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Citric Acid Cycle and Regulation, Glyoxylate Cycle

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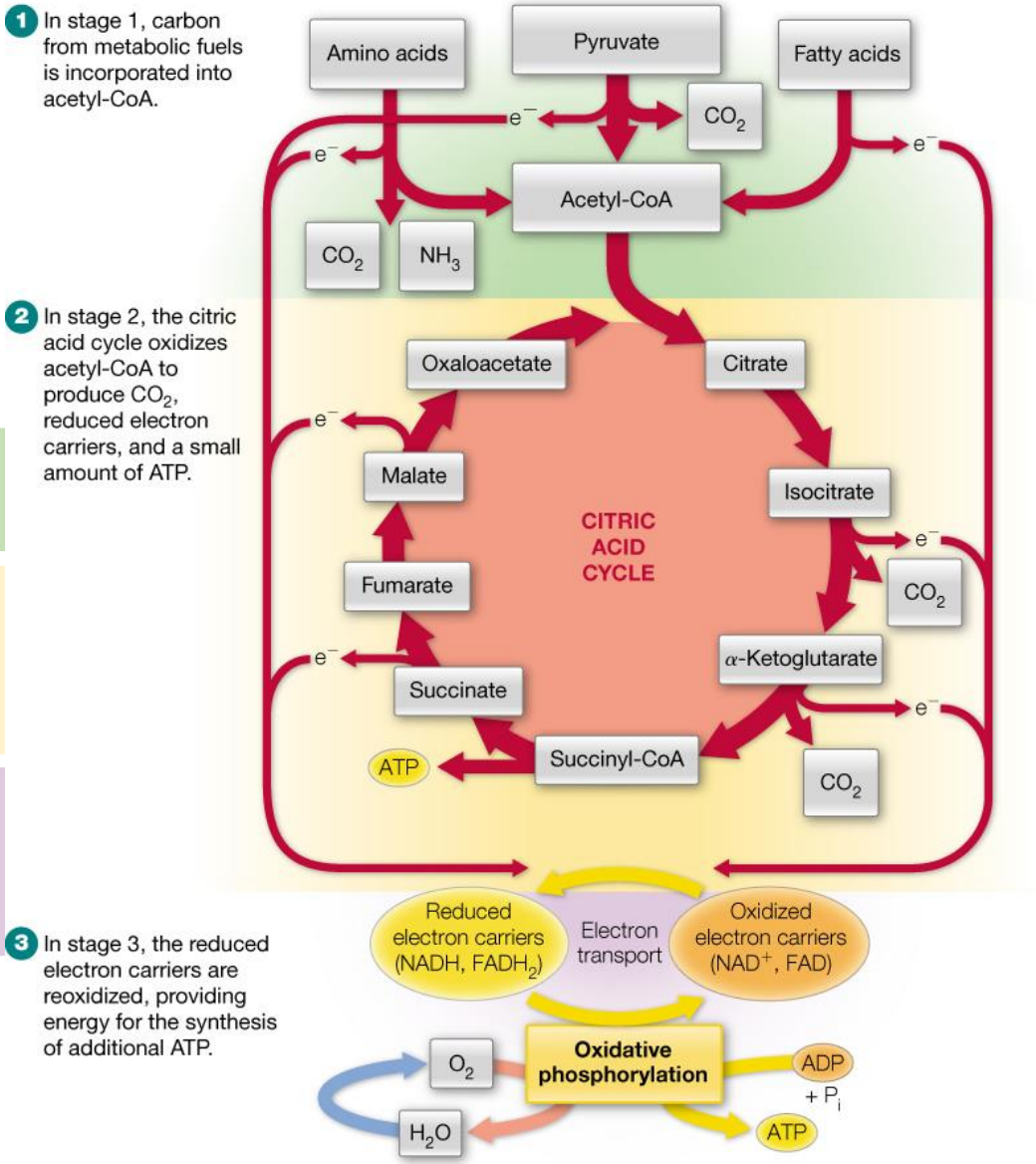
Objectives

- Citric acid cycle continued
  - Reactions before entering CAC (already done)
  - Reactions within CAC
- Regulation of CAC
- CAC's links to other metabolic pathways
- Glyoxalate cycle: a shortcut through CAC in plants

- Textbook Chap. 13

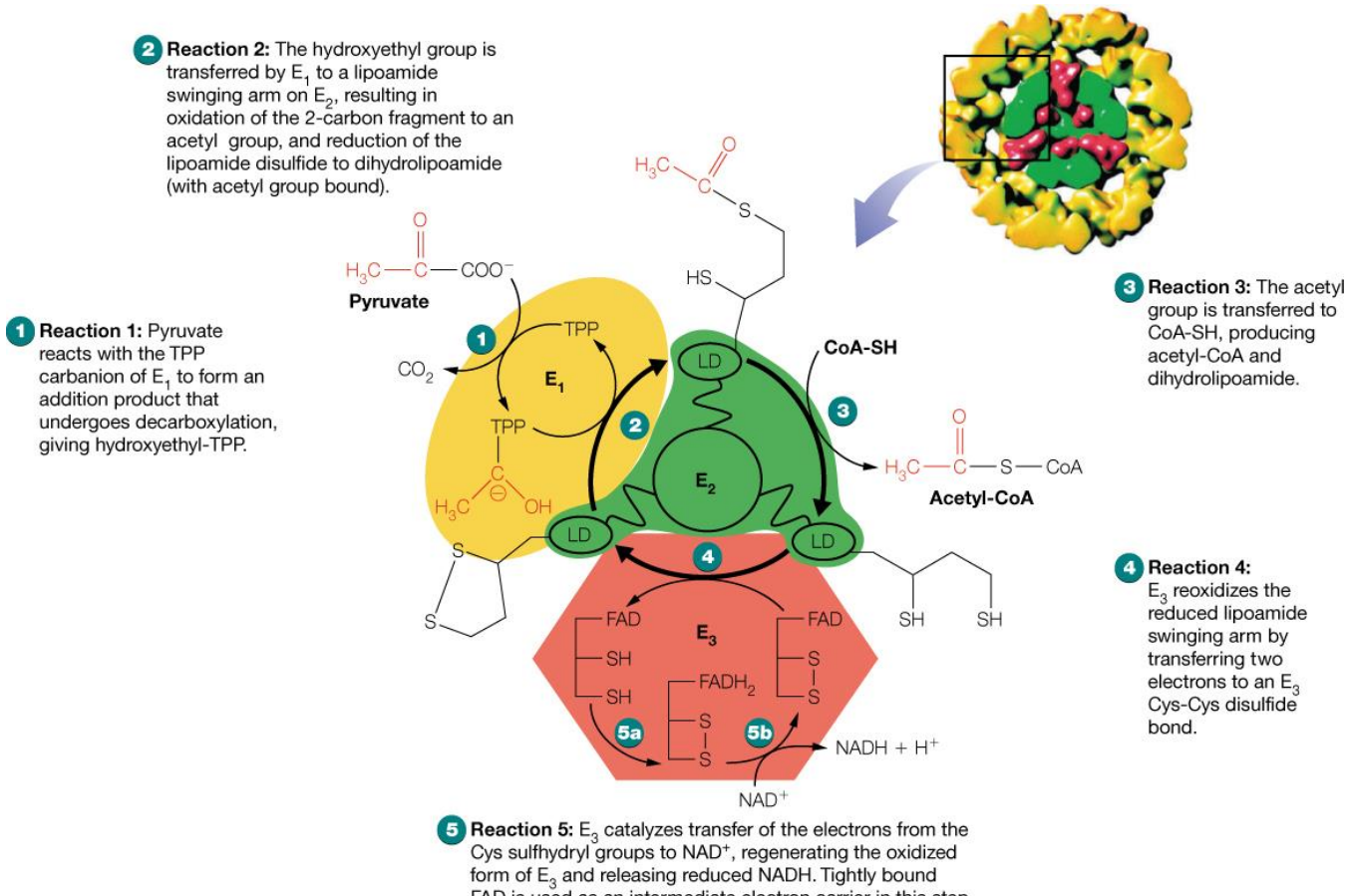
Stages of Cellular Respiration - recap

- Metabolic oxidation of organic substrates (cellular respiration) occurs in three stages:
  - In stage 1, carbon from metabolic fuels is incorporated into acetyl-CoA
  - In stage 2, the citric acid cycle oxidizes acetyl-CoA to produce CO_2, reduced electron carriers, and a small amount of ATP
  - In stage 3, the reduced electron carriers are reoxidized, providing energy for the synthesis of additional ATP
- In eukaryotic organisms, **these three stages are located in the mitochondria**.



Stage 1: Pyruvate dehydrogenase (PDH) multienzyme complex makes Acteyl-CoA for ernty into the Citric Acid Cycle (CAC)

$$\underset{3C}{\text{Pyruvate}} + \text{NAD}^+ + \text{CoA} \longrightarrow \underset{2C}{\text{Acetyl-CoA}} + \text{NADH} + \text{CO}_2$$



