Lecture2 Citric Acid Cycle Tricarboxylic acid cycle (TCA cycle or Krebs cycle)

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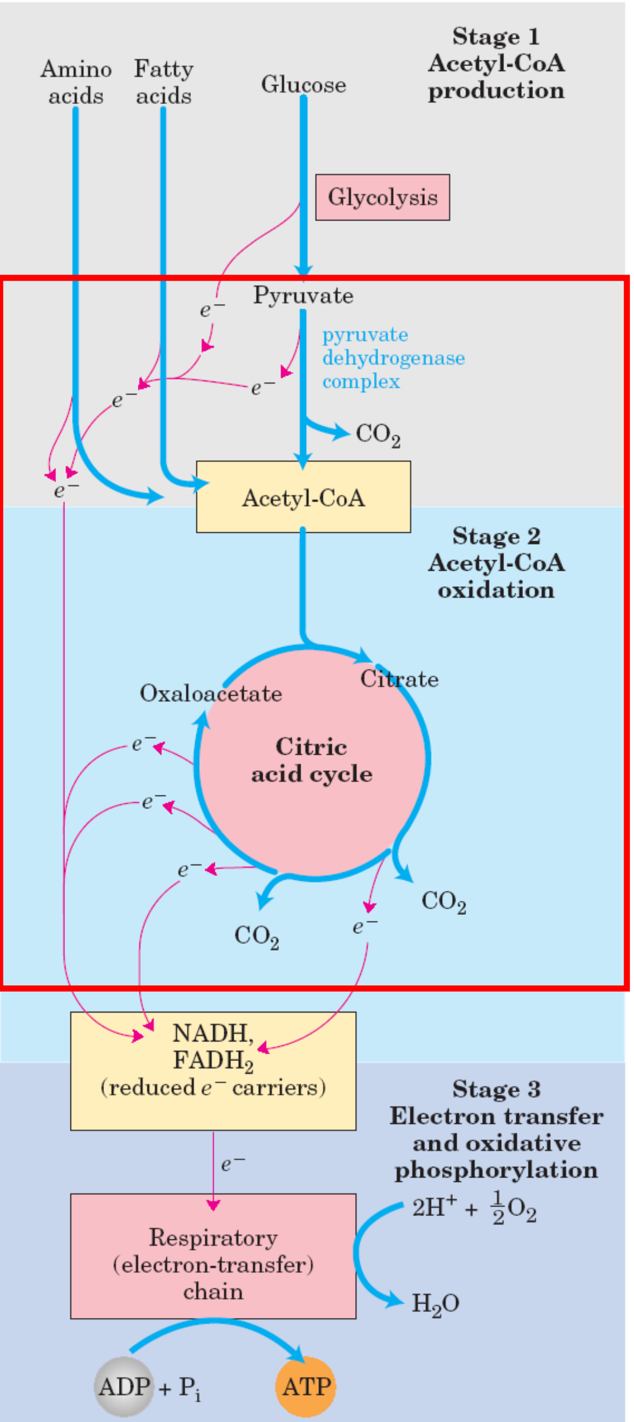

outline

1 Introduction

2 Production of Acetyl-CoA

3 Reactions of the Citric Acid Cycle

4 Regulation of the Citric Acid Cycle

5 The Glyoxylate Cycle(乙醛酸循环）

1. Introduction

The tricarboxylic acid (TCA) cycle is also known as the Krebs cycle or the citric acid cycle.

Hans Krebs, 1900–1981

- **1953 Nobel Laureate in Medicine**

  for his discovery of the citric acid cycle

- ***Background***

  1900-1981
  Place of Birth: Hildesheim, Germany
  Residence: Great Britain
  Affiliation: Sheffield University

HANS ADOLF KREBS

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acid cycle was
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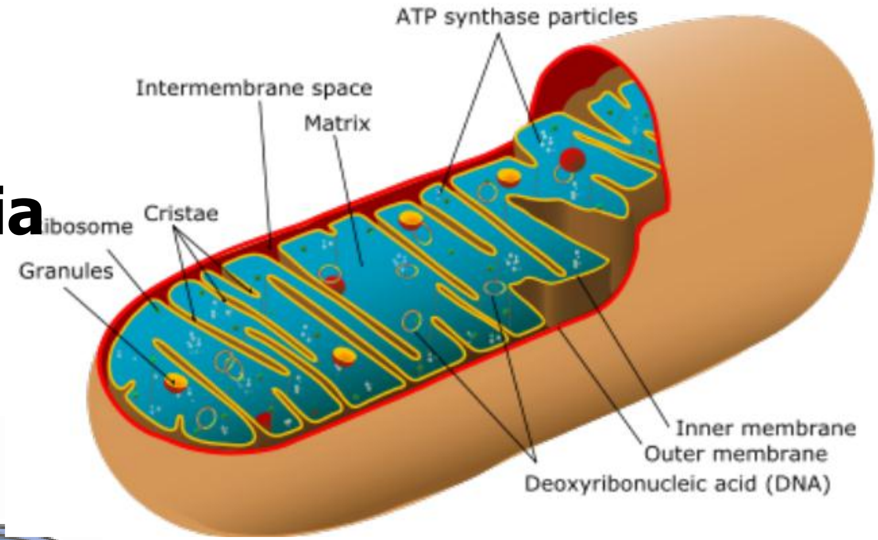
The Editor of NATURE presents his compliments to Mr. H. A. Krebs and regrets that as he has already sufficient letters to fill the correspondence columns of NATURE for seven or eight weeks, it is undesirable to accept further letters at the present time on account of the delay which must occur in their publication.

