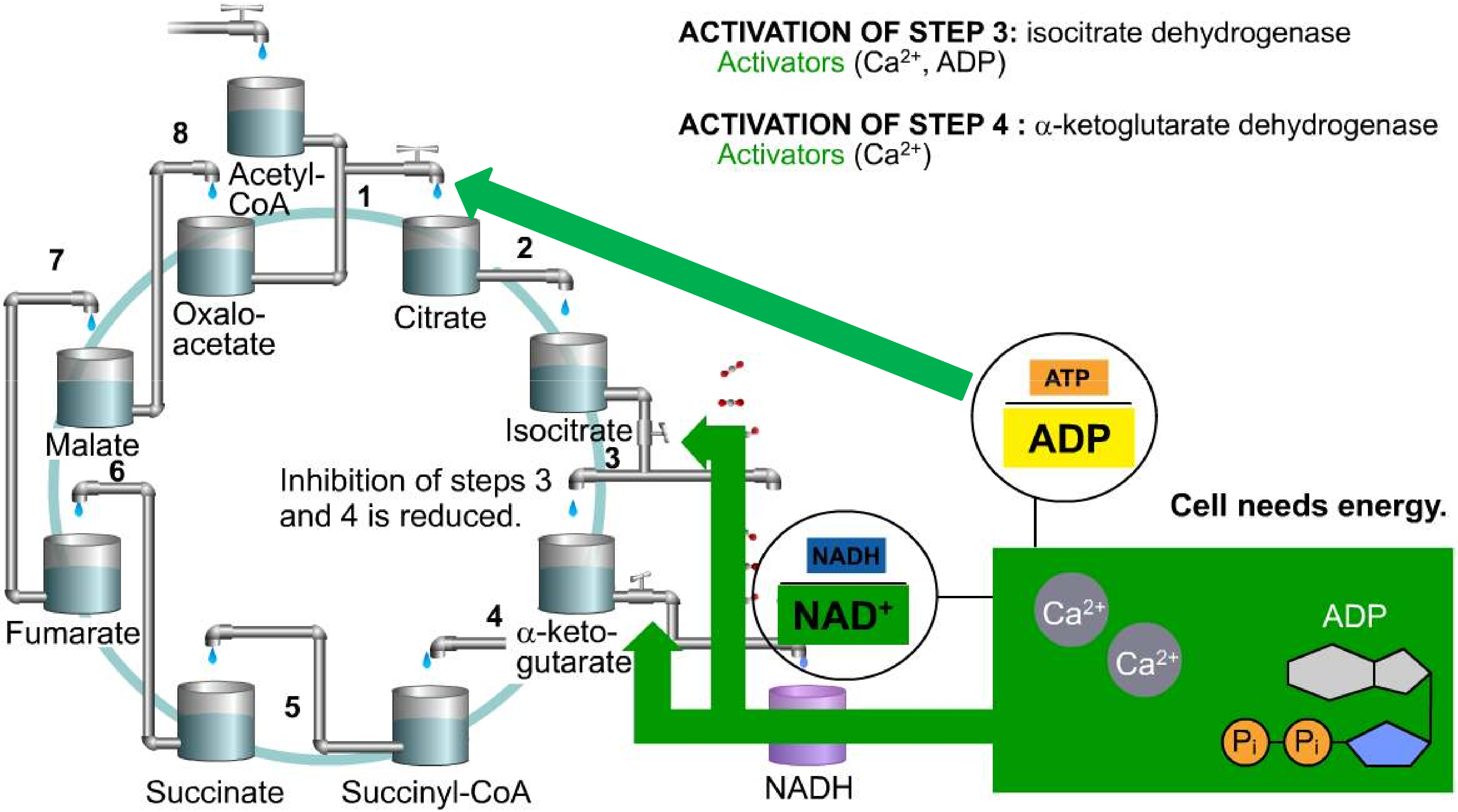
INHIBITION OF STEP 4: α-ketoglutarate dehydrogenase
- Product Inhibition (succinyl-CoA, NADH)
- Feedback Inhibition (NADH from pool generated by entire cycle)

Activation

ACTIVATION OF STEP 1: citrate synthase
Activator: ADP

ACTIVATION OF STEP 3: isocitrate dehydrogenase
Activators (Ca^{2+}, ADP)

ACTIVATION OF STEP 4 : α-ketoglutarate dehydrogenase
Activators (Ca^{2+})