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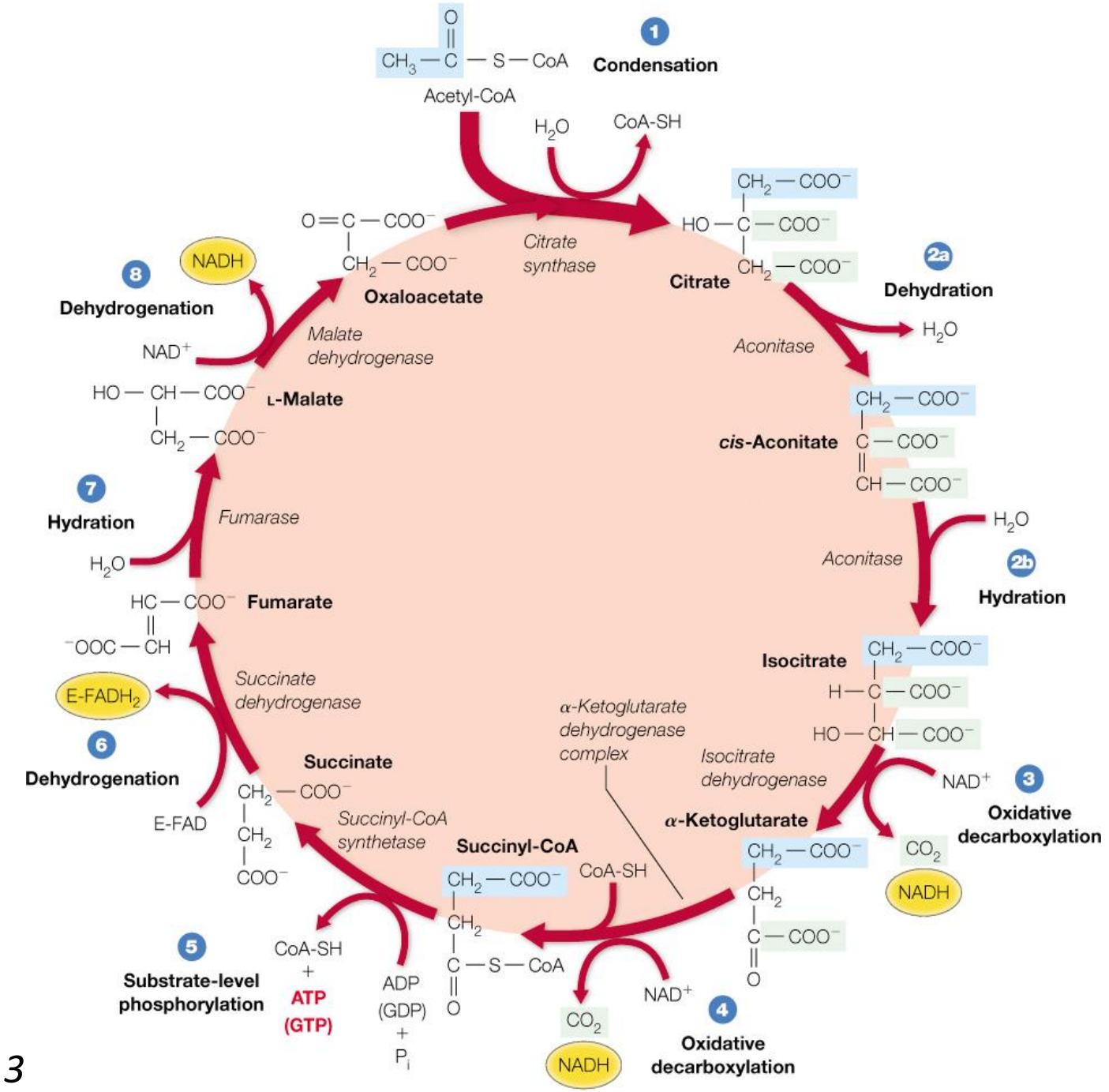
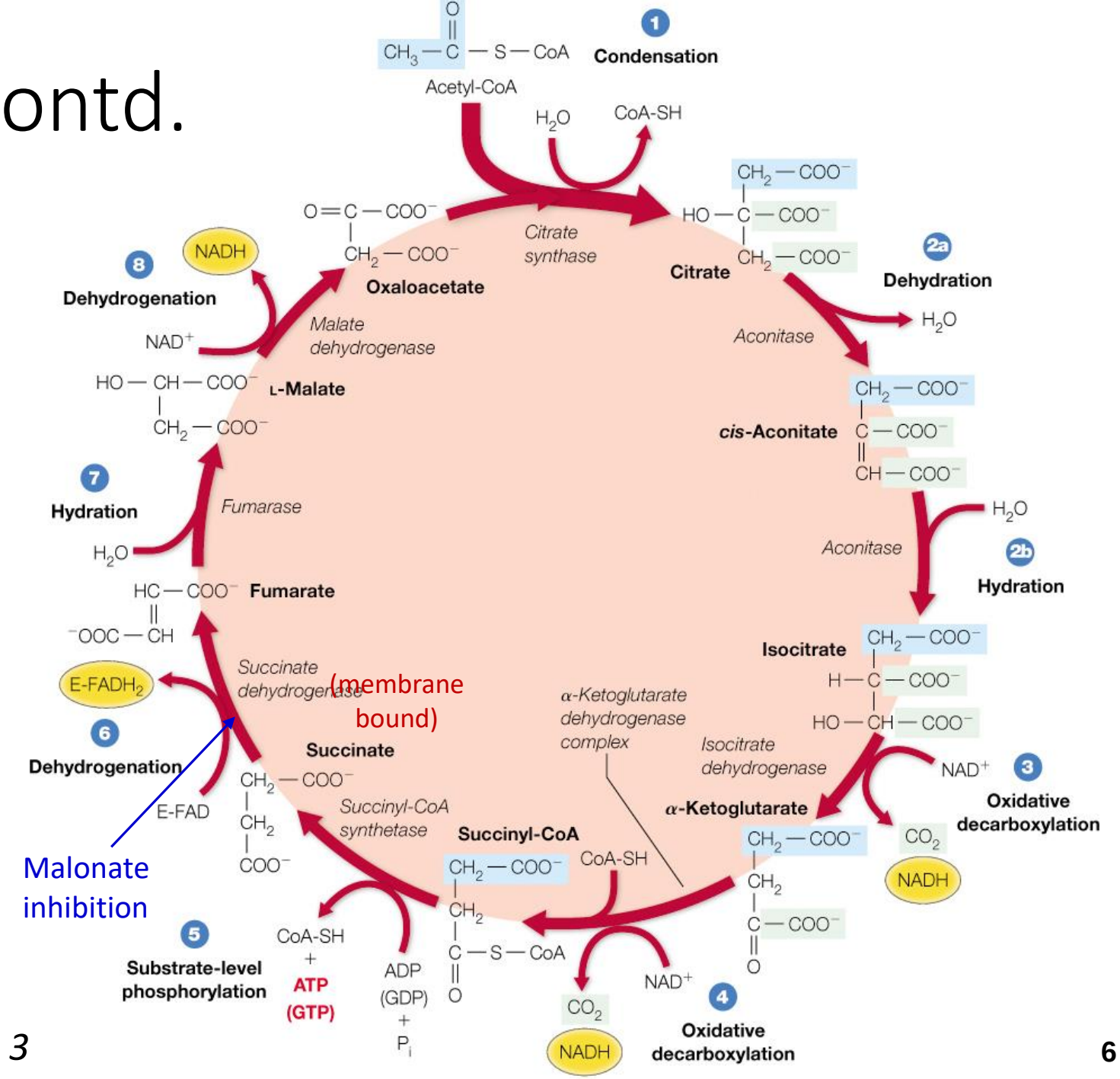
Stage 2: the CAC

- Starts with acetyl-CoA (2C) from pyruvate (3C) in Stage 1
- **Comprises 8 reactions**.
  - ➢ the product of the eighth reaction, **oxaloacetate**, and
  - ➢ the product of the PDH complex, **acetyl-CoA**, are the reactants for **the first reaction**
- The cycle generates **two CO_2, three NADH, and one $FADH_2$ molecule per turn.**
- Thus the **2 C's** (equivalent to the entering acetyl-CoA) **are oxidised.**

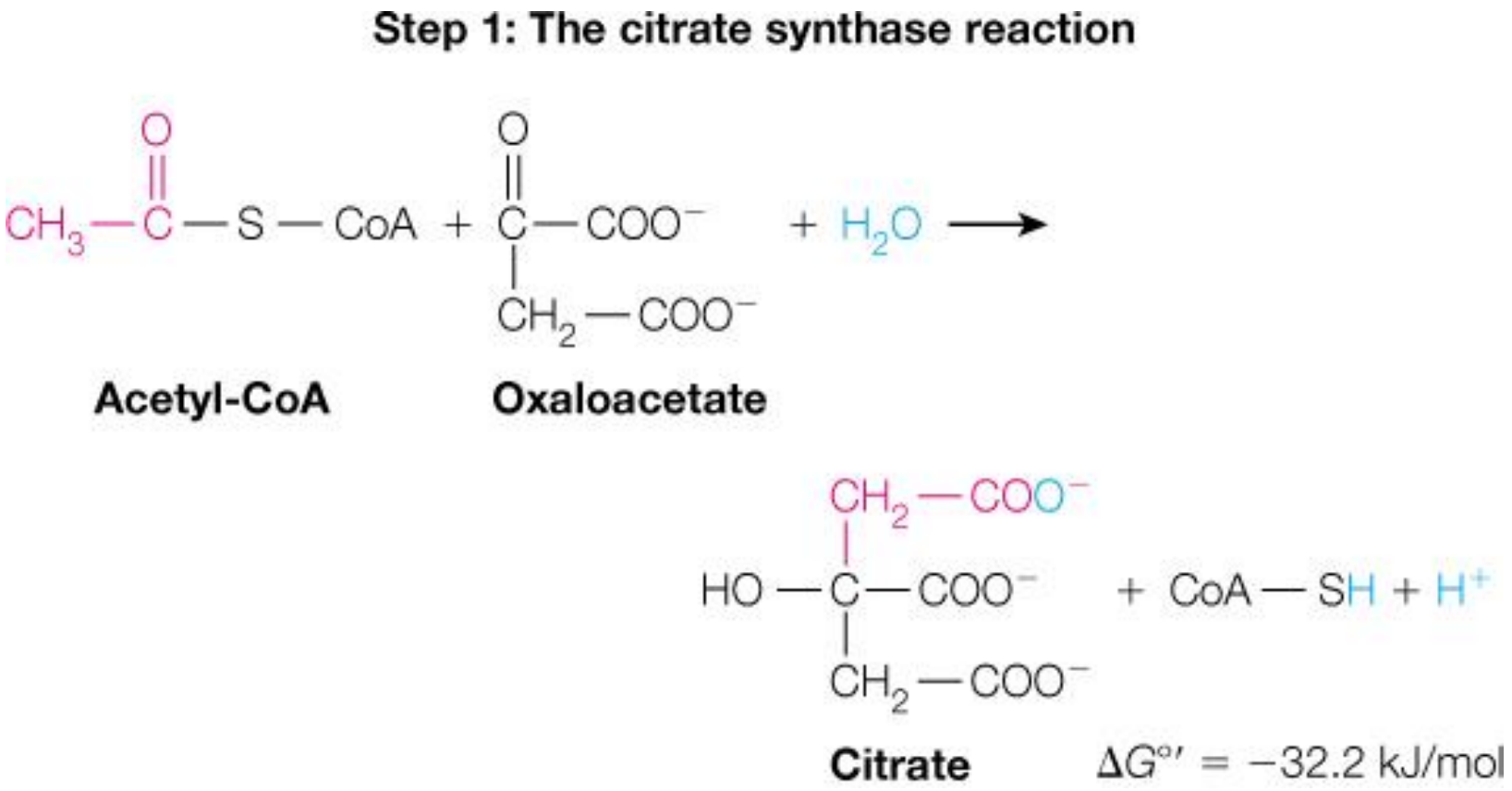


Stage 2: the CAC contd.

- Acetyl-CoA entering the citric acid cycle is highlighted (in blue) to show the fate of its two carbons through reaction 4.
- After reaction 5 , the intermediates, **succinate** and **fumarate** are symmetrical molecules – so no highlighting
  - Thus, C1 and C2 become indistinguishable from C3 and C4 beyond this point in the cycle.
- Carboxyl groups that leave the cycle as CO_2 in reactions 3 and 4 are highlighted in green.
- These **departing CO_2 groups** derive from the **two oxaloacetate carboxyl groups** that were incorporated as **acetyl-CoA in earlier turns of the citric acid cycle**.