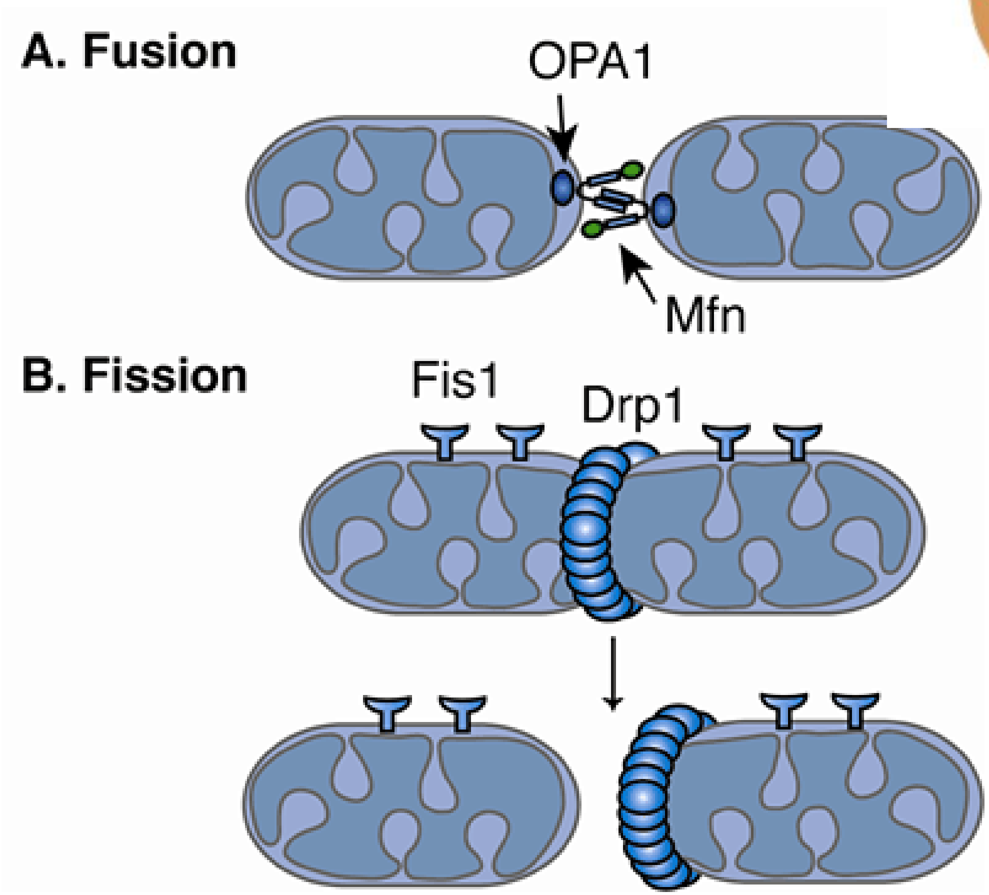
If Mr. Krebs does not mind such delay, the Editor is prepared to keep the letter until the congestion is relieved in the hope of making use of it. He returns it now, however, in case Mr. Krebs prefers to submit it for early publication to another periodical.

