Citric acid cycle

Chapter 19

The Central Role of the Citric Acid Cycle

3 processes play central roles in aerobic metabolism

- The citric acid cycle (TCA cycle)
- Electron transport (Chapter 20)
- Oxidative phosphorylation (Chapter 20)

- Metabolism consists of
  - **Catabolism:** the oxidative breakdown of nutrients
  - **Anabolism:** the reductive synthesis of biomolecules

- The citric acid cycle is **amphibolic** that is, it plays a role in both catabolism and anabolism. *It is the central metabolic pathway*

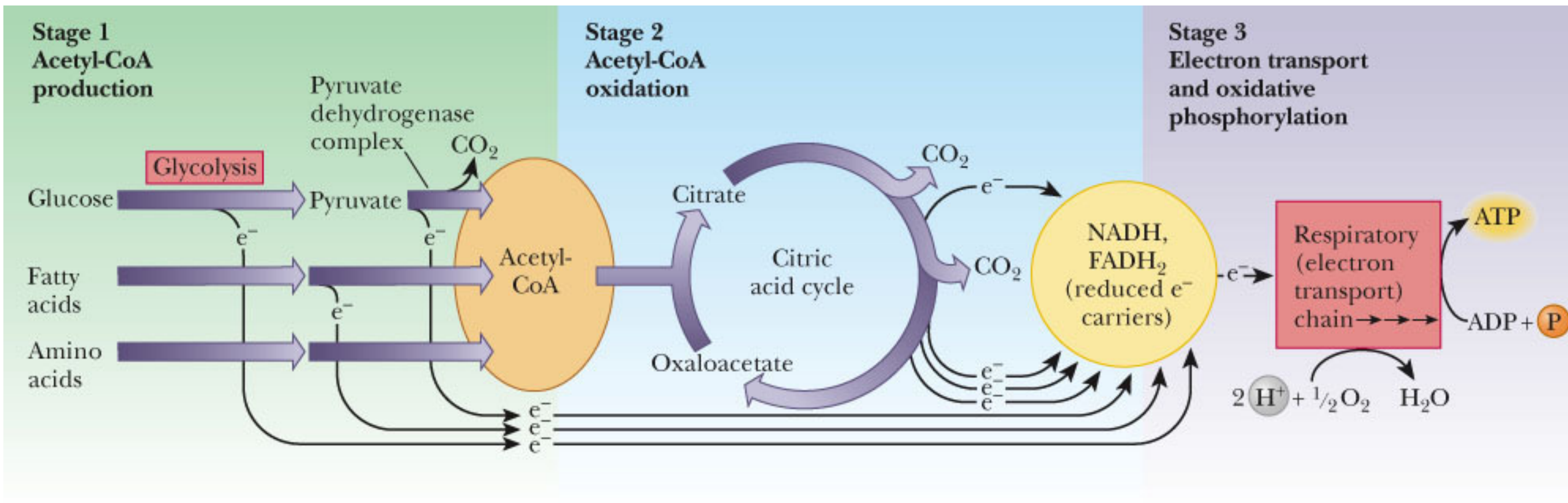
The Citric Acid Cycle

- **TCA CYCLE (**tricarboxylic acid cycle)
- **KREBS CYCLE**

- **Definition**:
  - Acetate in the form of *acetyl-CoA*, is derived from pyruvate and other metabolites, and is ***oxidized*** to CO_2

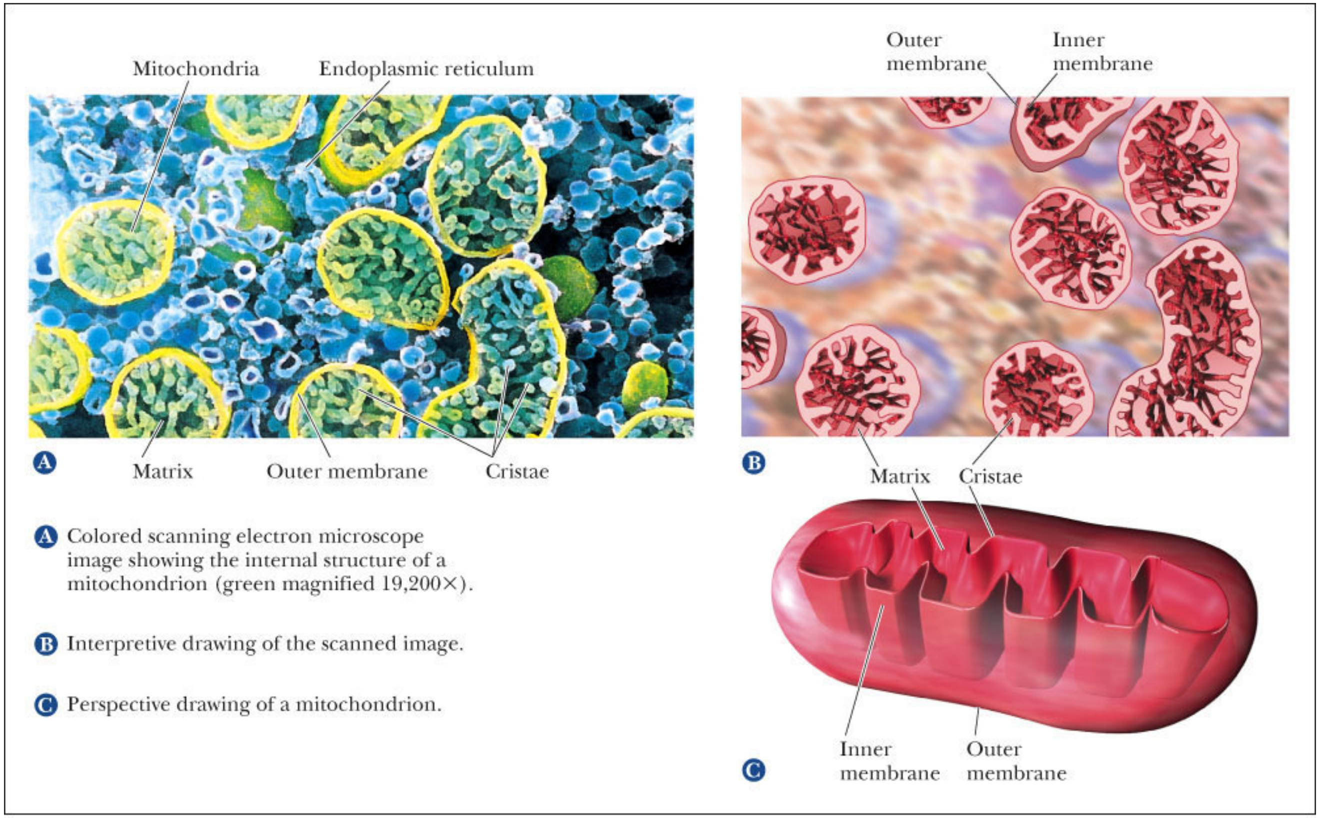
- The citric acid cycle is *central to all respiratory oxidation*
  - oxidizing acetyl-CoA from glucose, lipid and protein catabolism into aerobic respiration to maximize energy gain.

Relationship of TCA Cycle to Catabolism



Where does the Citric Acid Cycle Take Place?

- The cycle also supplies some *precursors* for biosynthesis.
- In eukaryotes, All enzymes are in the mitochondrial matrix or inner mitochondrial membrane