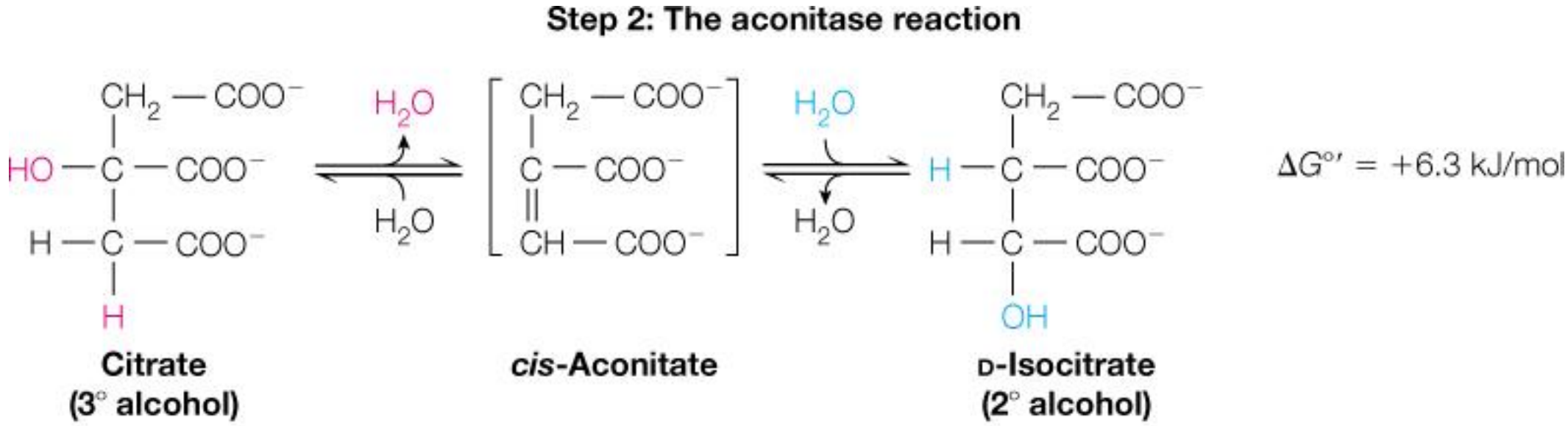
Reaction 1 – Citrate Synthase



Hydrolysis of the thioester makes the forward reaction highly exergonic, ensuring the CAC can proceed even at low concentrations of oxaloacetate

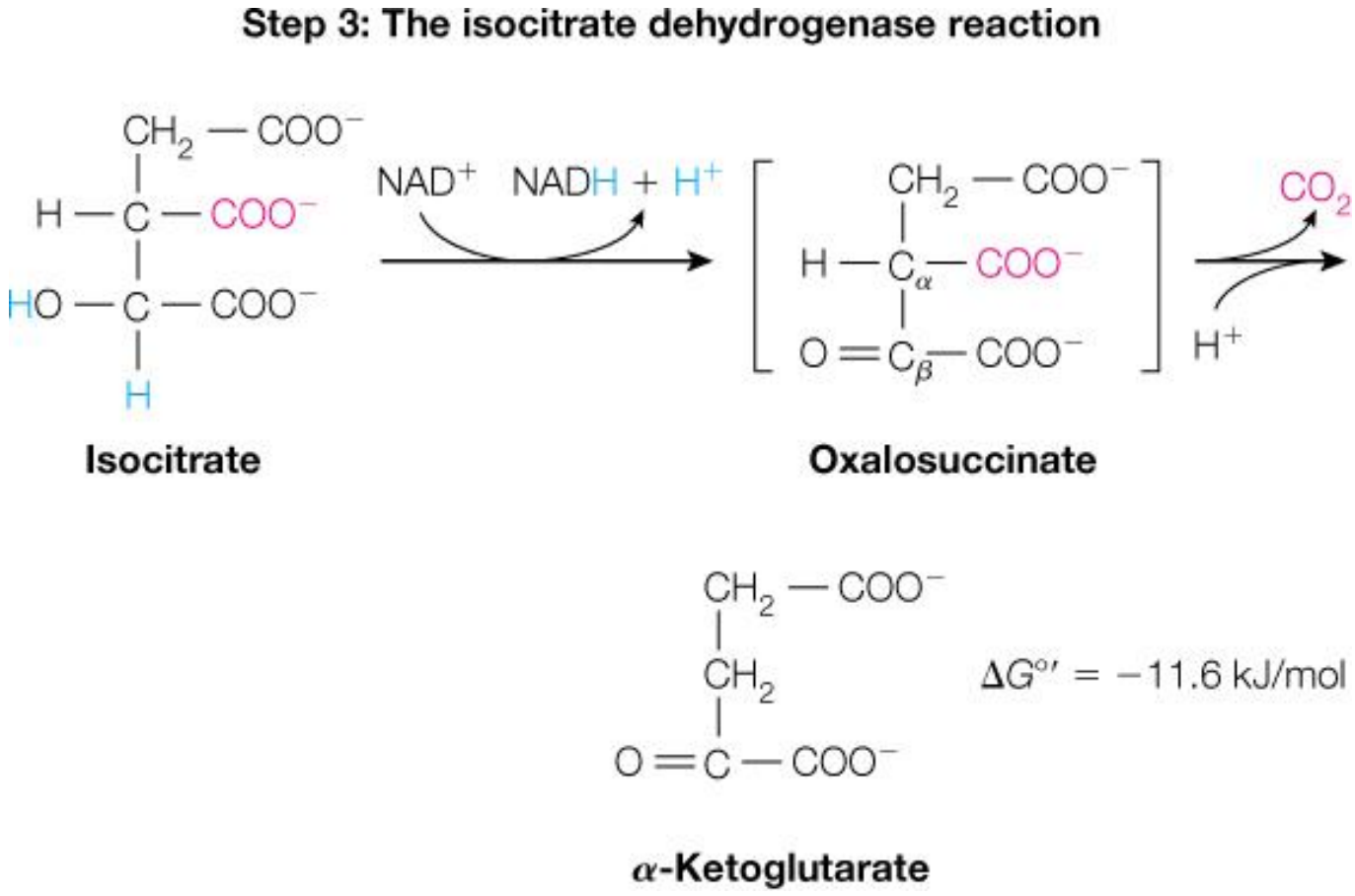
Reaction 2 – Aconitase

- converts the prochiral citrate to the chiral D-isocitrate in a two-step **isomerization reaction**
  - Step (a) is a dehydration reaction that forms *cis*-aconitate
  - Step (b) is a hydration reaction that forms D-isocitrate

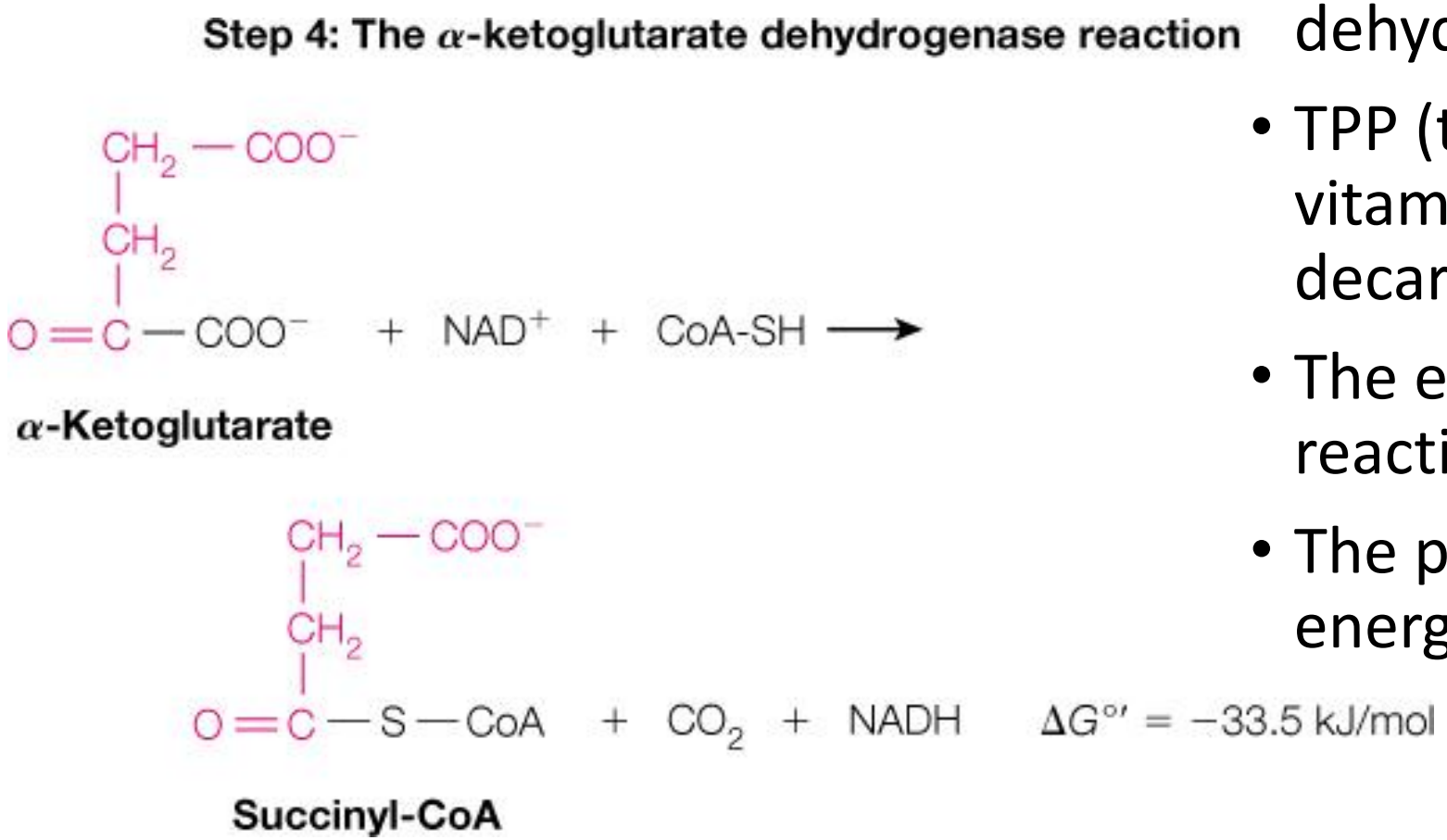


Reaction 3 – Isocitrate Dehydrogenase

- catalyzes an **oxidative decarboxylation**
  - **NAD^+ oxidizes** the 2° **alcohol** of isocitrate to form a **keto** group β to the carboxyl group that will be removed as **CO_2**
  - The β-keto group of oxalosuccinate acts as an electron sink to stabilize the transition state anion
  - Energy released in the oxidative decarboxylation is stored as **NADH**



Reaction 4 – α-Ketoglutarate Dehydrogenase Complex



- Reaction is similar to the pyruvate dehydrogenase complex
- TPP (thiamine pyrophosphate; vitamin B1) is required for the decarboxylation of the α-keto acid
- The energy of the oxidation reaction is conserved as NADH
- The product, succinyl-CoA, is an energy-rich molecule

Reaction 5 – Succinyl-CoA Synthetase

- performs a substrate level phosphorylation

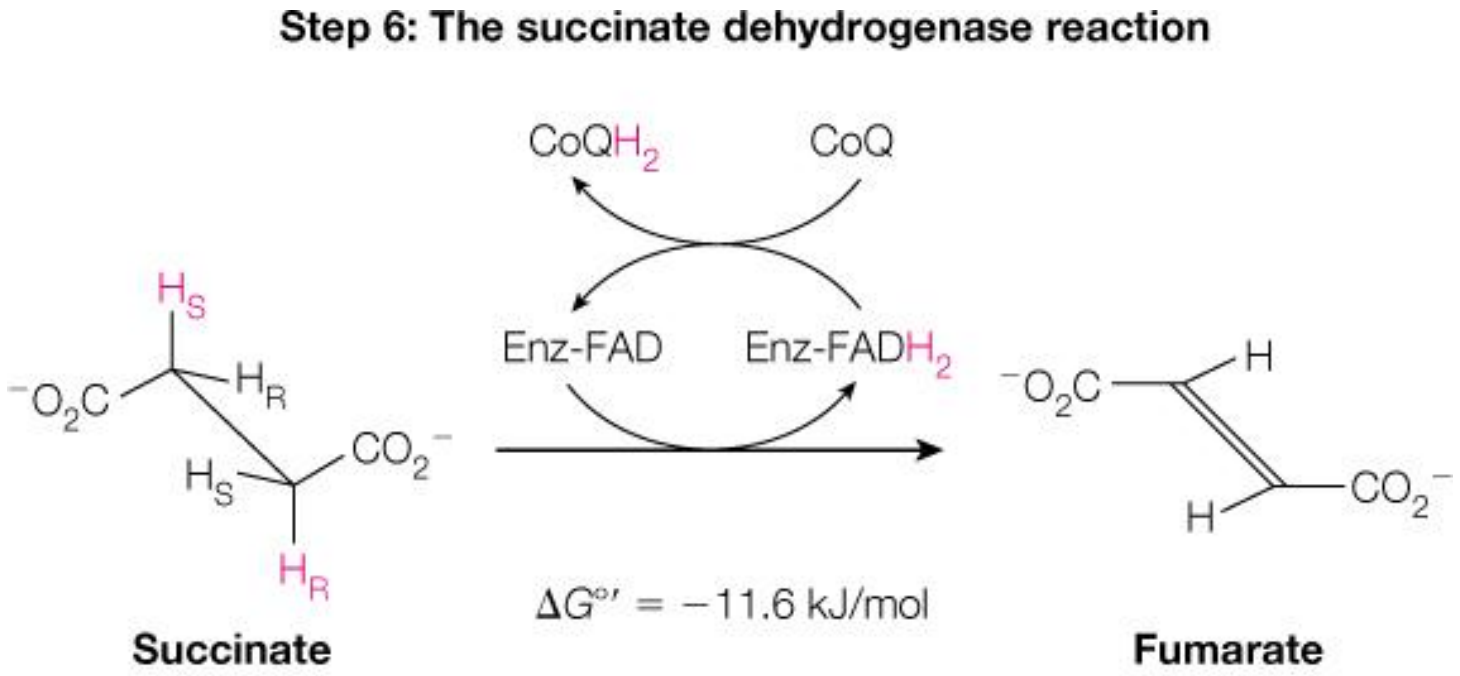
Step 5: The succinyl-CoA synthetase reaction