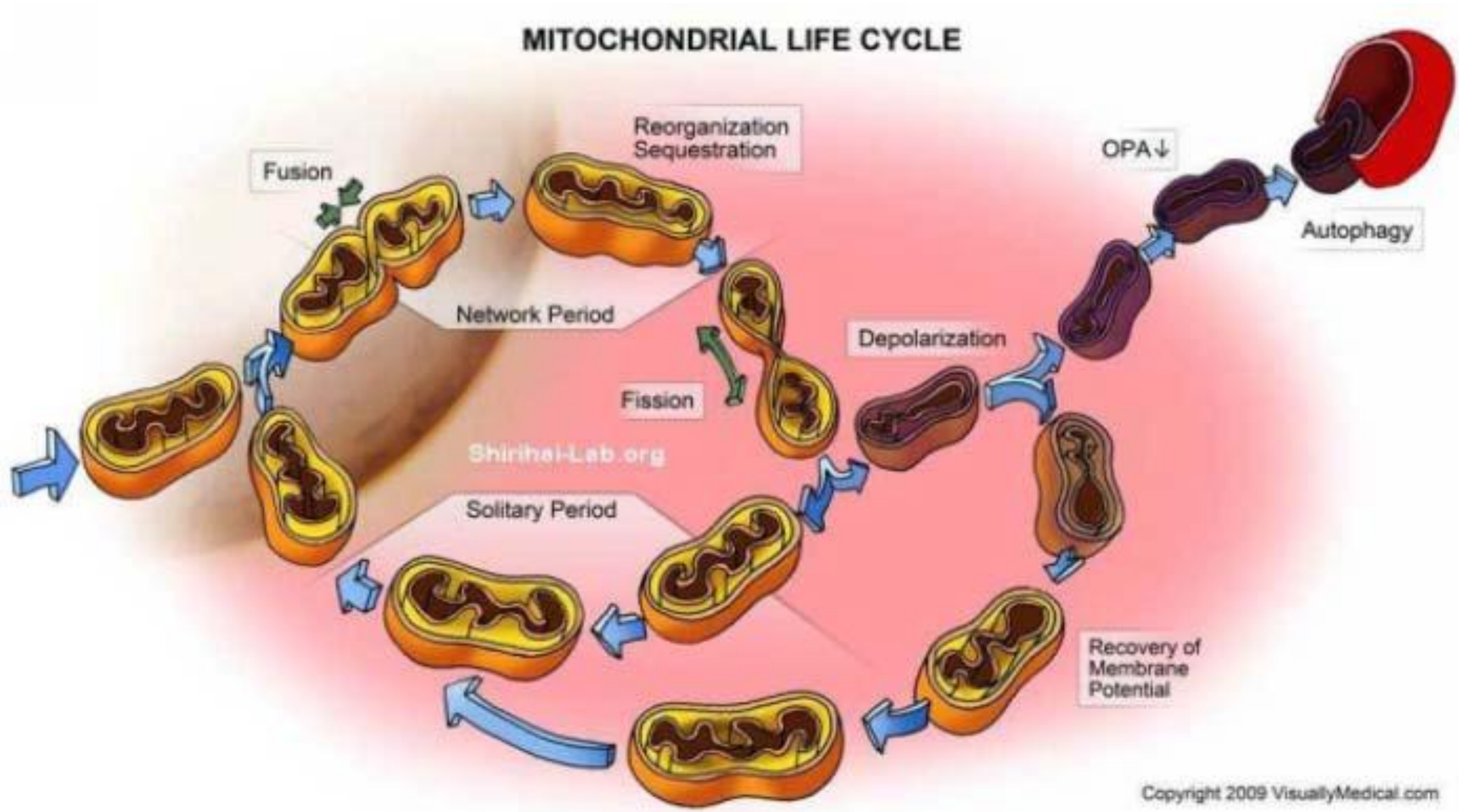
TCA Take place——

Eukaryotes: mitochondria

Prokaryotes: cytosol






MITOCHONDRIAL LIFE CYCLE
Fusion
Reorganization
Sequestration
Network Period
Fission
Depolarization
OPA↓
Autophagy
Shirihai-Lab.org
Solitary Period
Recovery of
Membrane
Potential
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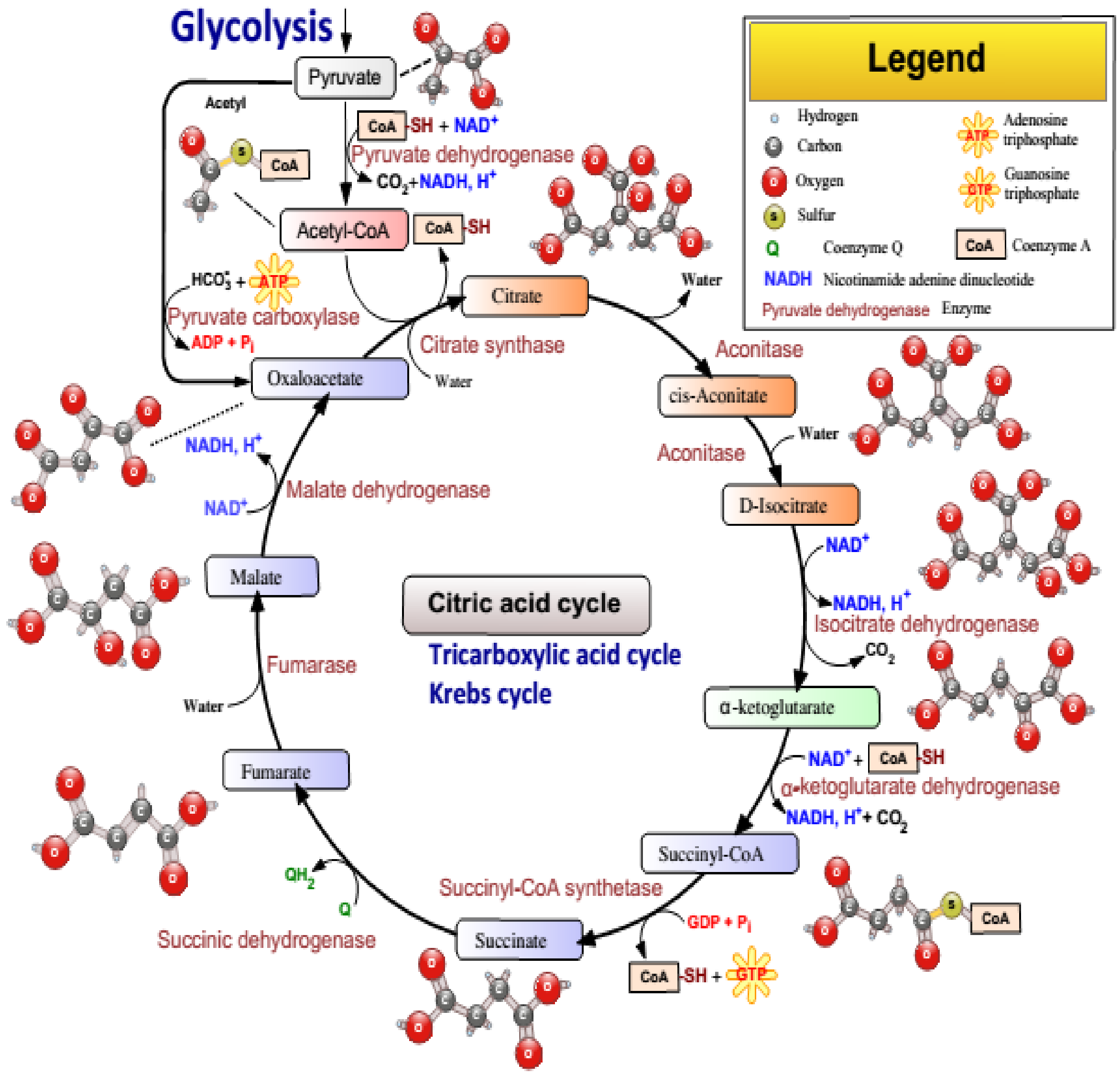
TCA is the **second of three steps** in cellular respiration, between **glycolysis** & **oxidative phosphorylation**