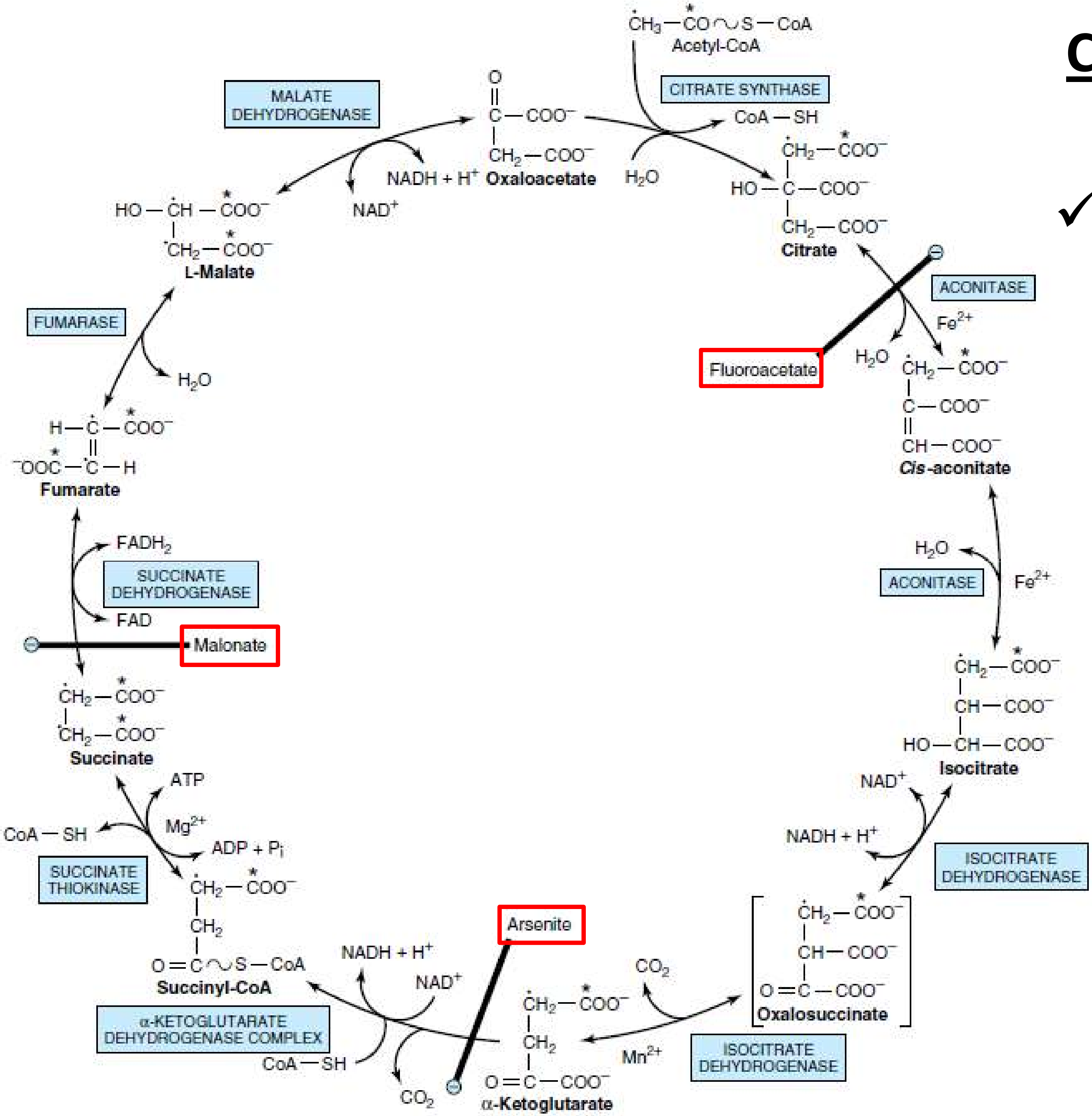
In muscle tissue, Ca^{2+} signals contraction and stimulates energy-yielding metabolism to replace the ATP consumed by contraction!

Citric acid cycle inhibitors:

✓ fluoroacetate

✓ arsenite

✓ malonate



Citric Acid Cycle Animation
http://www.wiley.com/legacy/college/boyer/0470003790/animations/tca/tca.htm

Literature:

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5. R. K. Murray i sur.: Harperova ilustrirana biokemija, prijevod 28. izdanja; Medicinska naklada, Zagreb, 2011.
6. http://www.wiley.com/college/pratt/0471393878/instructor/animations/citric_acid_cycle/index.html
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HOMEWORK: Questions to be answered

1. Briefly explain the metabolic role of citric acid cycle in:
(a) catabolism, particularly “energy metabolism”;
(b) anabolism!

2. Write the net (sum) chemical equation of the citric acid cycle!

3. Define the term anaplerotic reaction. Represent by structural formulas the most common anaplerotic reaction related to the citric acid cycle.

4. Briefly explain the regulation of citric acid cycle !