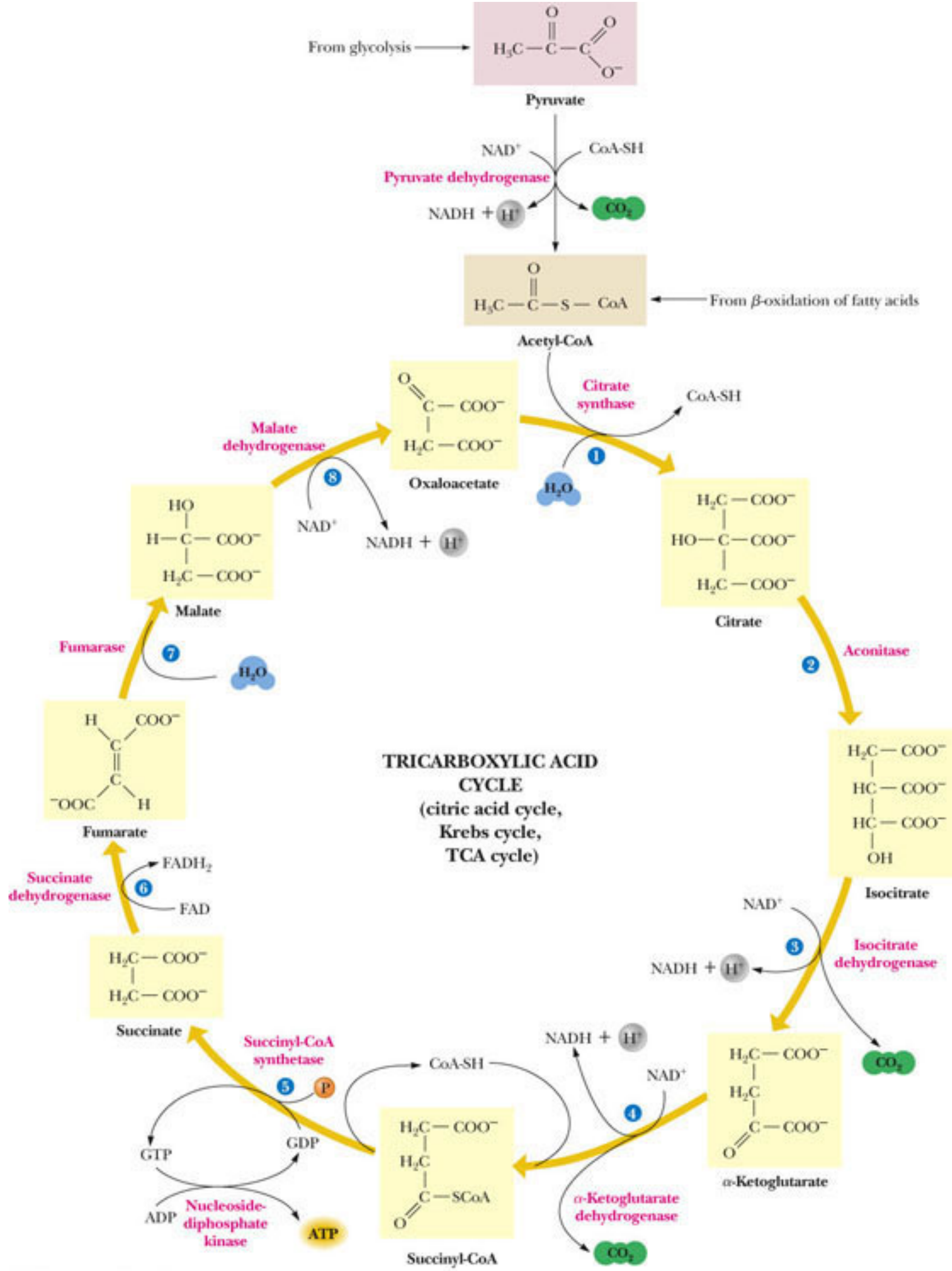


A Colored scanning electron microscope image showing the internal structure of a mitochondrion (green magnified 19,200×).

B Interpretive drawing of the scanned image.

C Perspective drawing of a mitochondrion.

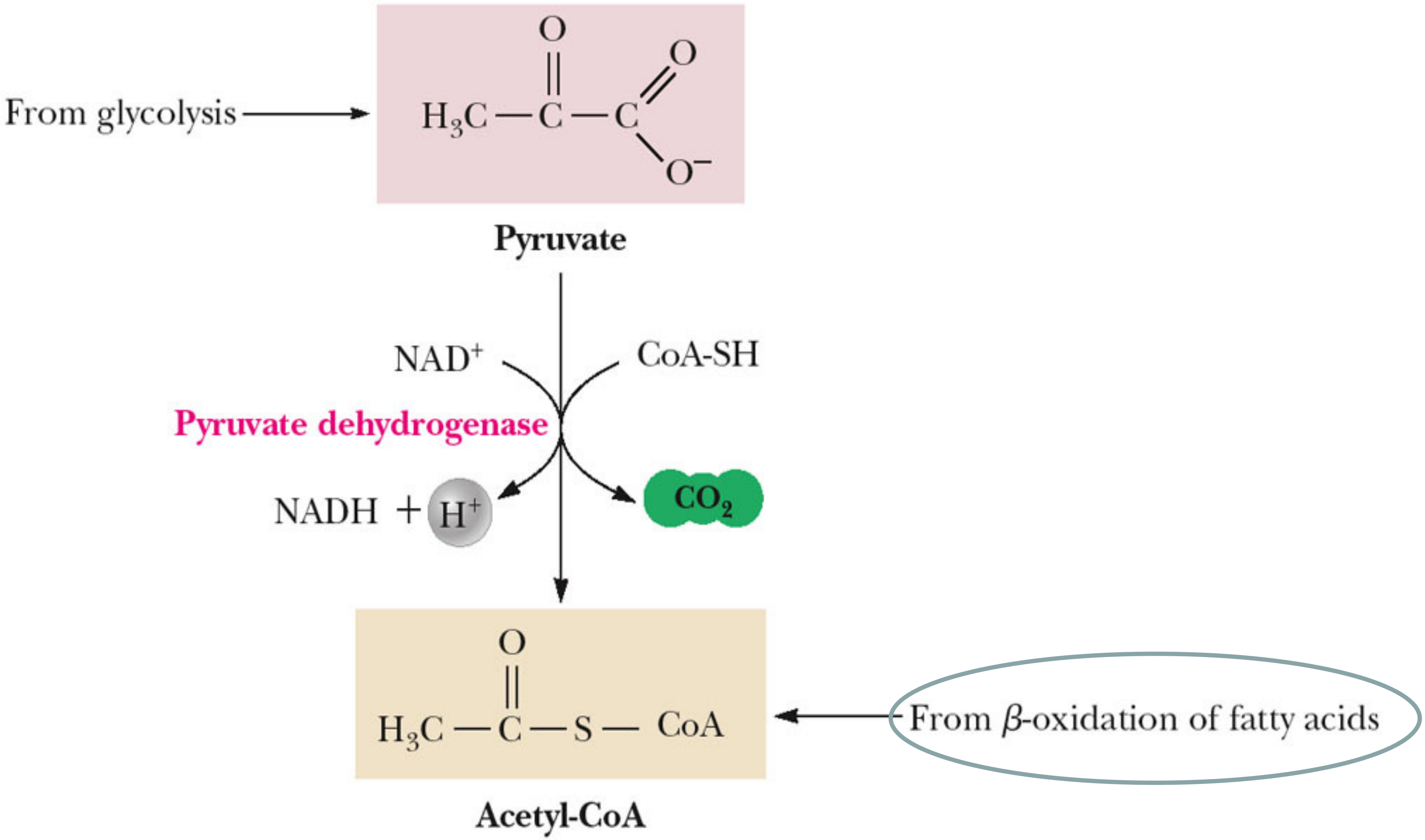
Features of TCA Cycle



The Citric Acid Cycle

- **Before cycle**
  - Conversion of pyruvate to acetyl-CoA

- **Stage 1 setup**
  - Enzyme = Pyruvate dehydrogenase complex
  - Location = Mmitochondrial matrix
    - irreversible means acetyl-CoA cannot flow backward to pyruvate