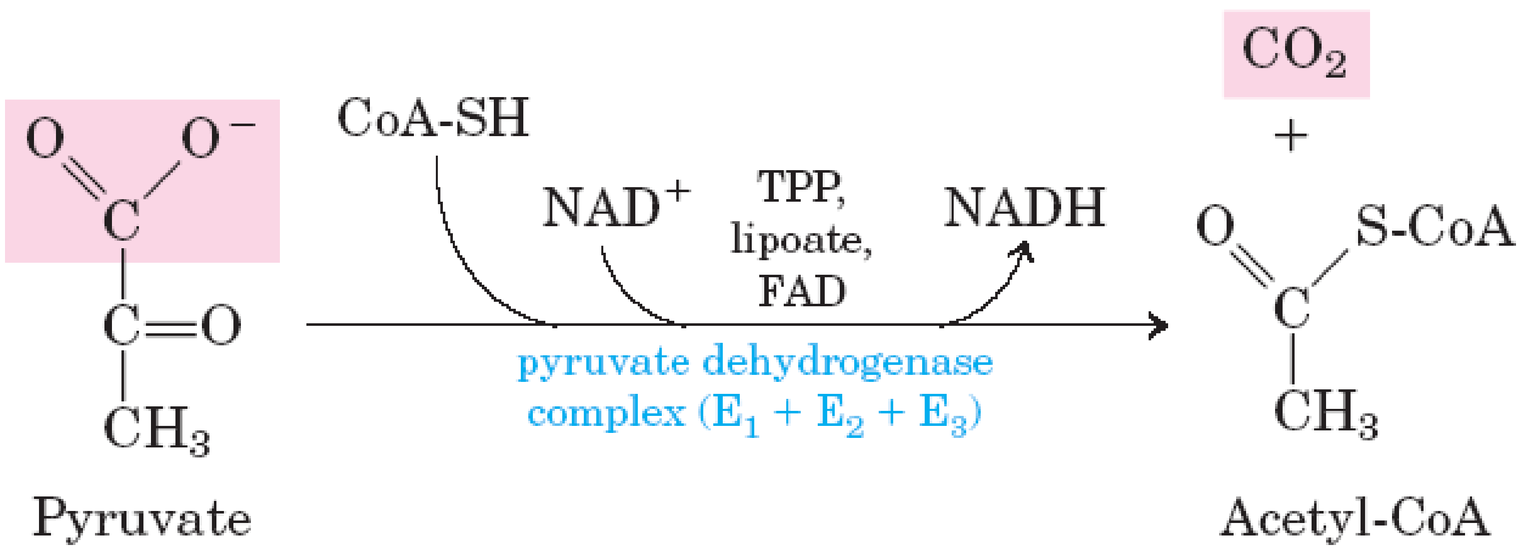


2. Production of Acetyl-CoA

-----Pyruvate Oxidation

A Major Entry Route for Carbon into the TCA

Pyruvate Dehydrogenase Complex 丙酮酸脱氢酶系



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

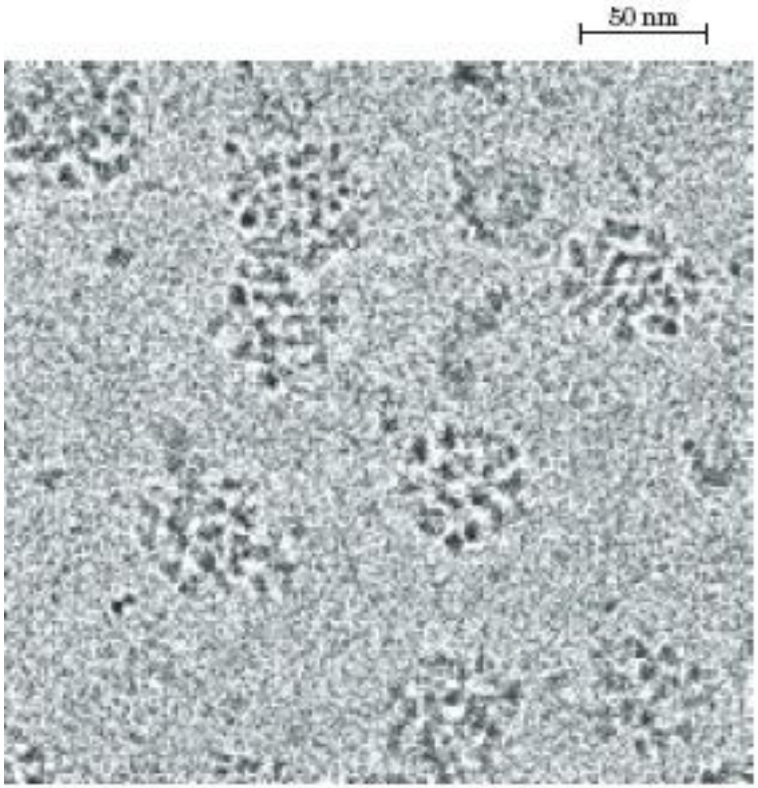
Pyruvate Dehydrogenase Complex （PDC）

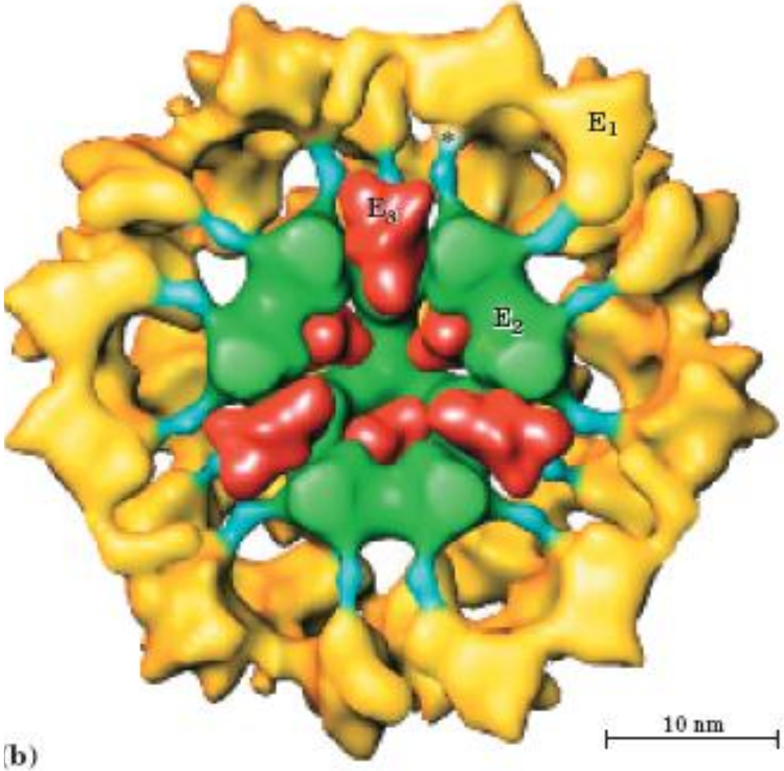