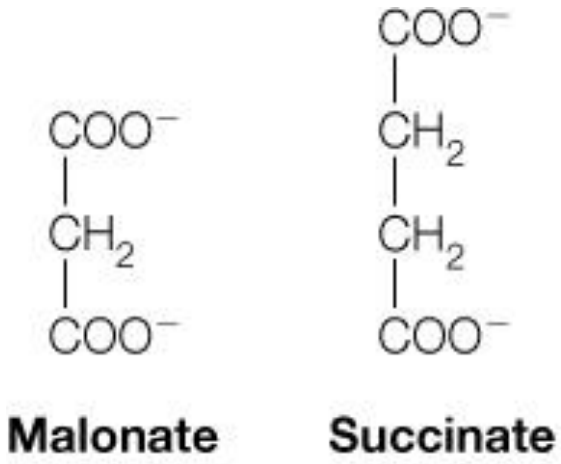
$$\text{Succinyl-CoA} + \text{P}_\text{i} + \text{ADP}\left(\text{GDP}\right) \rightleftarrows \text{succinate} + \text{ATP}\left(\text{GTP}\right) + \text{CoA-SH} \quad \Delta G^{\text{o}'} = -3.8 \text{ kJ/mol}$$

- The potential energy stored in the **thioester** (succinyl-CoA) is used to form **a nucleoside triphosphate (ATP or GTP)**

Reaction 6 – Succinate Dehydrogenase

- catalyzes the dehydrogenation of two saturated carbons to a double bond using **enzyme-bound FAD**
- is **membrane-bound** and delivers electrons directly into the **mitochondrial electron transport system** *via* **coenzyme Q**

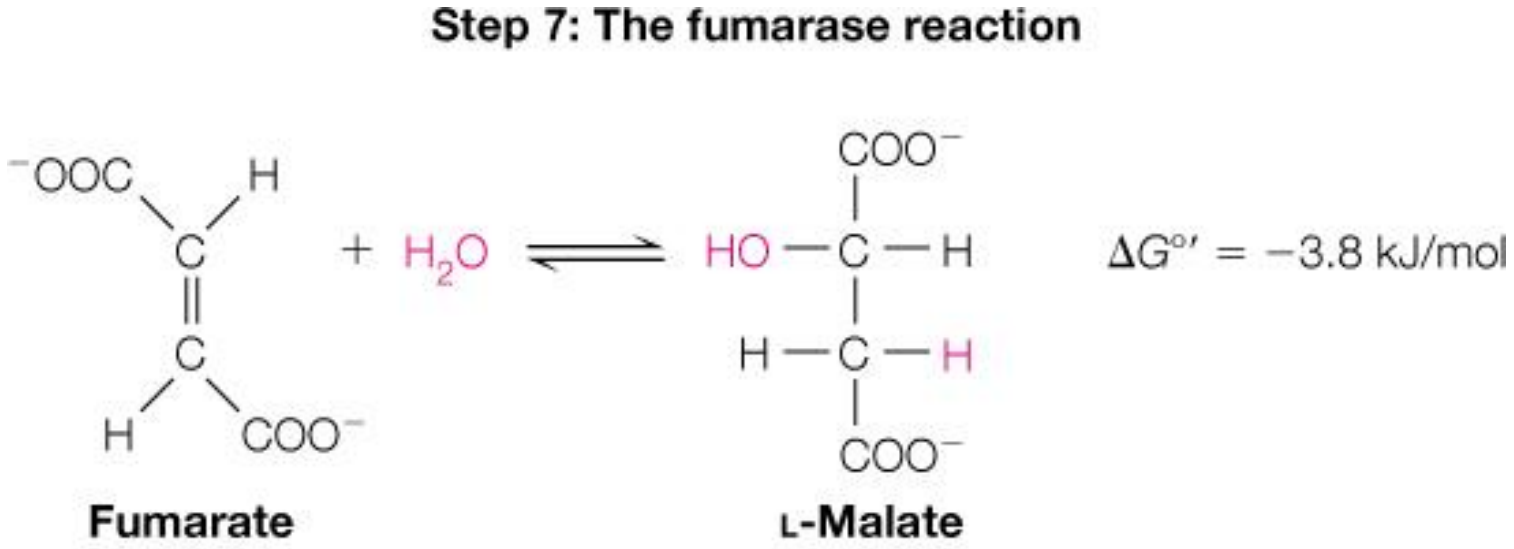




- is **inhibited** by **malonate**, a structural analog of succinate
- This inhibition assisted in figuring out the order of reactions in the CAC.

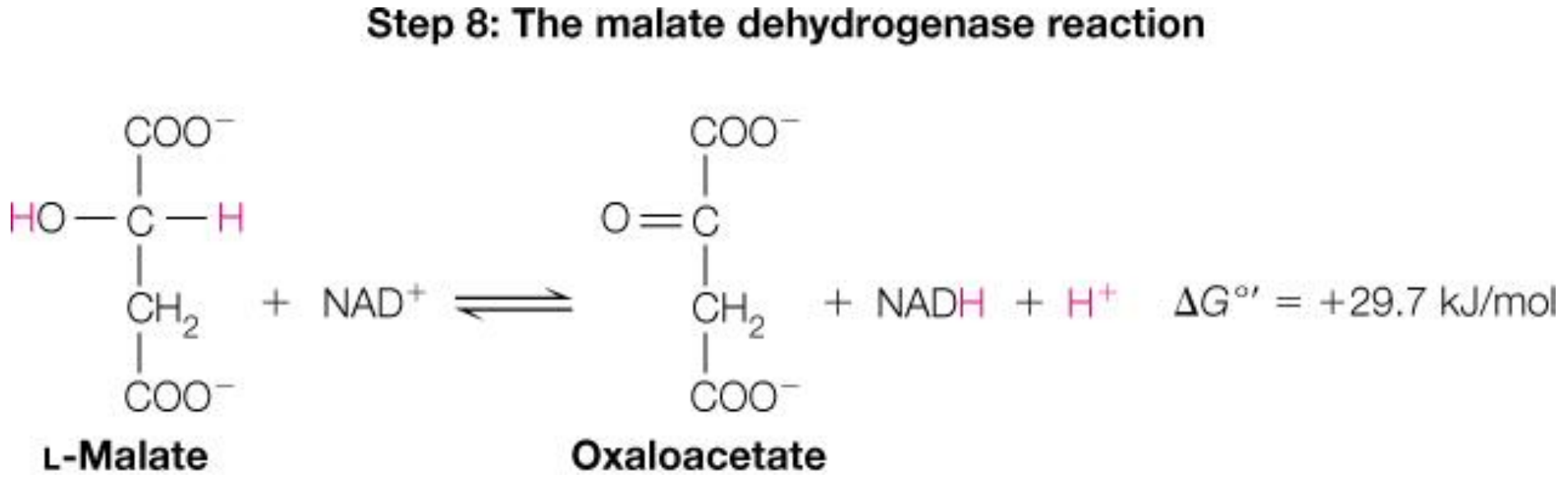
Reaction 7 – Fumarase

- performs a hydration reaction
- is highly stereospecific
  - the *trans* double bond of fumarate is specifically attacked, and only L-malate is formed
  - D-malate cannot be used as a substrate in the reverse reaction



Reaction 8 – Malate Dehydrogenase

- Although malate dehydrogenase has a large standard positive free energy change (+29.7 kJ/mol), the reaction proceeds to the right *in vivo* because the highly exergonic citrate synthase keeps oxaloacetate (and the electron transport chain keeps NADH) levels very low

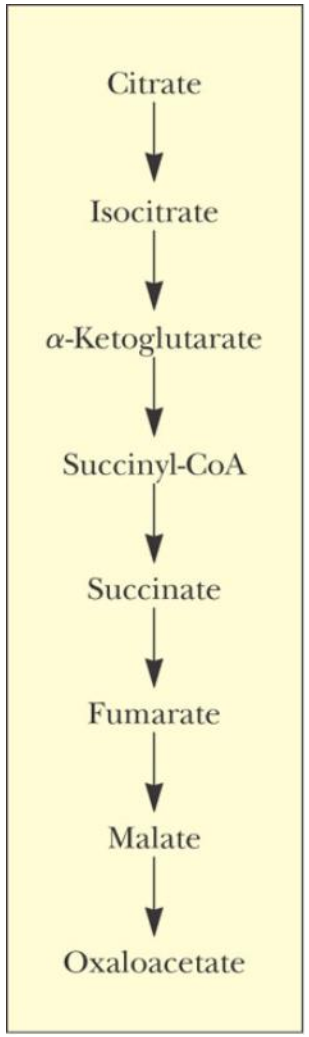


Summary of One Turn of CAC

- One turn of the citric acid cycle generates:
  - ➢ 1 high-energy phosphate (ATP or GTP) through substrate-level phosphorylation, plus
  - ➢ reduced cofactors (electron carriers): 3 NADH and 1 $FADH_2$ (equivalent to 8 electrons)
- The energy released as these electron carriers are reoxidized in the **electron transport chain** is used to drive the synthesis of ATP from ADP + P_i
- Sum of the eight reactions in one turn of the cycle:

$$\text{Acetyl-CoA} + 2H_2O + 3NAD^+ + \text{E-FAD} + \text{ADP} + P_i \longrightarrow$$