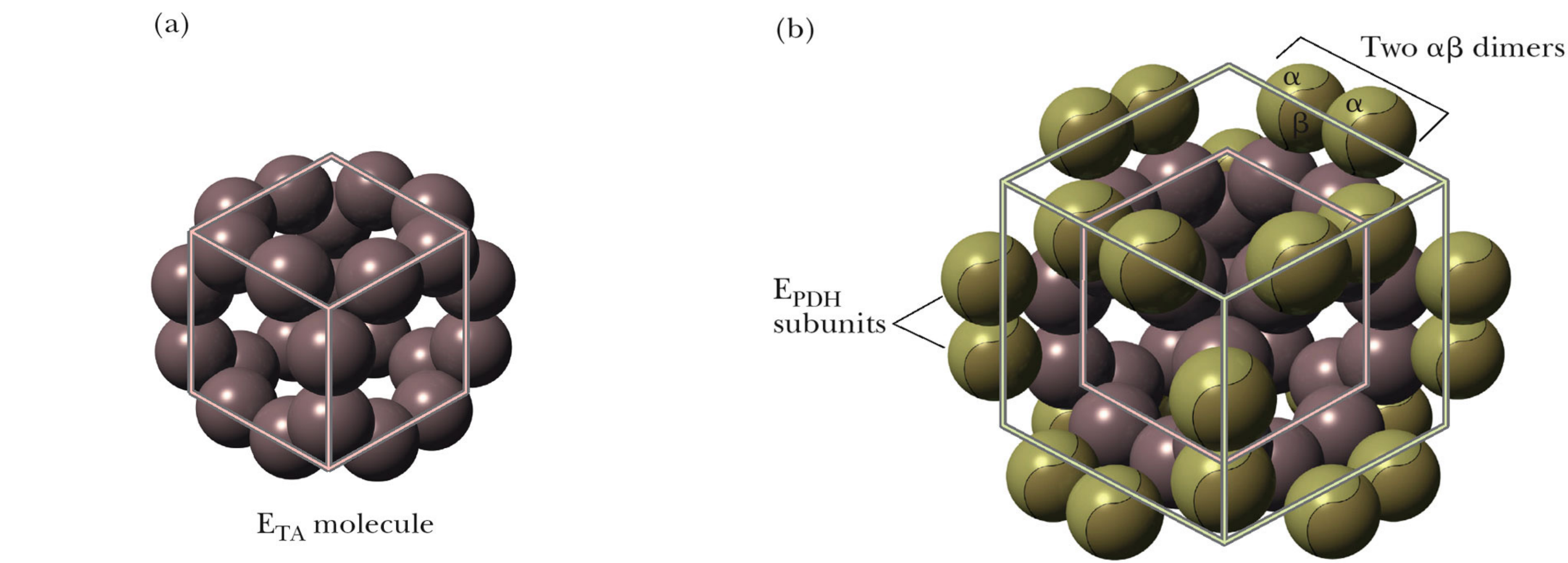


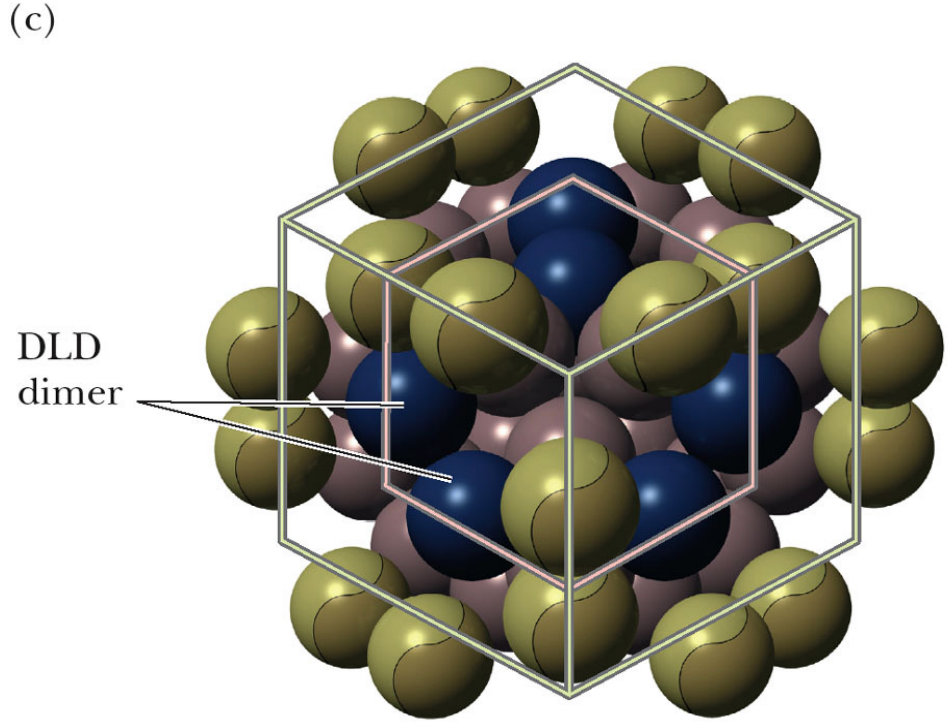
An overview of the citric acid cycle:
Note the names of the enzymes. The loss of CO2 is indicated, as is the phosphorylation of GDP to GTP. The production of NADH and FADH2 is also indicated.

Pyruvate is Converted to Acetyl-CoA

- Pyruvate dehydrogenase complex is responsible for the conversion of pyruvate to CO_2 and the acetyl portion of acetyl-CoA

- **Five enzymes in complex:**
  - Pyruvate dehydrogenase (=**E1**) has
    - Coenzyme = ***Thiamine pyrophosphate*** (TPP)
      - *Pyruvate dehydrogenase kinase*
      - *Pyruvate dehydrogenase phosphatase*
  - Dihydrolipoyl transacetylase (=**E2**)
    - Coenzymes ***lipoate*** and CoA
  - Dihydrolipoyl dehydrogenase (= **E3**)
    - Coenzymes ***FAD*** and ***NAD+***





The structure of the pyruvate dehydrogenase complex.:

(a) 24 dihydrolipoyl transacetylase (TA) subunits.

(b) 24 αβ dimers of pyruvate dehydrogenase are added to the cube two per edge).

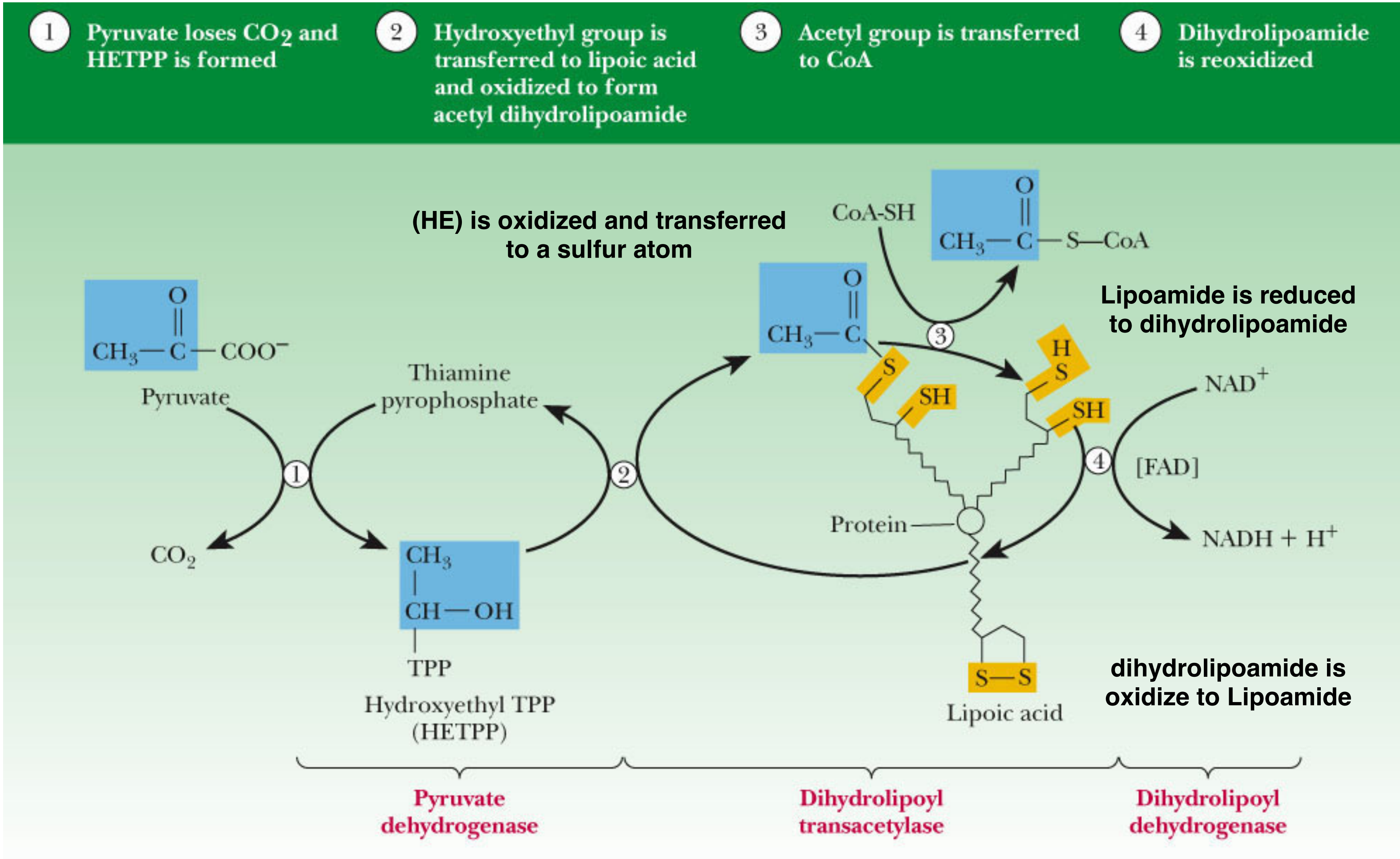
(c) Addition of 12 dihydrolipoyl dehydrogenase subunits (two per face) completes the complex.

(d) Total = 60 subunits

Pyruvate is Converted to Acetyl-CoA

- First, pyruvate loses CO_2 and hydroxyethylTPP (HETPP) is formed
- In the second step, the active form of lipoic acid is bound to the enzyme, **dihydrolipoyl transacetylase**, by an amide bond
  - The hydroxyethyl group (HE) is oxidized and transferred to a sulfur atom of the reduced form of lipoamide
  - Lipoamide is reduced to **dihydrolipoamide**
- In step 3, the acetyl group is transferred to the sulfur group of **coenzyme A**
  - Next, dihydrolipoamide is oxidized to **lipoamide**