three enzymes (E1, E2 and E3):

1. Pyruvate Dehydrogenase (E1) **丙酮酸脱氢酶**
2. Dihydrolipoyl transacetylase (E2) **二氢硫辛酰转乙酰基酶**
3. Dihydrolipoyl dehydrogenase (E3) **二氢硫辛酰脱氢酶**

five coenzymes:

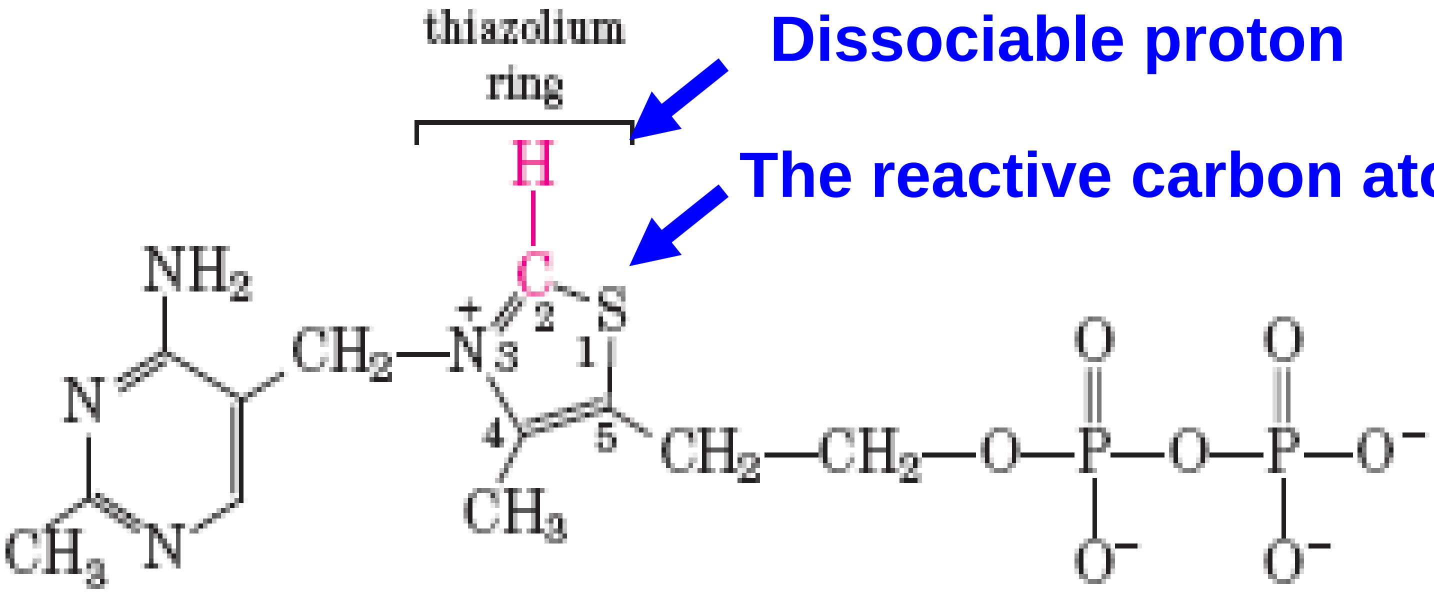
1. Thiamine Pyrophosphate 焦磷酸硫胺素, 辅羧酶(TPP) …VB1
2. Lipoic Acid硫辛酸- lipoamide硫辛酰胺
3. CoASH …泛酸
4. NAD^+… VPP
5. FAD… VB2