$$2CO_2 + 3NADH/H^+ + \text{E-FADH}_2 + \text{HS-CoA} + \text{ATP}$$



Energetics of CAC reactions

TABLE 13.2 Reactions of the citric acid cycle

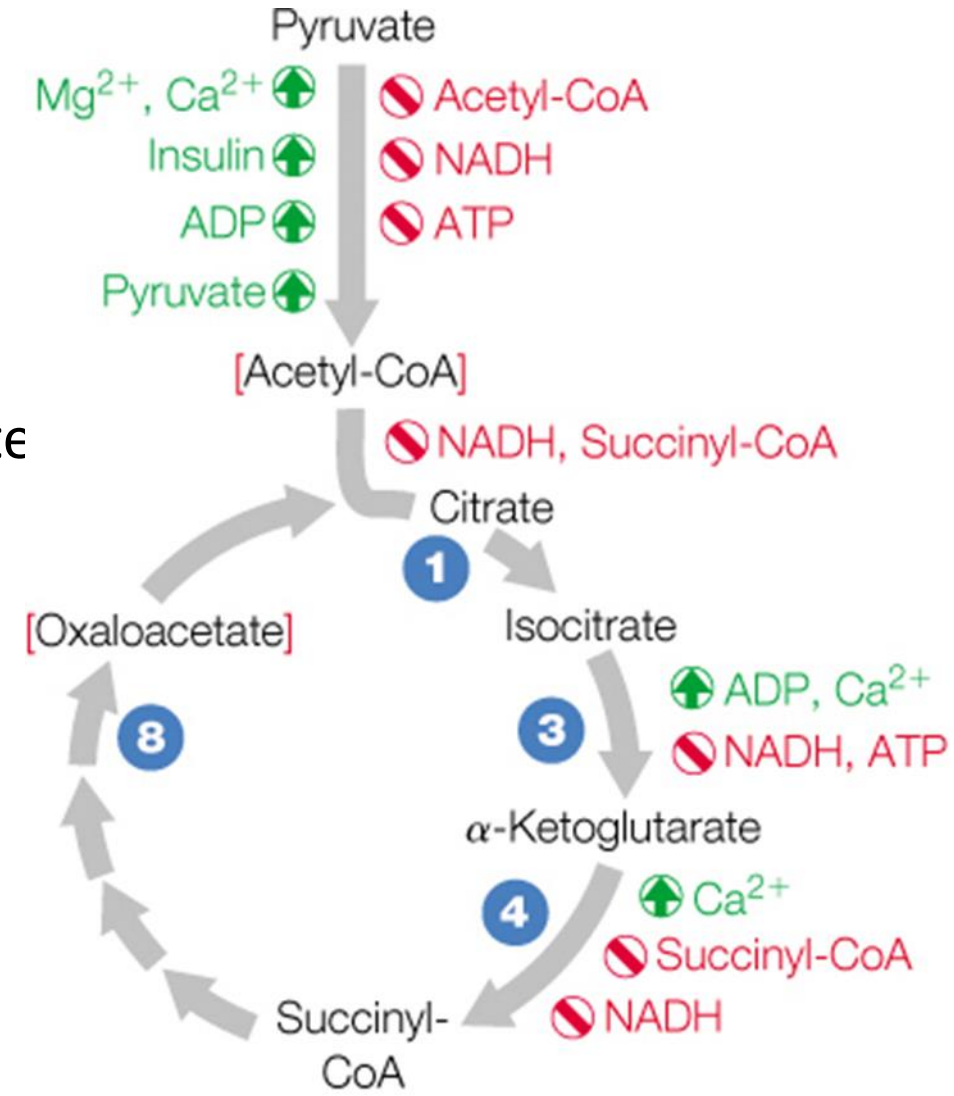
	Reaction	Enzyme	$\Delta G^{\circ\prime}$ (kJ/mol)	ΔG (kJ/mol)
1.	Acetyl-CoA + oxaloacetate + H_2O ⟶ citrate + CoA-SH + H^+	Citrate synthase	−32.2	~−55
2a.	Citrate ⇌ *cis*-aconitate + H_2O	Aconitase	+6.3	~0
2b.	*cis*-Aconitate + H_2O ⇌ isocitrate	Aconitase		
3.	Isocitrate + NAD^+ ⇌ α-ketoglutarate + CO_2 + NADH	Isocitrate dehydrogenase	−11.6	~−20
4.	α-Ketoglutarate + NAD^+ + CoA-SH ⇌ succinyl-CoA + CO_2 + NADH	α-Ketoglutarate dehydrogenase complex	−33.5	~−40
5.	Succinyl-CoA + P_i + ADP (GDP) ⇌ succinate + ATP (GTP) + CoA-SH	Succinyl-CoA synthetase	−3.8	~0
6.	Succinate + FAD (enzyme-bound) ⇌ fumarate + $FADH_2$ (enzyme-bound)	Succinate dehydrogenase	0	~0
7.	Fumarate + H_2O ⇌ L-malate	Fumarase	−3.8	~0
8.	L-Malate + NAD^+ ⇌ oxaloacetate + NADH + H^+	Malate dehydrogenase	+29.7	~0
		Net	−48.0	~−115

Note: $\Delta G^{\circ\prime}$ value for reaction 3 was calculated from the $E^{\circ\prime}$ values for α-ketoglutarate/isocitrate (−0.38 V) and NAD/NADH (−0.32 V).

- **3 enzyme reactions (1., 3. and 4.: catalyzed by citrate synthase, isocitrate dehydrogenase and α-ketoglutarate dehydrogenase)** control the rate of the CAC.
- However, all CAC metabolites are present both in the mitochondria and the cytosol – so equilibrium conditions are assumed within the compartments.
- **The last reaction (8., catalyzed by malate dehydrogenase) is unfavourable**.
  - However it is coupled with the next one (**1., citrate synthase reaction**) is highly favourable and drives the CAC forward.

Regulation of CAC

- Regulation occurs mainly by controlling entry into the cycle (**PDH complex** and **citrate synthase**) and by controlling key irreversible reactions (**isocitrate dehydrogenase** and **α-ketoglutarate dehydrogenase**)
  - ❖ substrates: acetyl-CoA, succinyl-CoA and oxaloacetate
  - ❖ product: NADH
- Cycle flux is primarily controlled by:
  - ❖ Allosteric activation of isocitrate dehydrogenase by ADP (energy state)
  - ❖ NAD^+/NADH ratio (redox state)
  - ❖ Inhibition of relevant enzymes by acetyl-CoA and succinyl-CoA (availability of energy-rich compounds)



Regulation of the PDH Complex

- The E_2 component of the PDH complex is inhibited by its product, acetyl-CoA
- The E_3 component is inhibited by its product, NADH
- The E_1 component is inactivated via phosphorylation by PDH kinase
- PDH kinase is activated by high levels of ATP, NADH, or acetyl CoA
- PDH phosphatase reactivates the PDH complex by removing the phosphate group

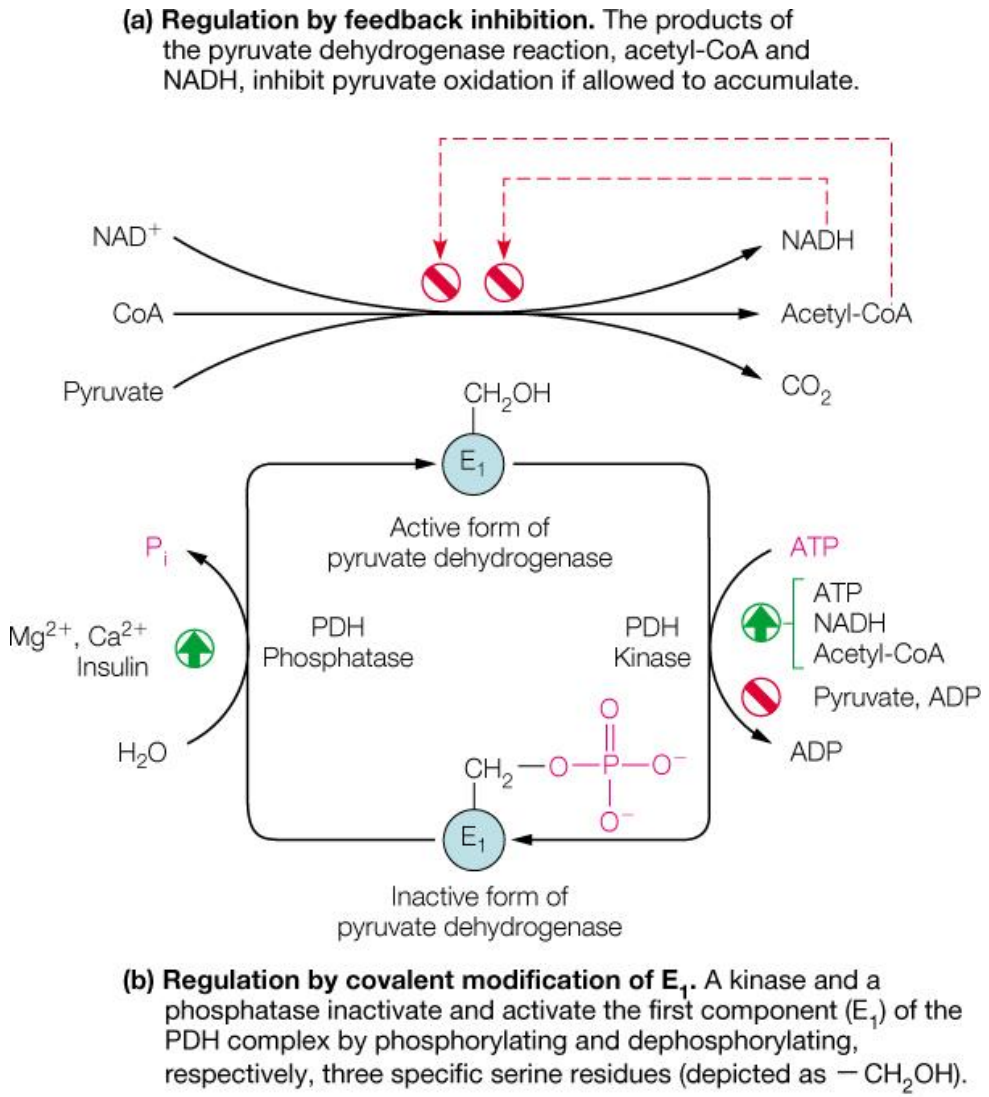


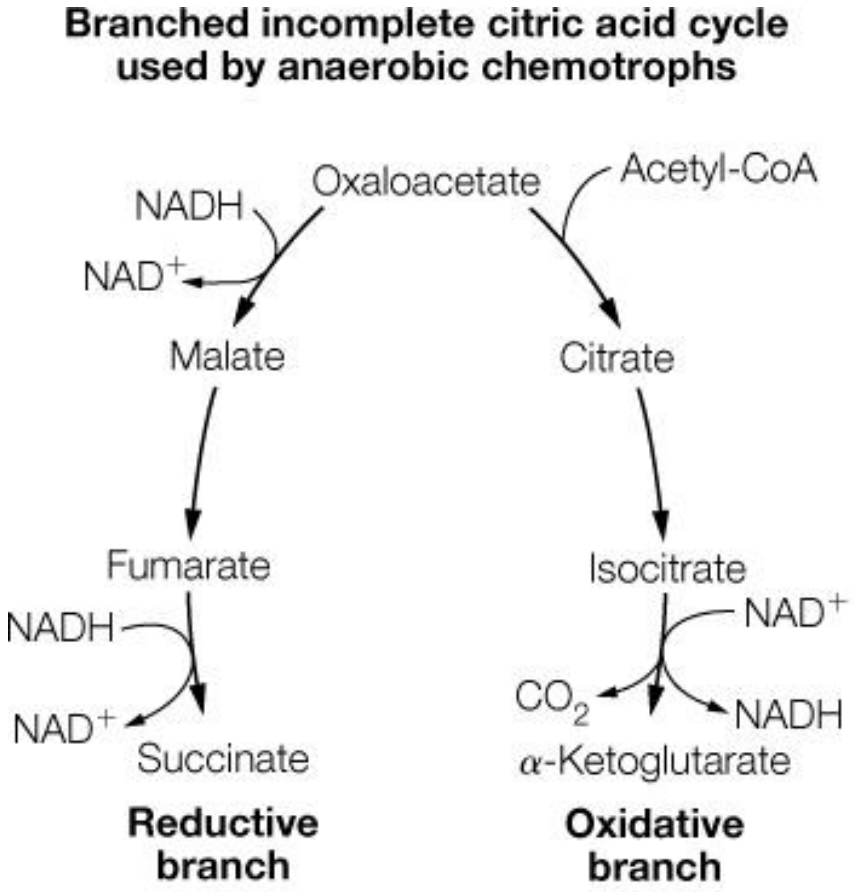
FIGURE 13.16 Regulation of the mammalian pyruvate dehydrogenase complex by feedback inhibition and by covalent modification of E_1.