Mechanism of the Pyruvate Dehydrogenase Complex

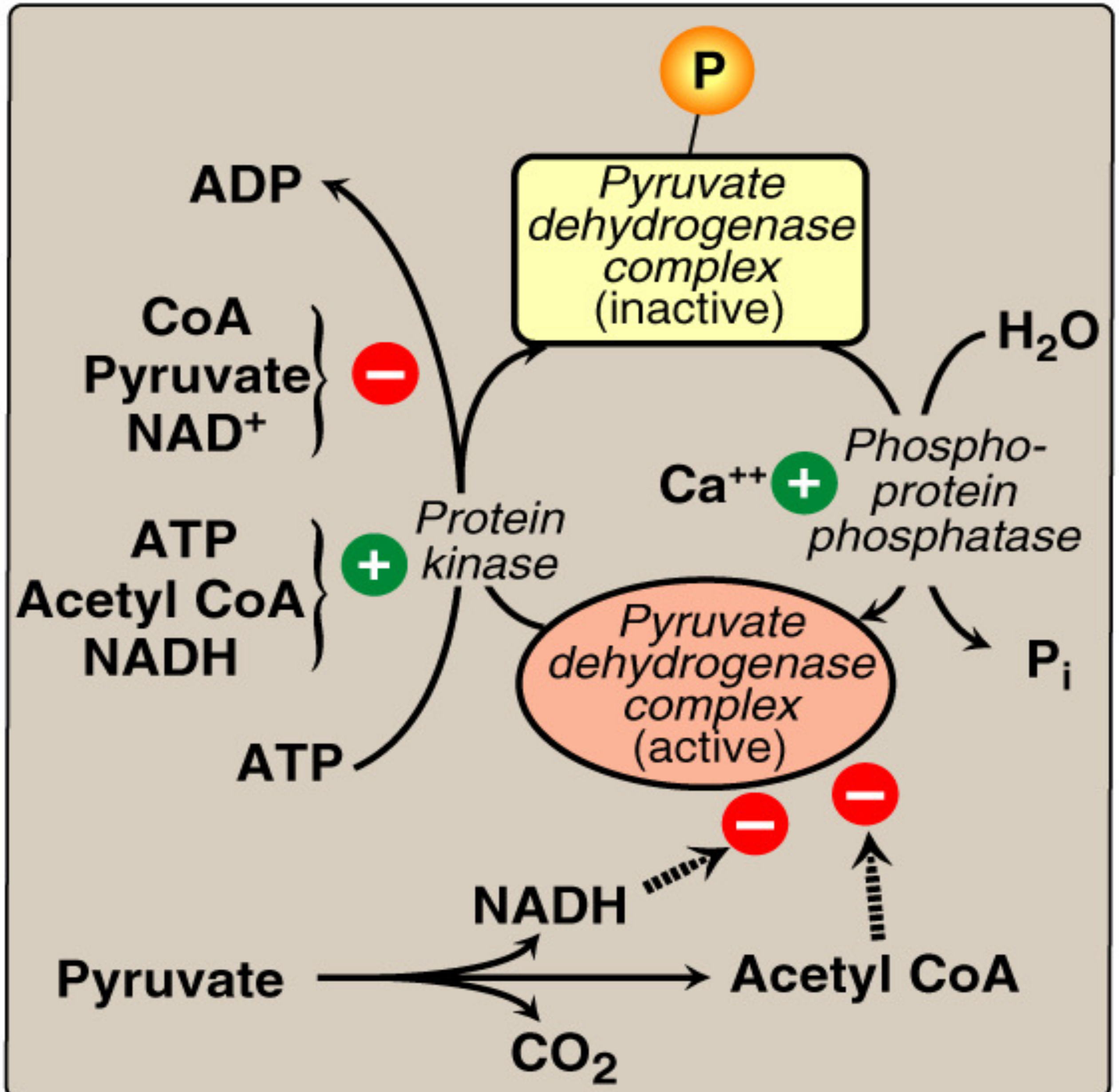


Regulation of Pyruvate Dehydrogenase

- Irreversible reaction must be tightly Controlled

- Three ways
  1. **Allosteric Inhibition**
     - Inhibited by products: acetyl-CoA, NADH &
     - high ATP
  2. **Allosteric activation by AMP**
     - Ratio ATP/AMP important
  3. **Phosphorylation - dephosphorylation** of E1 subunit

Pyruvate Dehydrogenase Regulation



Summary

- The two-carbon unit needed at the start of the citric acid cycle is obtained by converting pyruvate to acetyl-CoA
    - This conversion requires the three primary enzymes of the pyruvate dehydogenase complex, as well as, the cofactors TPP, FAD, NAD+, and lipoic acid
- The overall reaction of the pyruvate dehydogenase complex is the conversion of pyruvate, NAD^+, and CoA-SH to acetyl-CoA, NADH + H^+, and CO_2

Krebs Cycle: Preparatory Step

- Pyruvic acid is converted to acetyl CoA in three main steps:
  - Decarboxylation
    - Carbon is removed from pyruvic acid
    - Carbon dioxide is released
  - Oxidation
    - Hydrogen atoms are removed from pyruvic acid
    - NAD+ is reduced to NADH + H+
  - Formation of acetyl CoA – the resultant acetic acid is combined with coenzyme A, a sulfur-containing coenzyme, to form acetyl CoA

Krebs Cycle

- An eight-step cycle in which acetic acid is decarboxylated and oxidized, generating:
  - **Three molecules of NADH + H^+**
  - **One molecule of $FADH_2$**
  - **Two molecules of CO_2**
  - **One molecule of GTP=ATP**

- For each molecule of glucose entering glycolysis, two molecules of acetyl CoA enter the Krebs cycle