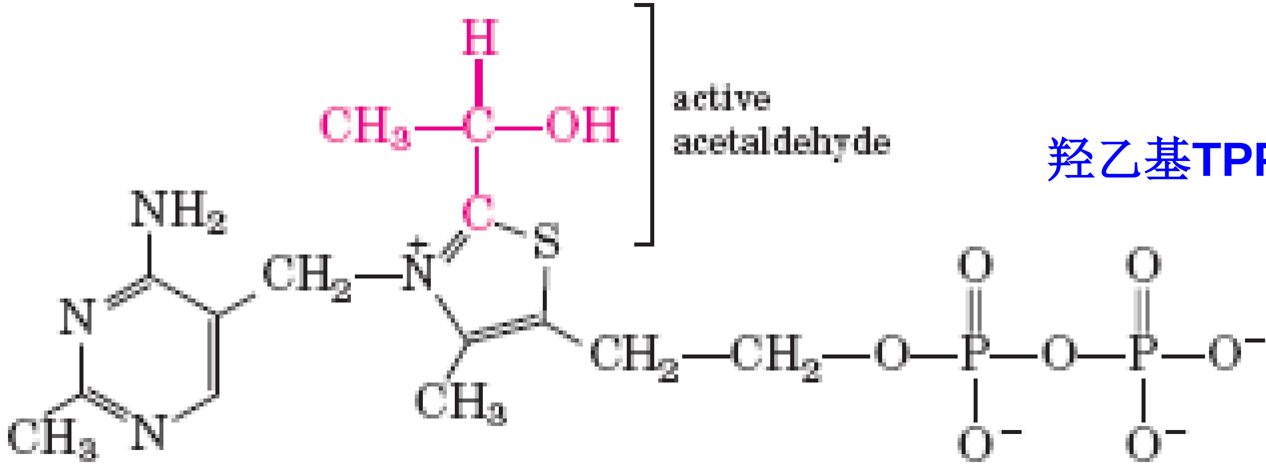


- The **core (green)**： 8 trimers 24 **E2**
- The lipoyl 硫辛酰基 (**blue**) of **E2** reaches to touch the active sites of **E1**
- The swinging arm on E2 can reach the active sites of E3 (**red**).