Summary: Regulation of Citric Acid Cycle

- The need for energy regulates CAC capacity at the pyruvate dehydrogenase step and at the three rate-controlling steps of the cycle.
- Regulatory mechanisms depend on:
  - substrate availability
  - product inhibition
  - covalent modification and
  - allosteric effects.

CAC Genes in All Three Domains of Life

- Genes encoding CAC enzymes have been found in organisms of all three domains of life (Bacteria, Archaea, and Eukarya), including anaerobic chemotrophs
- The latter harvest energy from glucose oxidation only, but still use an incomplete CAC (e.g., to generate biosynthetic precursors)



Effects of Defects in CAC Enzymes

- A complete lack of **any** of the at least eight CAC enzyme activities would be **lethal for humans**
- However, **specific mutations in CAC genes** leading to **defective enzymes** have been linked to
  - a number of neurodegenerative diseases (such as Leigh syndrome or other encephalopathies) and
  - tumors (e.g., uterine and renal cell cancer, and malignant gliomas, a common type of brain tumor).

CAC is key to cellular metabolism

- CAC is both catabolic and anabolic: amphibolic!
- **Catabolic** because it involves degradation and is a major free-energy conservation system in most organisms – here intermediates are required only in small quantities to maintain the cycle.
- ➢ Red arrows also show which ***anapletrotic pathways*** replenish CAC intermediates.
- **Anabolic** because many biosynthetic pathways use CAC intermediates

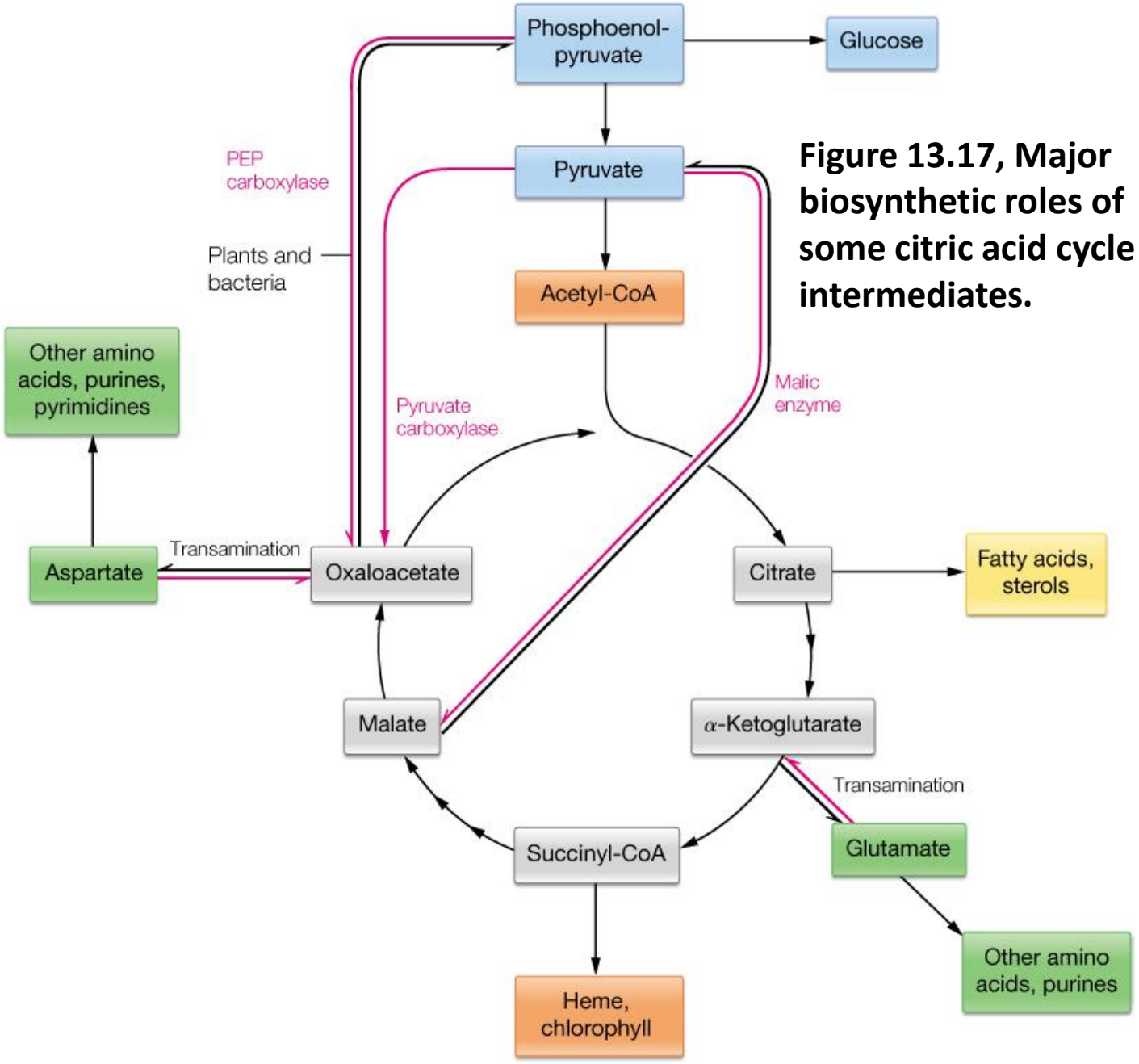


Figure 13.17, Major biosynthetic roles of some citric acid cycle intermediates.

Other pathways use CAC intermediates

1. **Glucose biosynthesis or gluconeogenesis (cytosol):** uses ***oxaloacetate*** (OAA).
   - Since CAC is cyclical, any of its intermediates can be converted to OAA for use in gluconeogenesis.
2. **Fatty acid and steroid biosynthesis:** requires ***acetyl-CoA.***
3. **Heme/chlorophyll synthesis: uses succinyl-CoA**
3. **Amino acid biosynthesis (and purines/pyrimidines):** uses
   - **α-ketoglutarate** and
   - **oxaloacetate**

   as starting materials, with enzymes using either NADH or NADPH (later lectures).

On the whole these pathways are called <u>cataplerotic</u>: as they "empty" CAC intermediates.

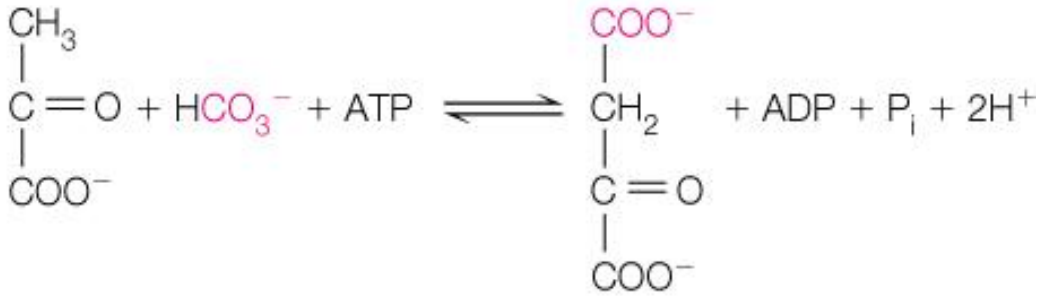
Reactions that replenish CAC intermediates: anaplerotic or “fill up”

1. During exercise, some pyruvate is directed to other pathways to generate more ATP.

1st step of <u>gluconeogenesis</u>: pyruvate converted to **oxaloacetate** (CAC intermediate) by **pyruvate carboxylase** which is in the <u>mitochondria</u> of the liver (requires biotin as a prosthetic group and ATP)

Also **phosphoenolpyruvate to oxalaoacetate** in heart and skeletal muscle by **PEPCK.**

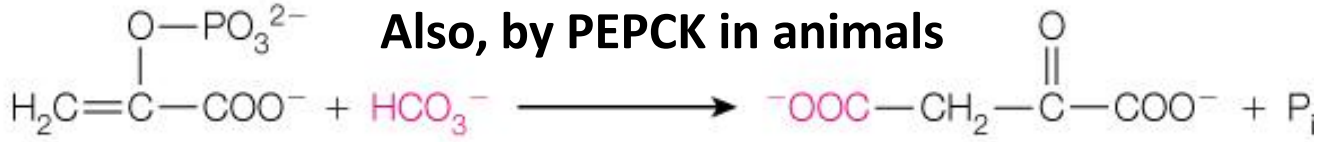
Replenishment of oxaloacetate in animals: The pyruvate carboxylase reaction



Pyruvate **Oxaloacetate**

Replenishment of oxaloacetate in plants and bacteria: The PEP carboxylase reaction

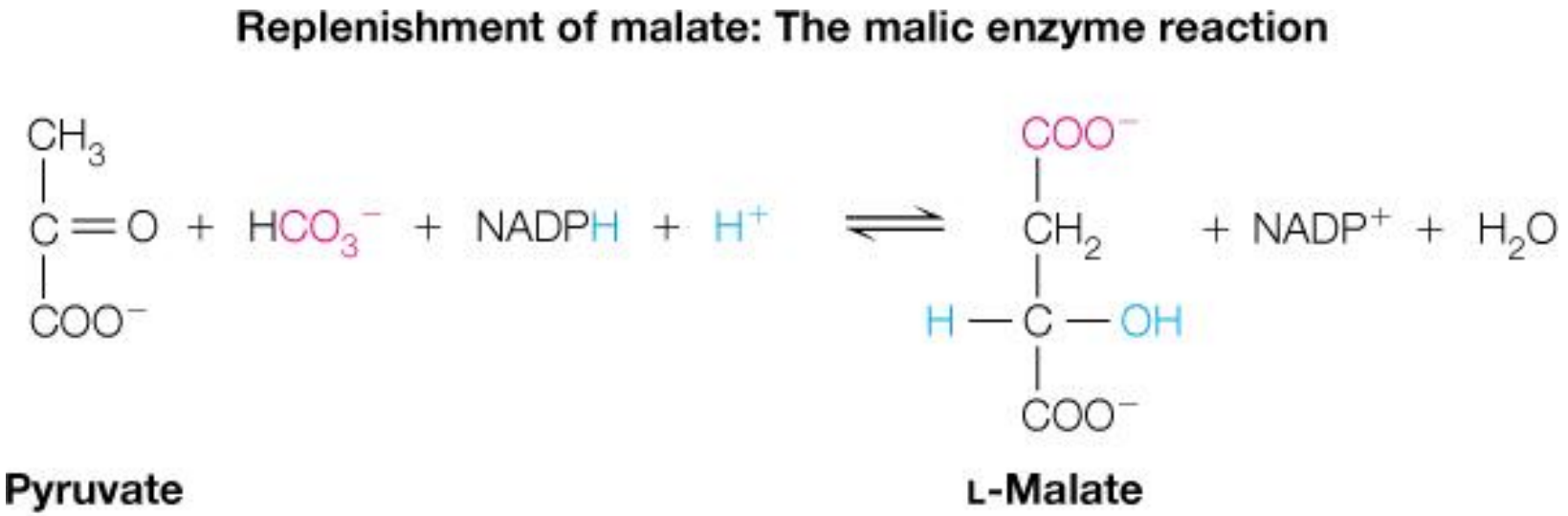
Also, by PEPCK in animals



Phosphoenolpyruvate (PEP) **Oxaloacetate**

Reactions that replenish CAC intermediates: anaplerotic or “fill up”