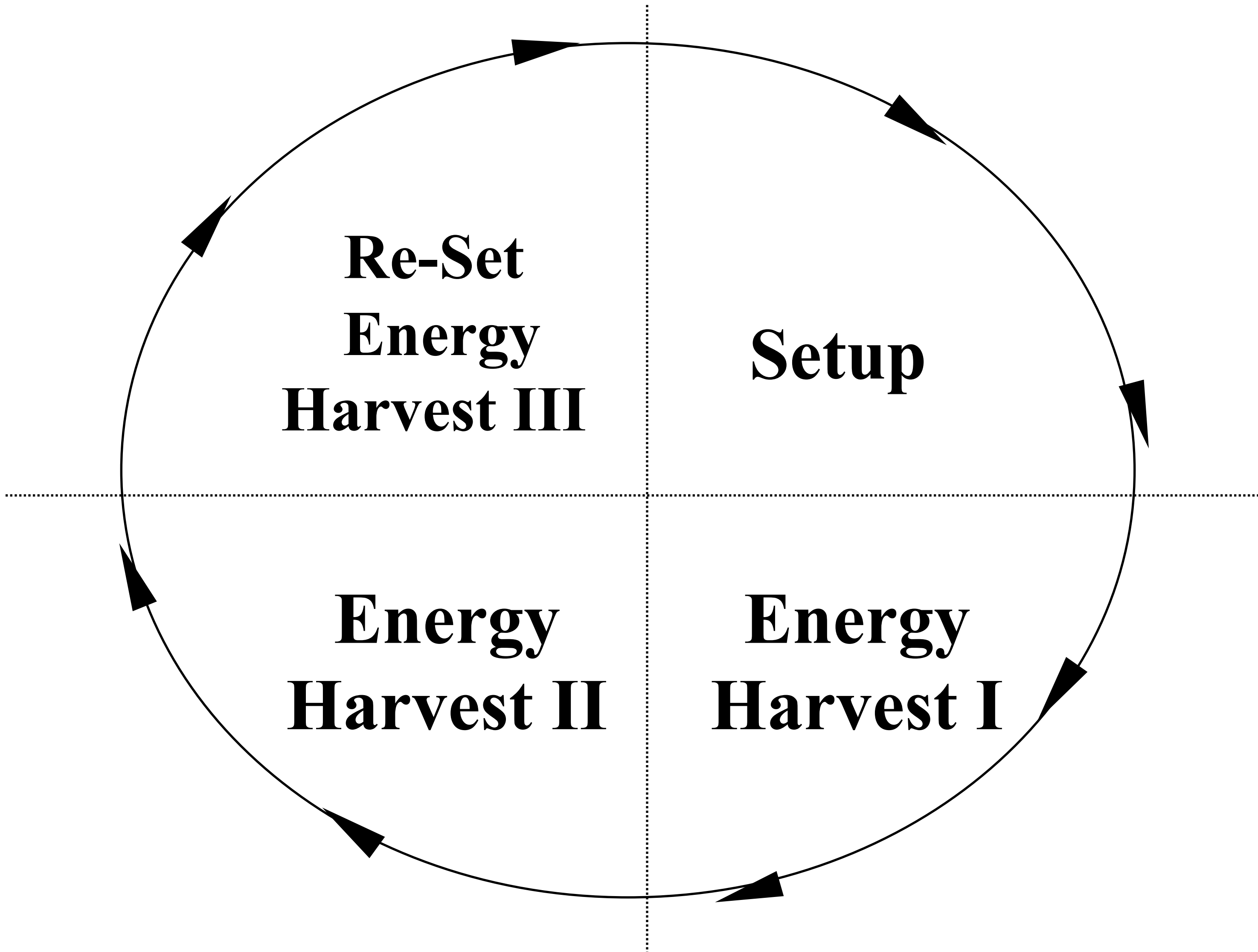
General Features

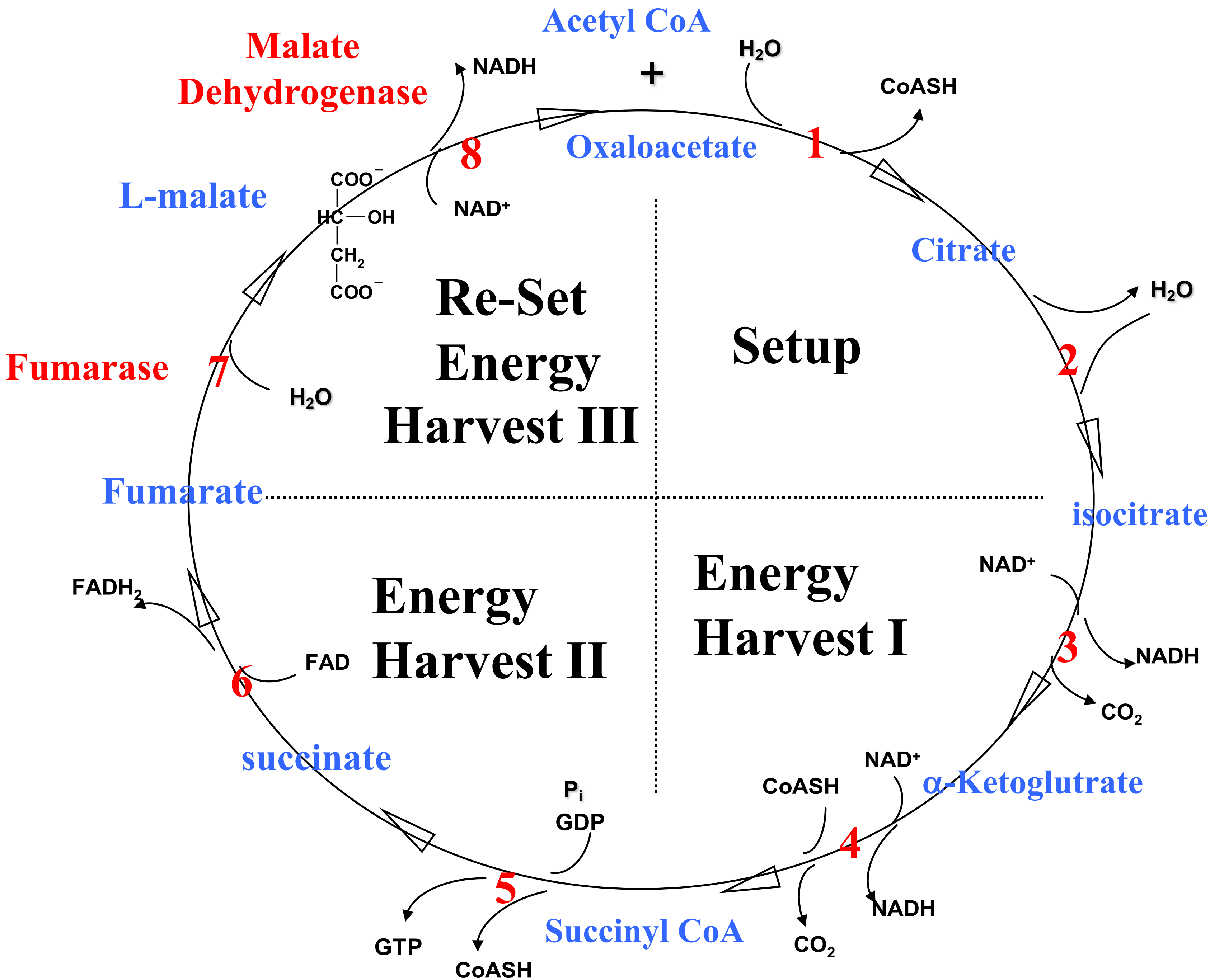
- Location
  - Eukaryotes
    - mitochondrial matrix and membrane (one step only)
  - Prokaryotes
    - Plasma membrane
- Overall reaction

$$\mathrm{CH_3}\underset{\underset{\mathrm{O}}{\|}}{\mathrm{C}}\mathrm{SCoA} + \mathbf{3\ NAD^+} + \mathbf{FAD} + \mathbf{GDP} + \mathbf{P_i} + \mathbf{2\ H_2O}$$

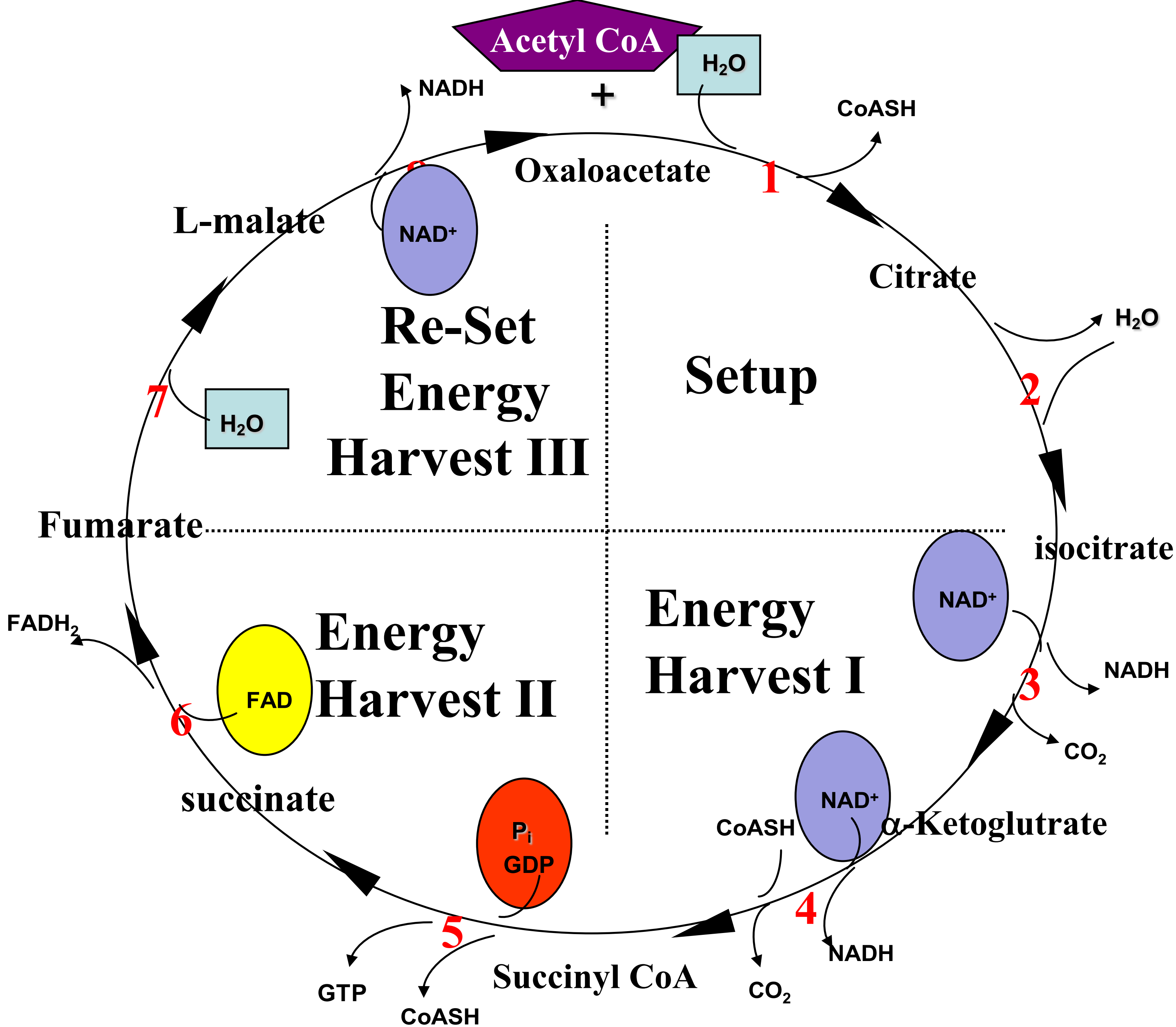
$$\Downarrow$$

$$2\ \mathrm{CO_2} + \mathrm{HSCoA} + \mathbf{3\ NADH} + \mathbf{FADH_2} + \mathbf{GTP} + \mathbf{2\ H^+}$$