Pyruvate Dehydrogenase (E1) 丙酮酸脱氢酶

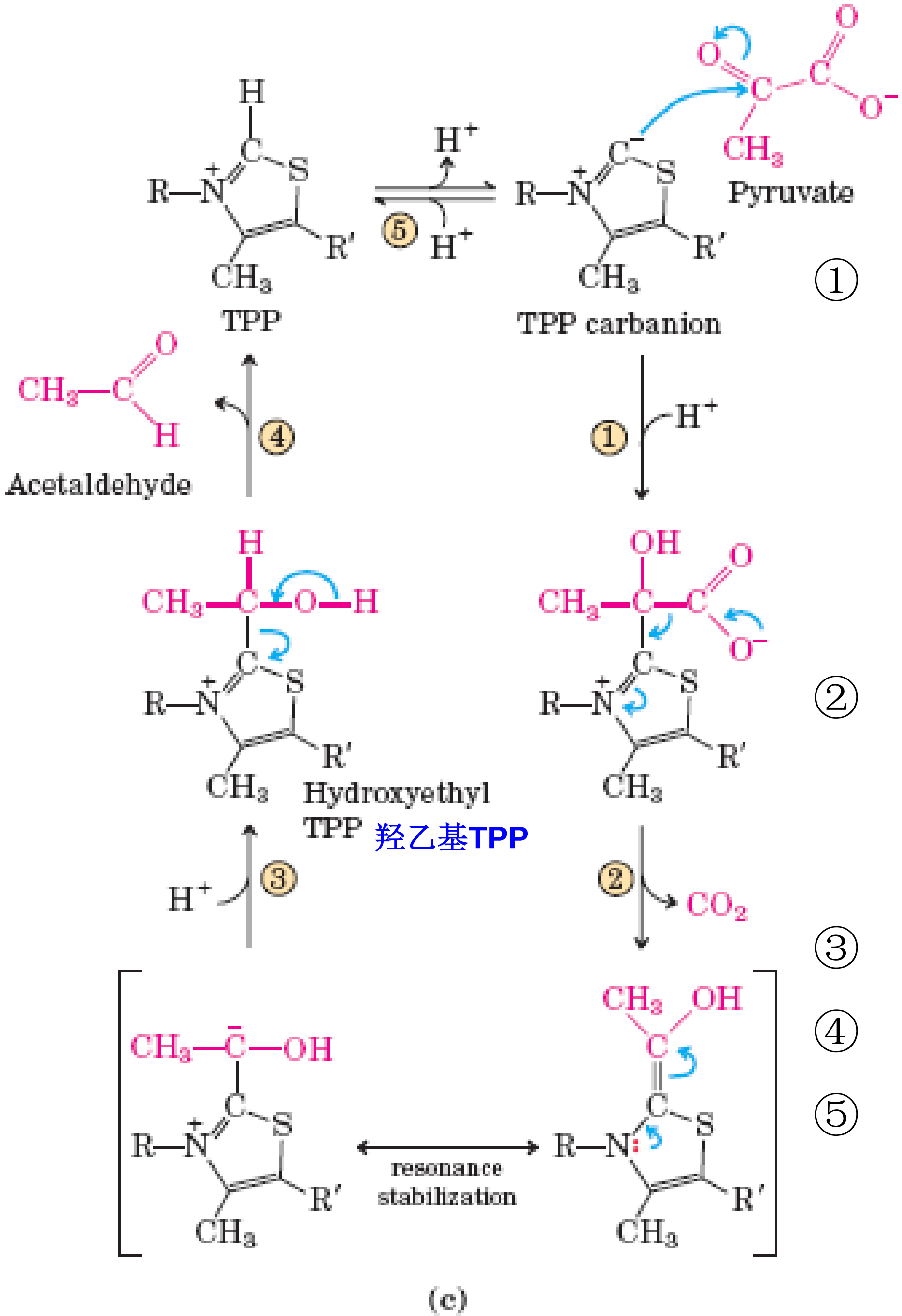


Thiamine pyrophosphate (TPP) **is the coenzyme form of VB1**



羟乙基**TPP（HETPP)**

Hydroxyethyl thiamine pyrophosphate



① The TPP carbanion (负碳离子) acts as a nucleophile(亲核), attacking the carbonyl group of pyruvate.

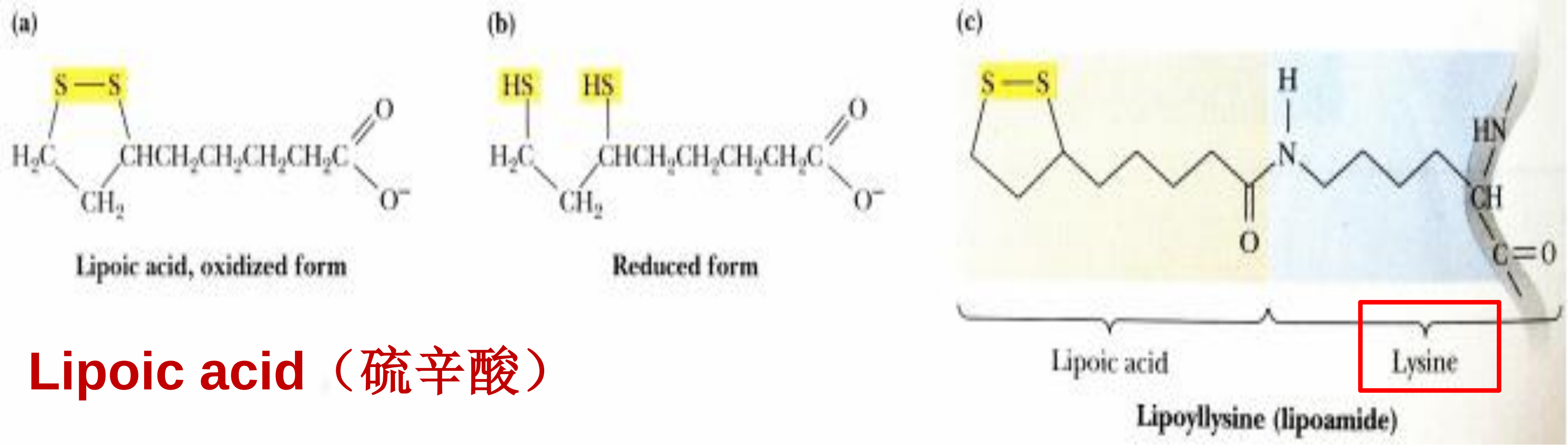
② Decarboxylation produces a carbanion that is stabilized by the thiazolium (噻唑) ring.

③ Protonation to form hydroxyethyl TPP

④ release of acetaldehyde.

⑤ A proton dissociates to regenerate the carbanion.

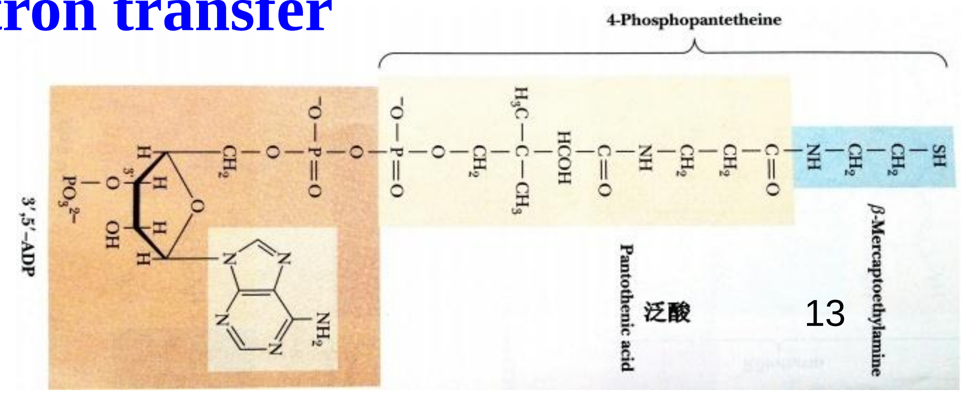
Dihydrolipoyl transacetylase (E2)二氢硫辛酰转乙酰基酶