2. Malic enzyme (aka **malate dehydrogenase**) directly makes **malate** from **pyruvate, using NADPH as cofactor:**

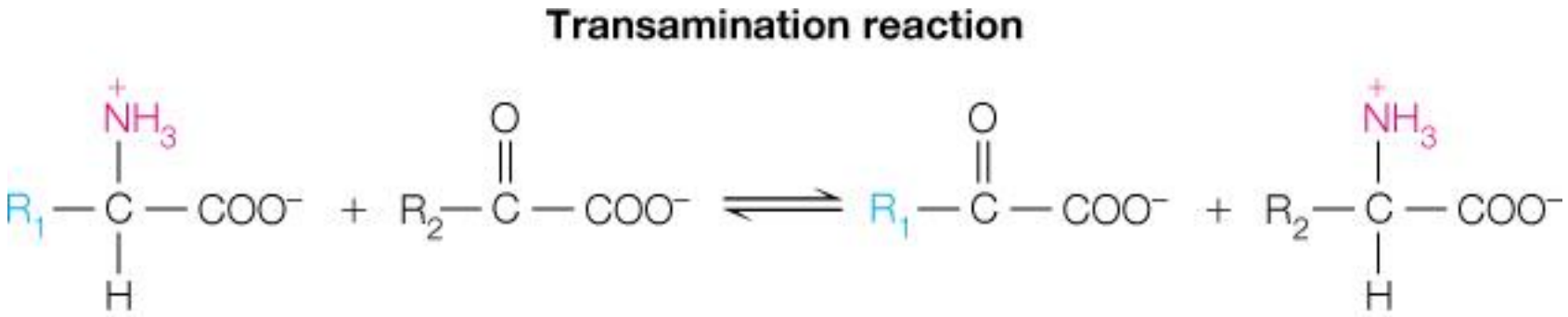


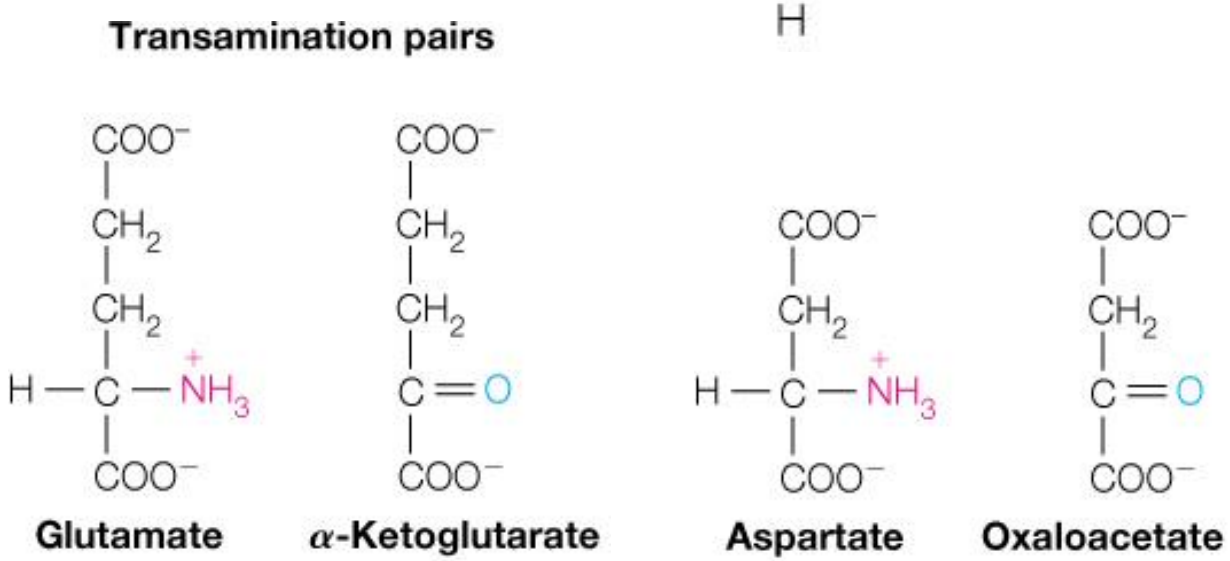
The reverse reaction importantly makes NADPH for fatty acid biosynthesis.

Reactions that replenish CAC intermediates: anaplerotic or "fill up"

3. Amino acid reactions

a. Transamination





b. Glutamate dehydrogenase also makes **α-ketoglutarate from glutamate,** with **NAD^+ or $NADP^+$ as cofactor**, under protein-rich conditions.

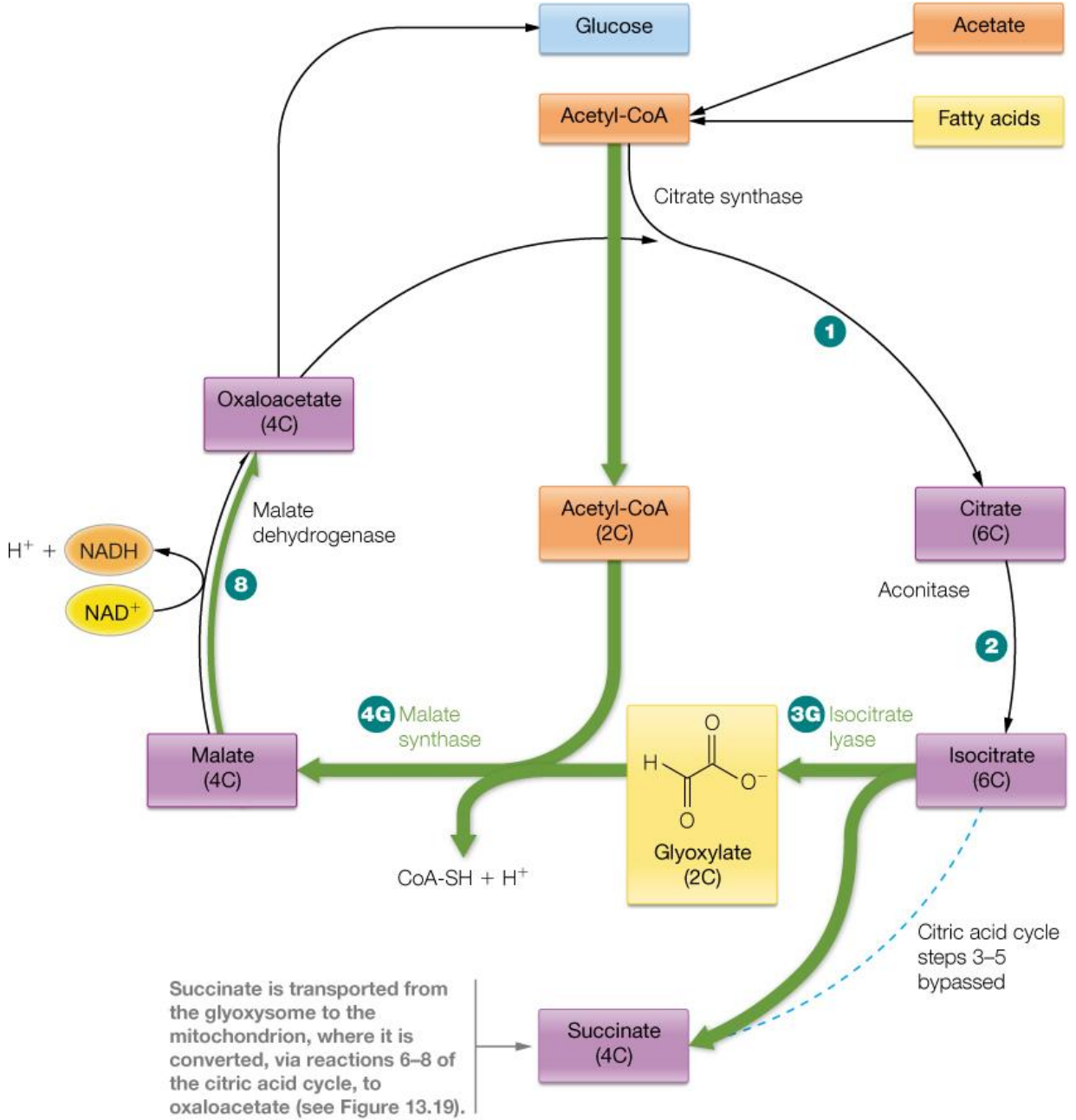
Conversion from Fats to Carbohydrates

- Vertebrates cannot convert acetyl-CoA (and hence fatty acids) into glucose (and other carbohydrates) via gluconeogenesis, without losing C atoms
- At least two highly exergonic reactions make conversions essentially irreversible:
  1) phosphoenolpyruvate (PEP) to pyruvate (during glycolysis)
  2) pyruvate to acetyl-CoA (acetyl-CoA production via PDH complex)
- Oxalacetate can be converted into PEP via PEP carboxykinase, but it has to be generated from acetyl-CoA via CAC, which involves two decarboxylations of a C2 body and therefore has no net gain
- The **glyoxylate cycle** allows the formation of glucose from acetyl-CoA by bypassing the two CAC decarboxylation steps (isocitrate and α-ketoglutarate dehydrogenases) in plants and some microorganisms.

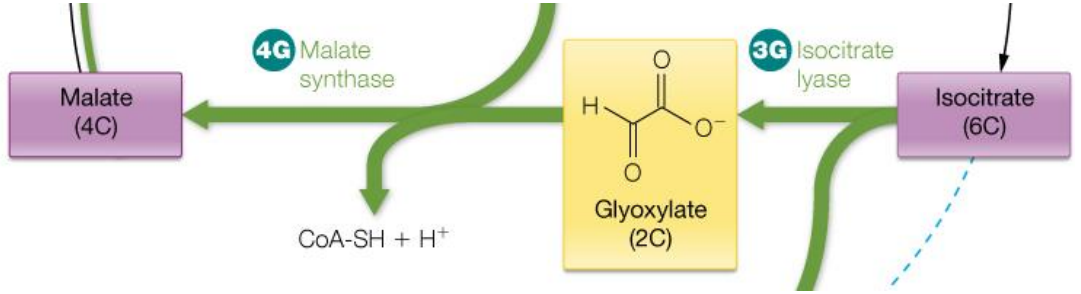
The Glyoxylate cycle

- The glyoxylate cycle consists of three CAC enzymes, citrate synthase, aconitase, and malate dehydrogenase, and two additional enzymes**, isocitrate lyase** and **malate synthase**
- In plant cells, the glyoxylate cycle is located in special organelles, glyoxysomes, usually in close proximity to lipid bodies and mitochondria
- Helps plants grow in the dark!
- Overall net outcome:

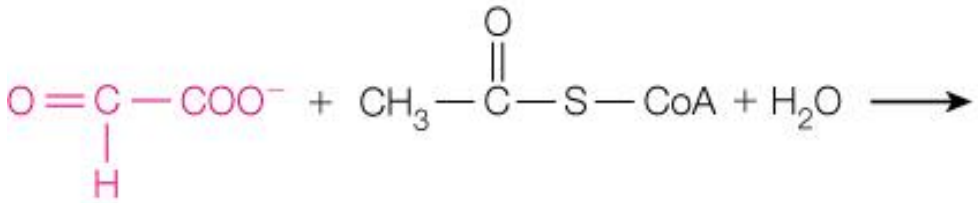
$$\underset{(2x2C)}{2\ \text{Acetyl-CoA} + \text{NAD}^+} \longrightarrow \underset{(4C)}{2\ \text{CoA} + \text{succinate} + \text{NADH} + \text{H}^+}$$