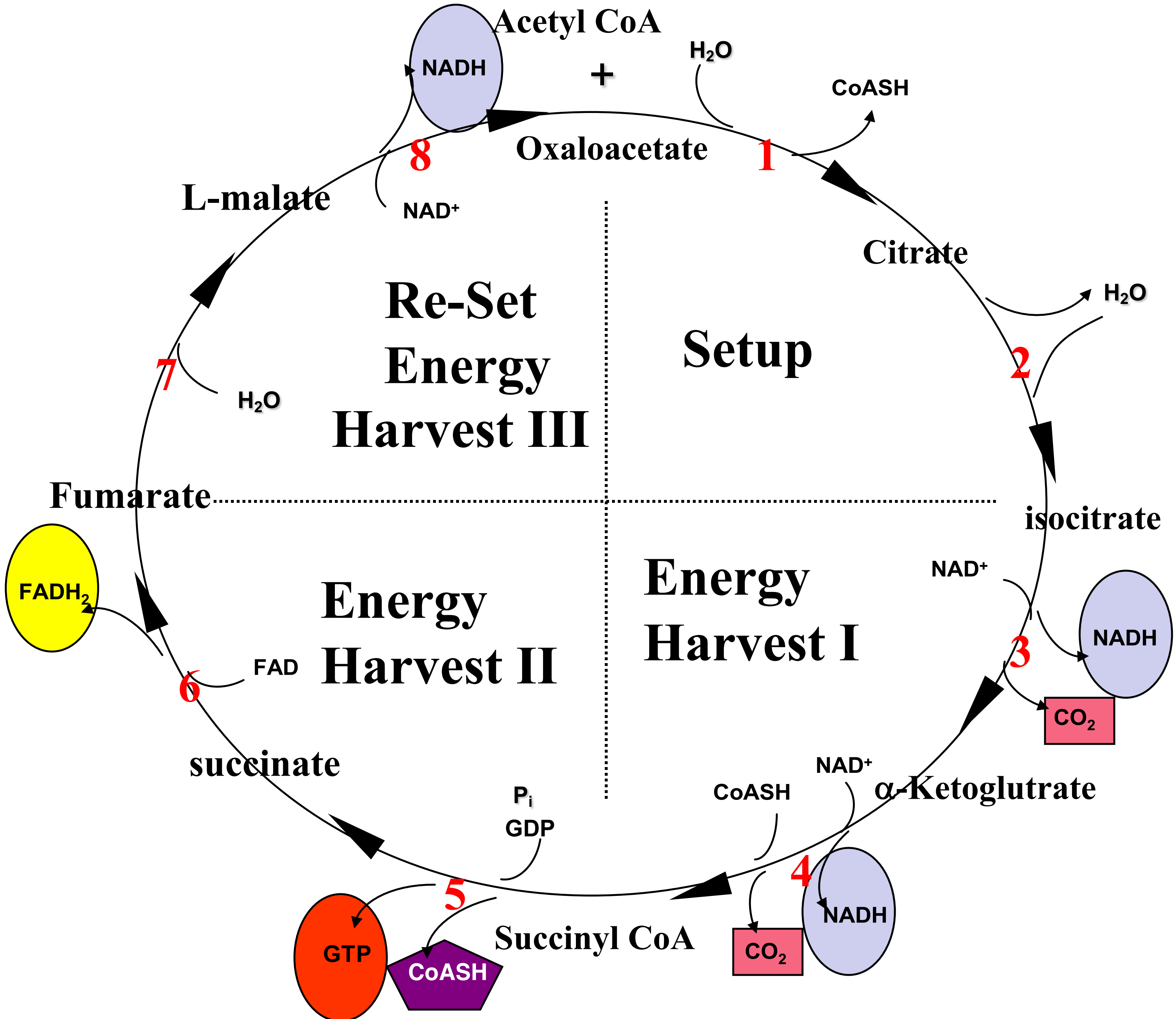

Re-Set
Energy
Harvest III
Setup
Energy
Harvest II
Energy
Harvest I



Acetyl CoA
+
H_2O
CoASH
1
Malate
Dehydrogenase
NADH
8
Oxaloacetate
NAD+
L-malate
COO⁻
HC—OH
CH_2
COO⁻
Citrate
H_2O
2
Re-Set
Energy
Harvest III
Setup
Fumarase
7
H_2O
Fumarate
isocitrate
NAD+
3
NADH
CO_2
$FADH_2$
FAD
6
Energy
Harvest II
Energy
Harvest I
succinate
P_i
GDP
CoASH
NAD+
α-Ketoglutrate
4
NADH
CO_2
5
GTP
CoASH
Succinyl CoA



Acetyl CoA
+
H2O
CoASH
Oxaloacetate
1
Citrate
H2O
2
isocitrate
NAD+
NADH
3
CO2
α-Ketoglutrate
NAD+
CoASH
4
CO2
NADH
Succinyl CoA
Pi
GDP
5
GTP
CoASH
succinate
FAD
6
FADH2
Fumarate
H2O
7
L-malate
NAD+
NADH
Setup
Energy
Harvest I
Energy
Harvest II
Re-Set
Energy
Harvest III


Acetyl CoA
+
NADH
H_2O
CoASH
Oxaloacetate
1
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2
Re-Set
Energy
Harvest III
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7
H_2O
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isocitrate
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NADH
3
CO_2
$FADH_2$
FAD
6
Energy
Harvest II
Energy
Harvest I
succinate
P_i
GDP
CoASH
NAD^+
α-Ketoglutrate
4
5
GTP
CoASH
Succinyl CoA
CO_2
NADH

Summary

- In the citric acid cycle and the pyruvate dehydrogenase reaction, one molecule of pyruvate is oxidized to three molecules of CO_2 as a result of oxidative decarboxylation

- The oxidations are accompanied by reductions involving NAD^+ to NADH & FAD to $FADH_2$

- GDP is phosphorylated to GTP

Oxidation of Pyruvate Forms CO_2 and ATP

Pyruvate dehydrogenase complex:

$$\text{Pyruvate} + \text{CoA-SH} + \text{NAD}^+ \rightarrow \text{Acetyl-CoA} + \text{NADH} + \text{CO}_2 + \text{H}^+$$

Citric acid cycle:

$$\text{Acetyl-CoA} + 3\text{NAD}^+ + \text{FAD} + \text{GDP} + \text{P}_i + 2\text{H}_2\text{O} \rightarrow$$

$$2\text{CO}_2 + \text{CoA-SH} + 3\text{NADH} + 3\text{H}^+ + \text{FADH}_2 + \text{GTP}$$

Overall reaction:

$$\text{Pyruvate} + 4\text{NAD}^+ + \text{FAD} + \text{GDP} + \text{P}_i + 2\text{H}_2\text{O} \rightarrow$$

$$3\text{CO}_2 + 4\text{NADH} + \text{FADH}_2 + \text{GTP} + 4\text{H}^+$$

Eventual ATP production per pyruvate:

$$4\text{NADH} \rightarrow 10\text{ATP} \ (2.5\text{ATP for each NADH})$$

$$1\text{FADH} \rightarrow 1.5\text{ATP} \ (1.5\text{ATP for each FADH}_2)$$

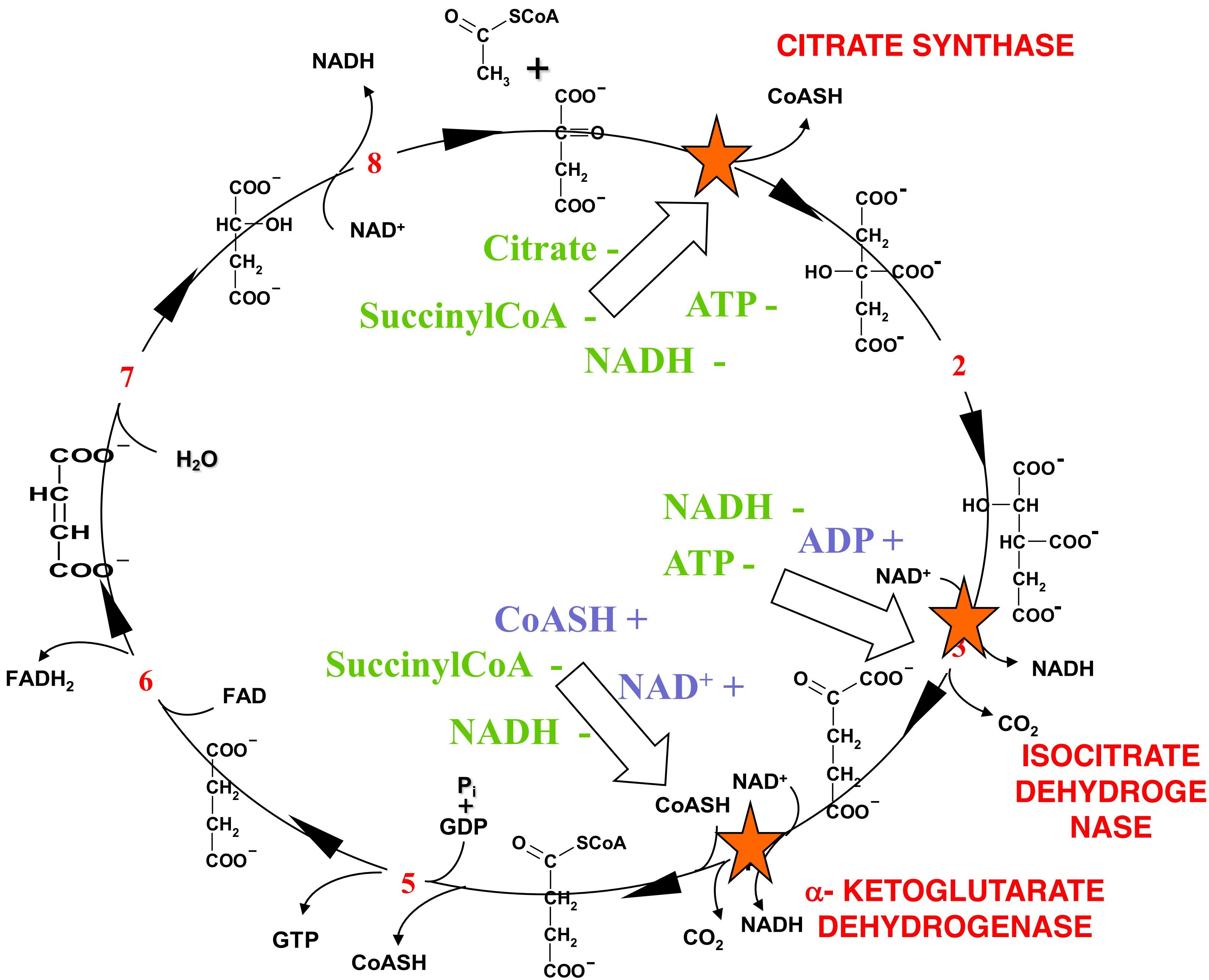
$$1\text{GTP} \rightarrow 1\text{ATP}$$

Total 12.5 ATP per pyruvate or 25 ATP per glucose

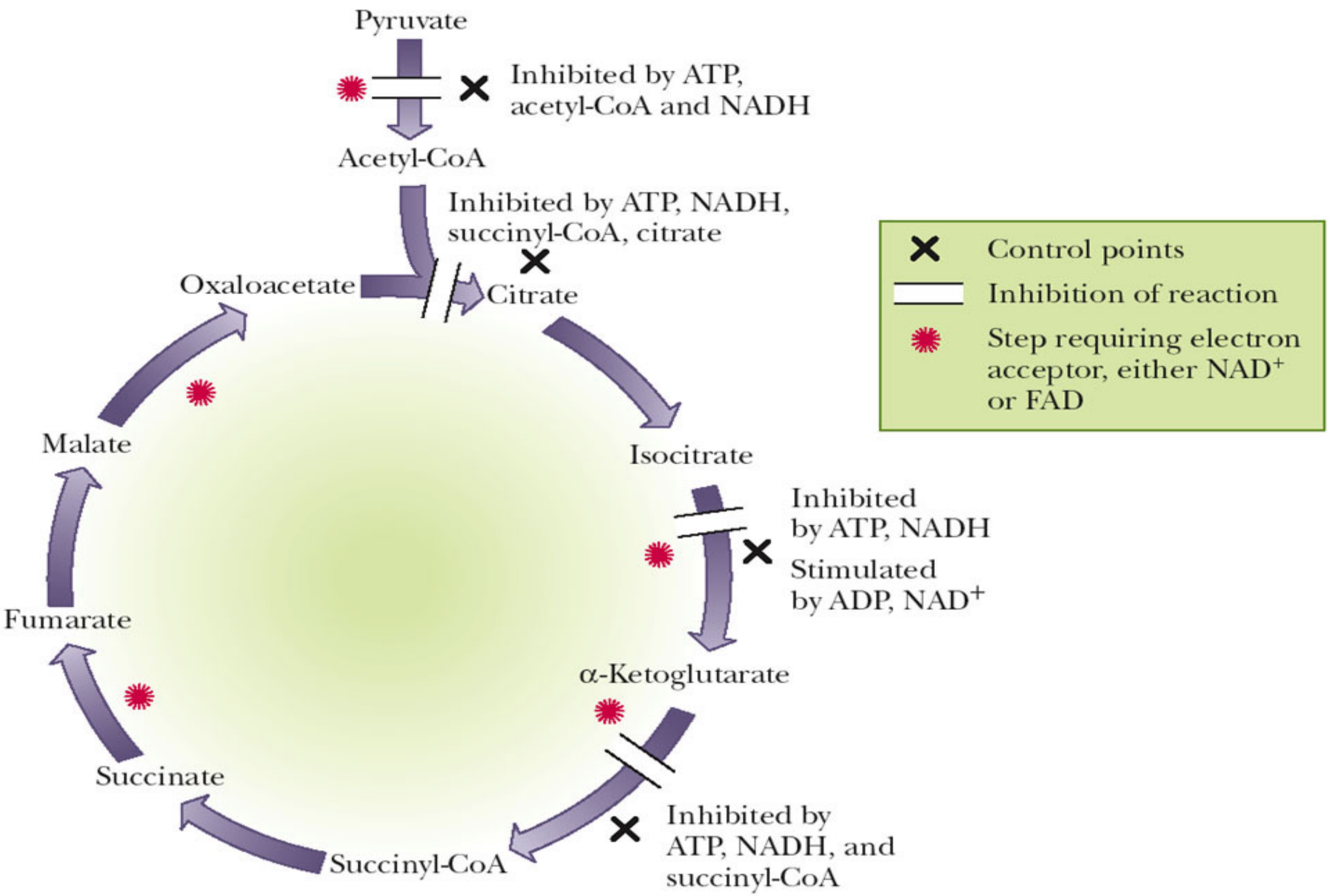
Control of the Citric Acid Cycle

- There are 3 points of control within the cycle:
  1. **Citrate synthase:** inhibited by ATP, NADH, and succinyl CoA; also product inhibition by citrate
  2. **Isocitrate dehydrogenase:** activated by ADP and NAD^+, inhibited by ATP and NADH
  3. **α-ketoglutarate dehydrogenase complex:** inhibited by ATP, NADH, and succinyl CoA; activated by ADP and NAD^+

- There is one control point outside the cycle
  - **Pyruvate dehydrogenase:** inhibited by ATP and NADH; also product inhibition by acetyl-CoA


CITRATE SYNTHASE
ISOCITRATE
DEHYDROGE
NASE
α- KETOGLUTARATE
DEHYDROGENASE
Citrate -
SuccinylCoA -
ATP -
NADH -
NADH -
ADP +
ATP -
CoASH +
SuccinylCoA -
NAD+ +
NADH -
CoASH
NADH
NAD+
FADH2
FAD
H2O
GTP
Pi
+
GDP
CO2
2
5
6
7
8

Control of the Citric Acid Cycle



Energetics of the Citric Acid Cycle

Table 19.2

The Energetics of Conversion of Pyruvate to CO_2

Step	Reaction	$\Delta G°'$ kJ mol^{-1}	$\Delta G°'$ kcal mol^{-1}
	Pyruvate + CoA-SH + NAD^+ → Acetyl-CoA + NADH + CO_2	–33.4	–8.0
1	Acetyl-CoA + Oxaloacetate + H_2O → Citrate + CoA-SH + H^+	–32.2	–7.7
2	Citrate → Isocitrate	+6.3	+1.5
3	Isocitrate + NAD^+ → α-Ketoglutarate + NADH + CO_2 + H^+	–7.1	–1.7
4	a-Ketoglutarate + NAD^+ + CoA-SH → Succinyl-CoA + NADH + CO_2 + H	–33.4	–8.0
5	Succinyl-CoA + GDP + P_i → Succinate + GTP + CoA-SH	–3.3	–0.8
6	Succinate + FAD → Fumarate + $FADH_2$	~0	~0
7	Fumarate + H_2O → L-Malate	–3.8	–0.9
8	L-Malate + NAD^+ → Oxaloacetate + NADH + H^+	+29.2	+7.0
Overall:	Pyruvate + 4NAD^+ + FAD + GDP + P_i + 2H_2O → CO_2 + 4NADH + $FADH_2$ + GTP + 4H^+	–77.7	–18.6

Control of the Citric Acid Cycle (Cont’d)

Table 19.3

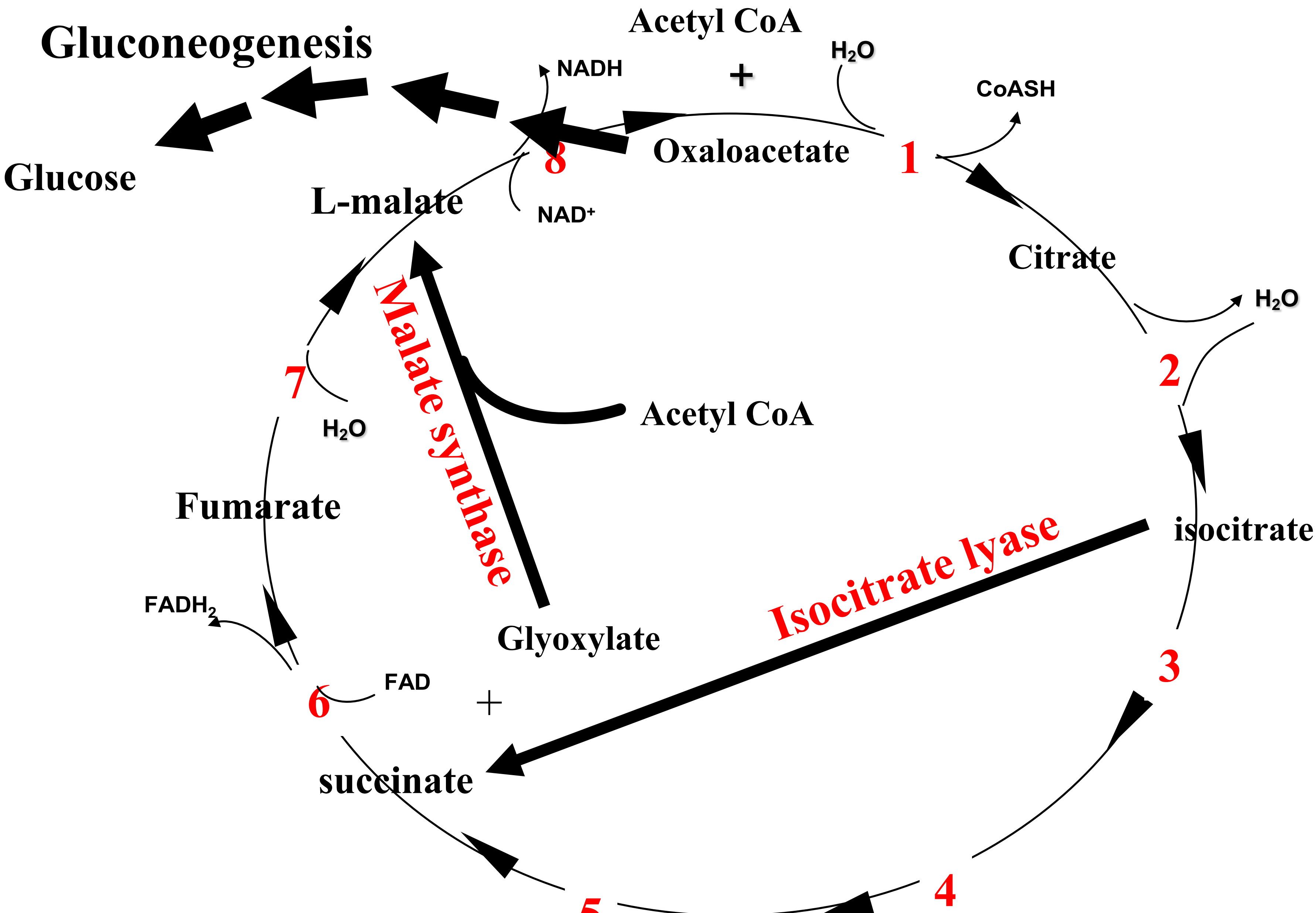
Relationship between the Metabolic State of a Cell and the ATP/ADP and $NADH/NAD^+$ Ratios