Reactions of the Glyoxylate Cycle

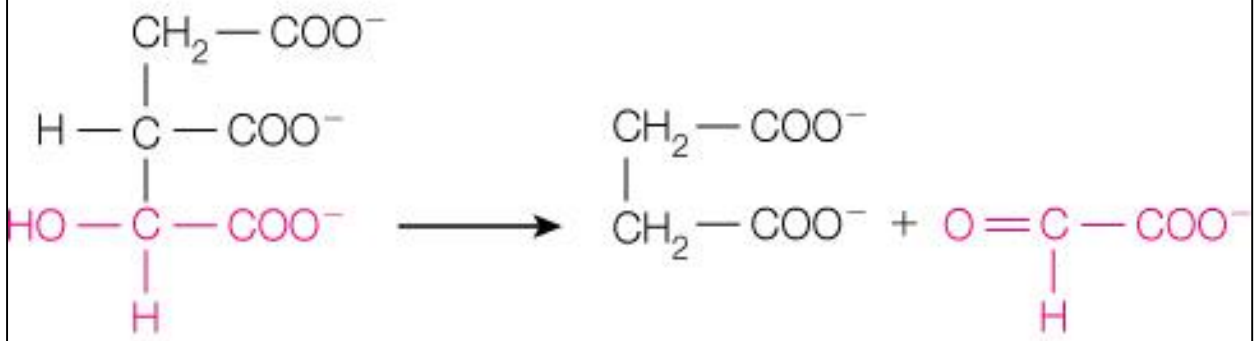


The malate synthase reaction

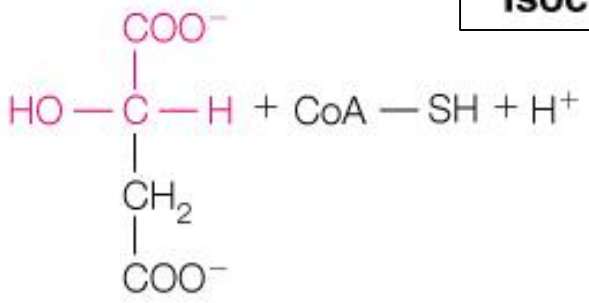


Glyoxylate **Acetyl-CoA**

The isocitrate lyase reaction

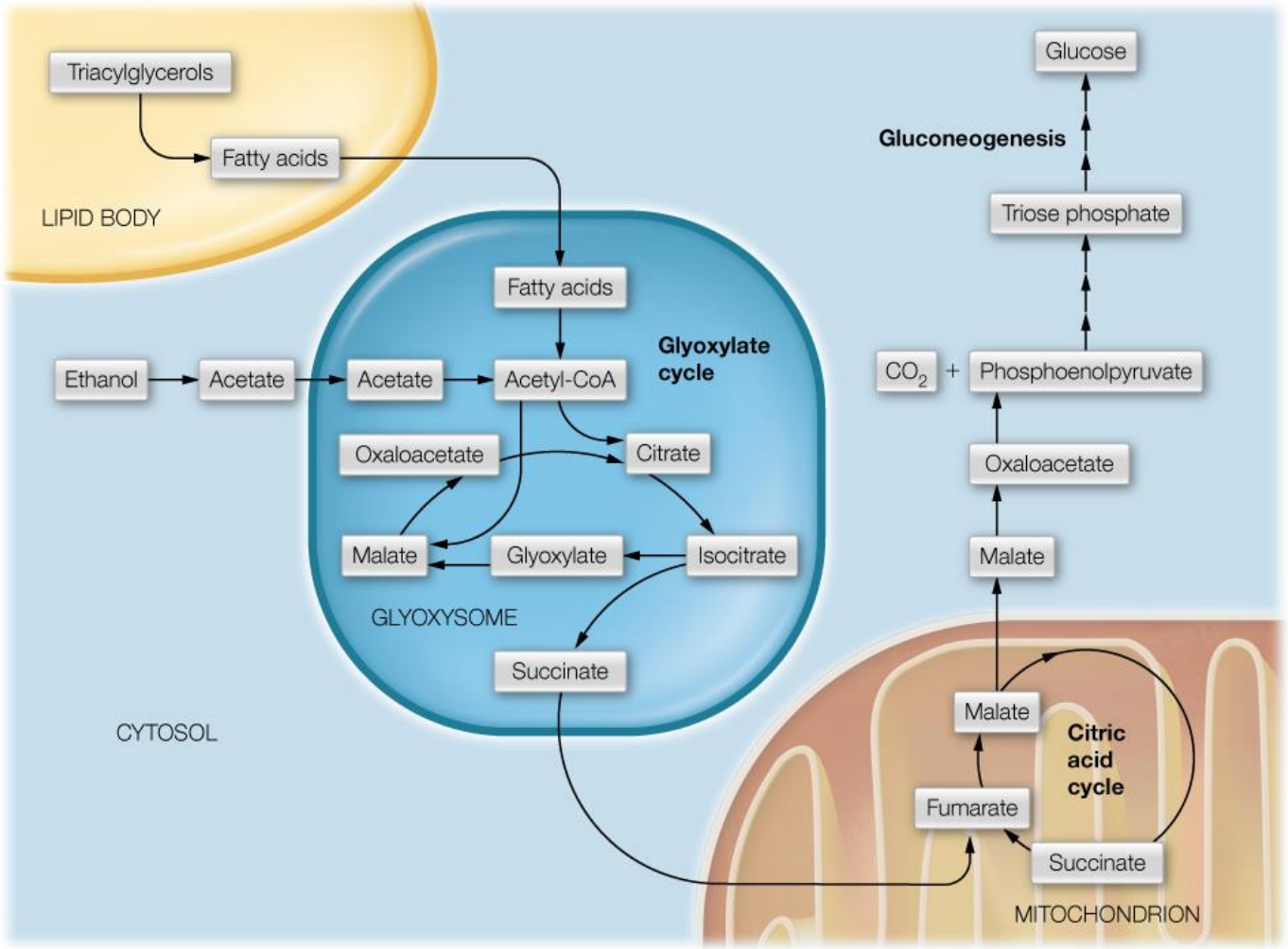


Isocitrate **Succinate** **Glyoxylate**



Malate

Intracellular Connection of the Glyoxylate Cycle in Plant Cells



Glyoxalate cycle summary

- Only in glyoxysomes in plants, yeast and some bacteria
- **No C lost as CO_2 in the cycle**
- Overall reaction:

$$2\ \text{Acetyl-CoA} + NAD^+ \longrightarrow 2\ \text{CoA} + \text{succinate} + NADH + H^+$$

- **Relevance for human health**: pathogens survive using this cycle in their hosts:
  - *Mycobacterium tuberculosis*, which causes tuberculosis, can persist for years in the lung. It survives on lipids, using CAC to produce precursors for amino acid synthesis and using the **glyoxylate cycle** to produce carbohydrate precursors.
  - Drugs have been designed to inhibit the bacterial ***isocitrate lyase in the glyoxalate cycle*** to combat the pathogen's survival.

Citric Acid Cycle is linked to Other Pathways

Summary

- The citric acid cycle is a central pathway for the oxidation of carbohydrates, lipids and proteins.
- The key entrant to the pathway is pyruvate, which is converted to acetyl-CoA by the pyruvate dehydrogenase complex.
- Each CAC turn involves the entry of a 2C unit as acetyl-CoA, with the loss of 2 others C's as CO_2, along with 1 ATP equivalent and reduced cofactors: 3 NADH and 1 $FADH_2$
- The CAC provides metabolites for gluconeogenesis, fatty acid synthesis, and amino acid synthesis.
- Citric acid cycle intermediates can be replenished by other pathways.
- Some organisms use the glyoxylate cycle, a variant of the citric acid cycle, for the net conversion of acetyl-CoA to oxaloacetate.

Summary of carbohydrate metabolism

- Glycolysis

$$\text{Glucose} + 2\ ADP + 2\ P_i + 2\ NAD^+ \rightarrow 2\ \text{Pyruvate} + 2\ ATP + 2\ NADH + 2\ H^+ + 2\ H_2O$$

- Pyruvate to acetyl-CoA

$$2\ \text{Pyruvate} + 2\ NAD^+ + 2\ \text{CoA} \rightarrow 2\ \text{Acetyl-CoA} + 2\ NADH + 2CO_2$$

- Acetyl-CoA in CAC

$$2\ \text{Acetyl-CoA} + 2\ ADP + 4\ H_2O + 6\ NAD^+ + 2\ FAD + 2\ P_i \rightarrow 2\ \text{CoA} + 2\ ATP + 6\ H^+ + 6\ NADH + 2\ FADH_2 + 4\ CO_2$$

Overall:

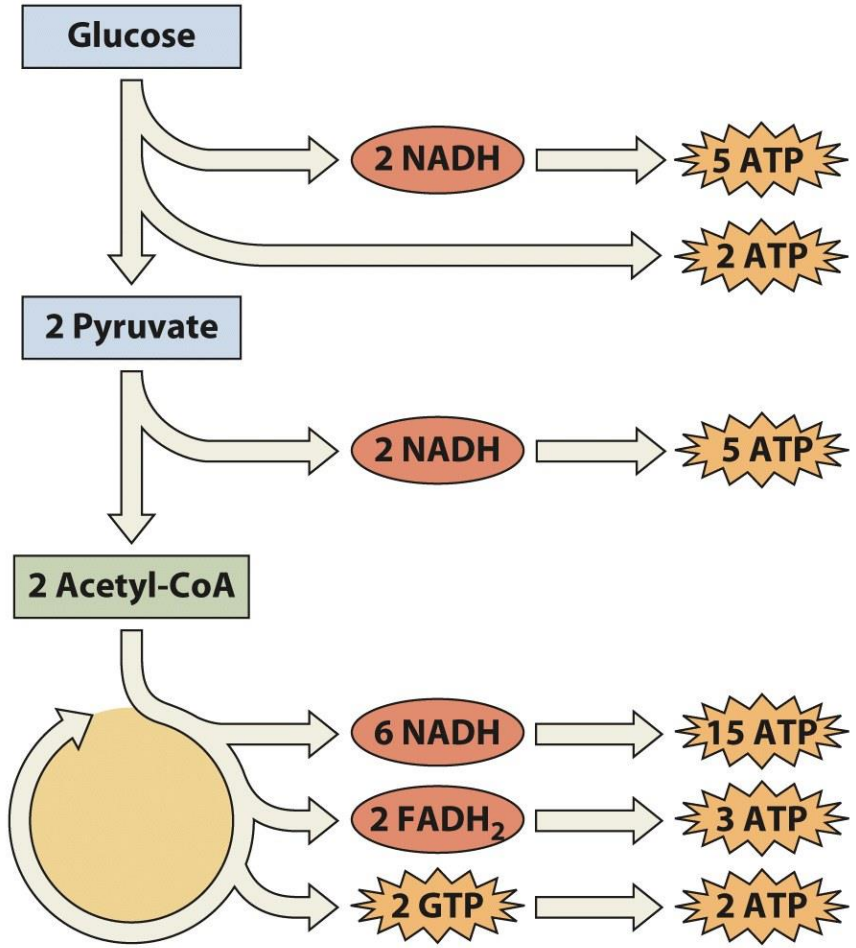
$$\text{Glucose} + 4\ ADP + 4\ P_i + 10\ NAD^+ + 2\ FAD + 2\ H_2O \rightarrow 6\ CO_2 + 10\ NADH + 2\ FADH_2 + 4\ ATP + 10\ H^+$$

Notes for exam prep

- Compare PPP and glyoxalate cycles, as both are shortcut pathways.
- Note which metabolic pathways each one is linked to.

Electrons are Funneled into ATP Synthesis from glycolysis

- From 1 molecule of glucose going through glycolysis followed by citric acid cycle to complete oxidation in ECR-OxPhos, the theoretical amount of energy produced is:32 ATPs, using:
  - ❖ 1 NADH ~ 2.5 ATPs
  - ❖ 1 $FADH_2$ ~ 1.5 ATPs
- Of these 20 ATPs (62.5%) come from CAC alone.
- So, although glycolysis and CAC are anaerobic, max. energy extraction comes for aerobic reactions.



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