Cells in a resting metabolic state
Need and use comparatively little energy
High ATP, low ADP levels imply high (ATP/ADP)
High NADH, low NAD^+ levels imply high ($NADH/NAD^+$)
Cells in a highly active metabolic state
Need and use more energy than resting cells
Low ATP, high ADP levels imply low (ATP/ADP)
Low NADH, high NAD^+ levels imply low ($NADH/NAD^+$)

The glyoxylate cycle

- In plants and some bacteria (NOT animals) acetyl Co-A can serve as the starting material for the biosynthesis of CHO
  - Animals can’t convert fats to CHO

- Important
  - In seed germination
    - Seeds are rich in lipids, which contain fatty acids
    - During germination, plants use the acetyl-CoA produced in fatty acid oxidation to produce oxaloacetate and other intermediates for carbohydrate synthesis
  - In some bacteria used during limitation of carbon source



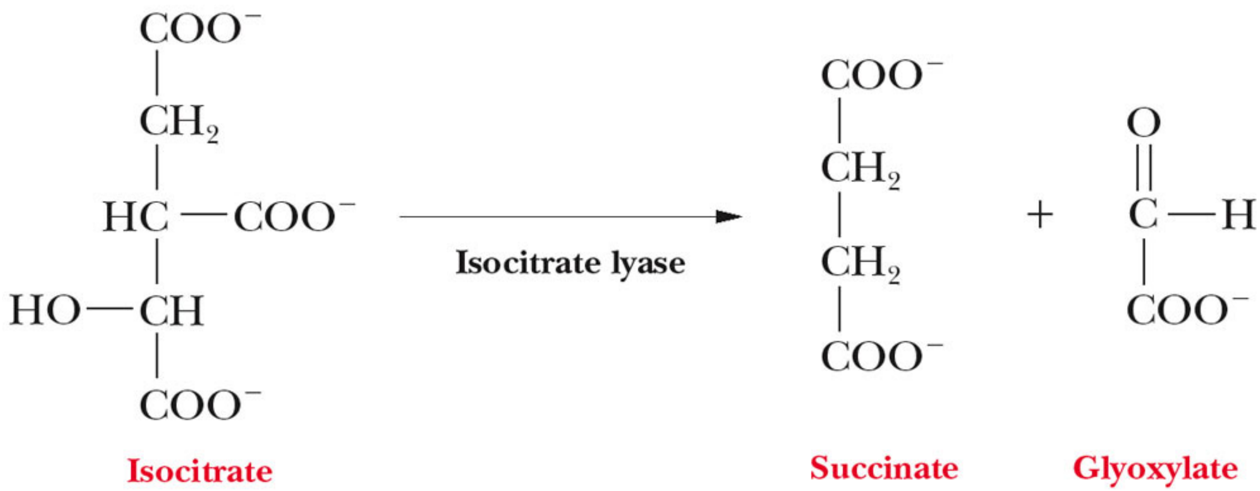
Gluconeogenesis
Glucose
Acetyl CoA
+
H_2O
NADH
CoASH
Oxaloacetate
1
8
L-malate
NAD^+
Citrate
H_2O
2
Malate synthase
Acetyl CoA
7
H_2O
isocitrate
Fumarate
Isocitrate lyase
$FADH_2$
Glyoxylate
3
6
FAD
+
succinate
4
5

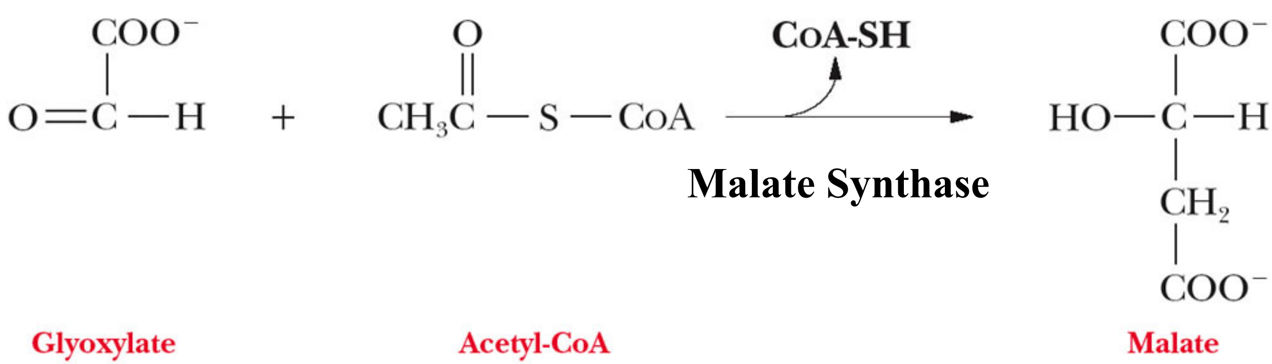
The Glyoxylate Cycle

- The glyoxylate cycle bypasses the two oxidative decarboxylations of the citric acid cycle
  - Instead, it routes isocitrate via glyoxylate to malate
  - Key enzymes in this cycle are
    - Isocitrate lyase
    - Malate synthase

- **The glyoxylate cycle takes place:**
  - In plant: in glyoxysomes, specialized organelles devoted to this cycle
  - In bacteria in the cytoplasm

The Glyoxylate Cycle



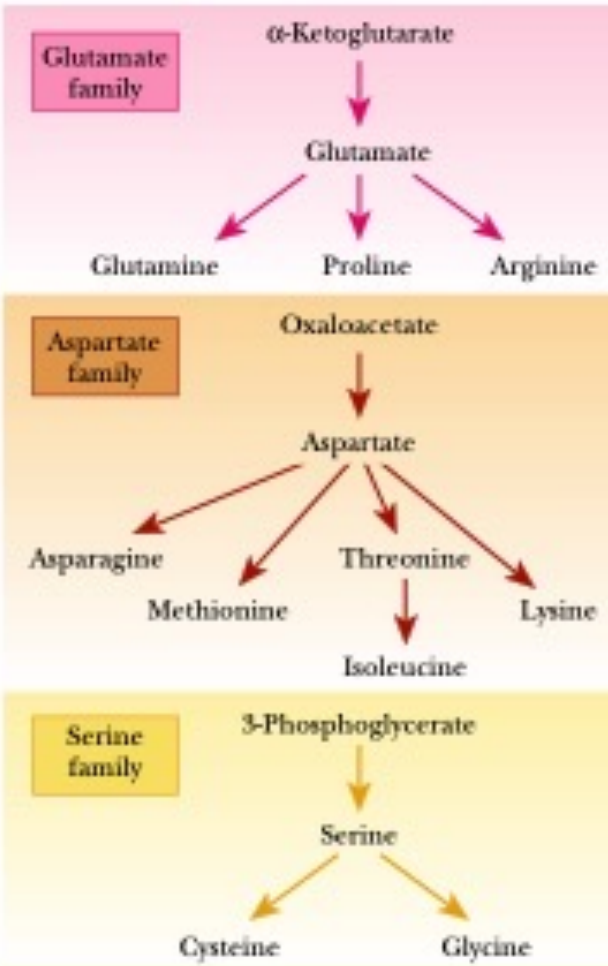


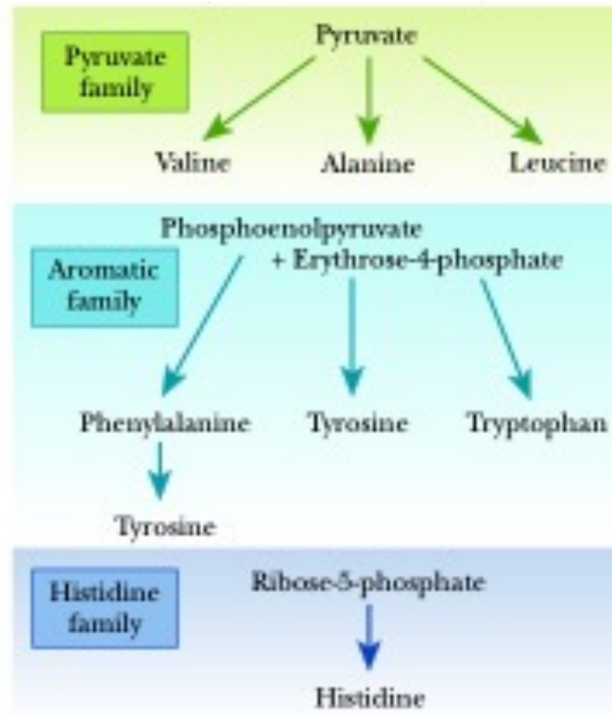
The link to oxygen

- The citric acid cycle is considered part of the aerobic metabolic process because of its link to the chemical nutrients and ATP

- **NADH and FADH$_2$**, two important cofactors generated by the citric acid cycle, ultimately pass their electrons to oxygen

The link to amino acid metabolism





The Link To Amino Acid Metabolism