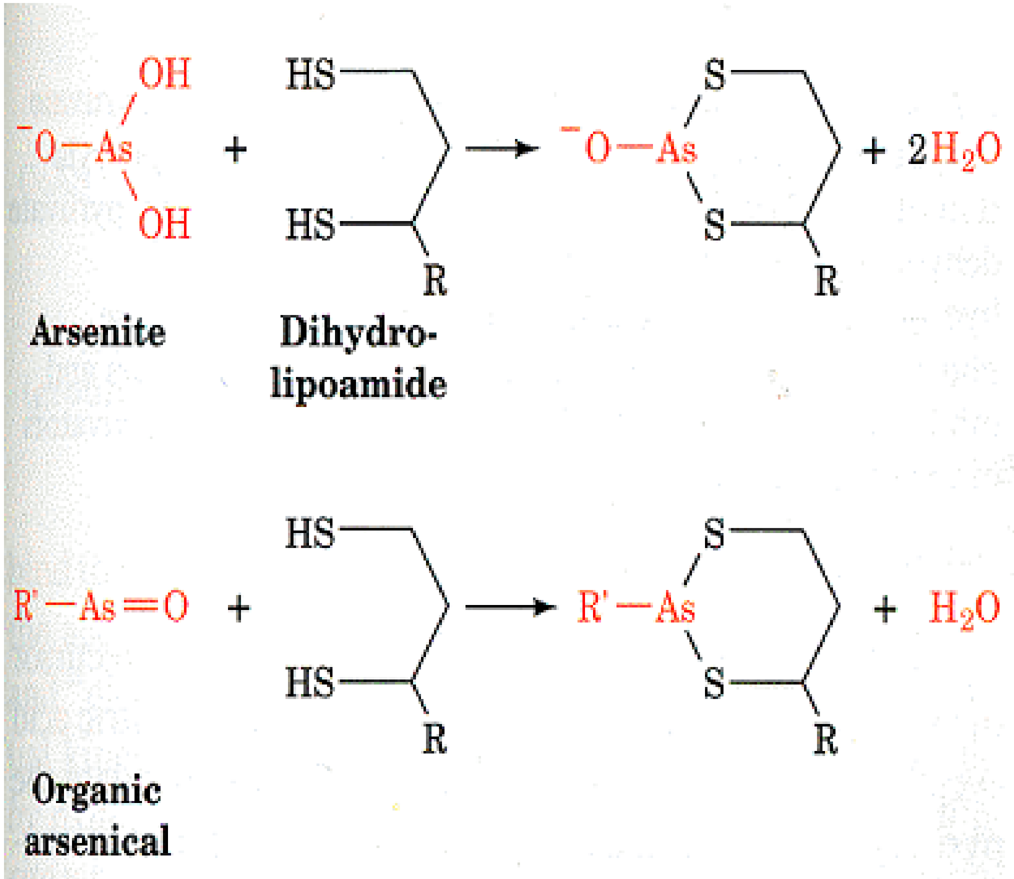


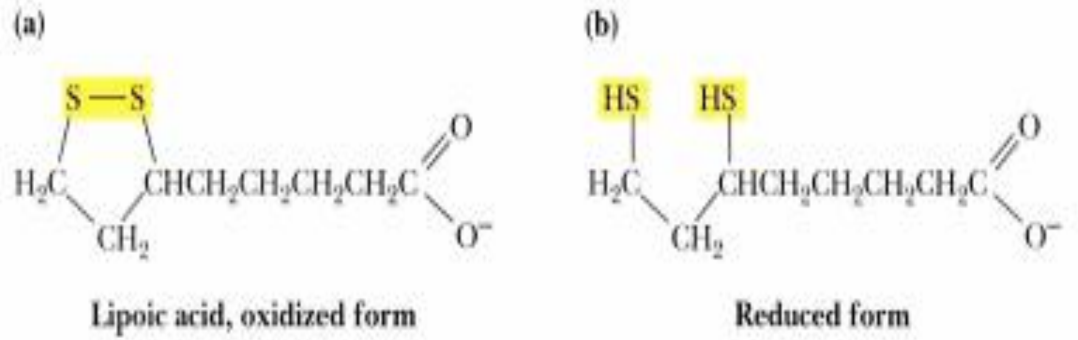
Lipoic acid（硫辛酸）

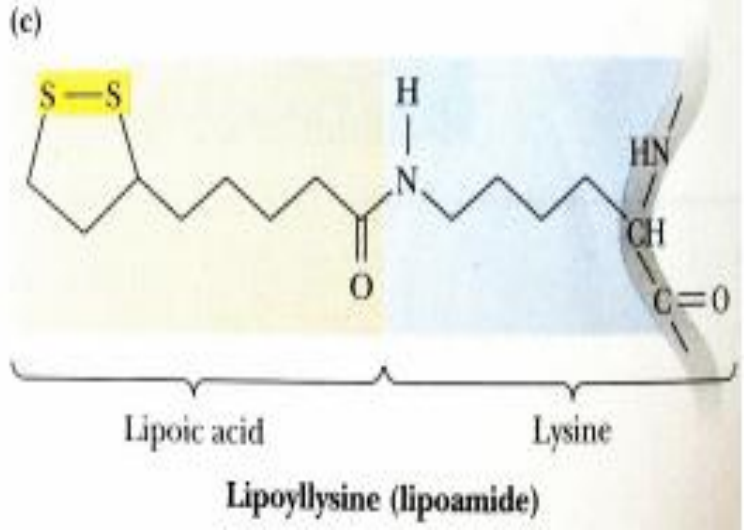
- ➢**Pyruvate dehydrogenase and α-ketoglutarate dehydrogenase**
- ➢ **Covalently bound with Lys- ε-NH_2**
- ➢ **Acyl-group transfer and electron transfer**

Coenzymes A



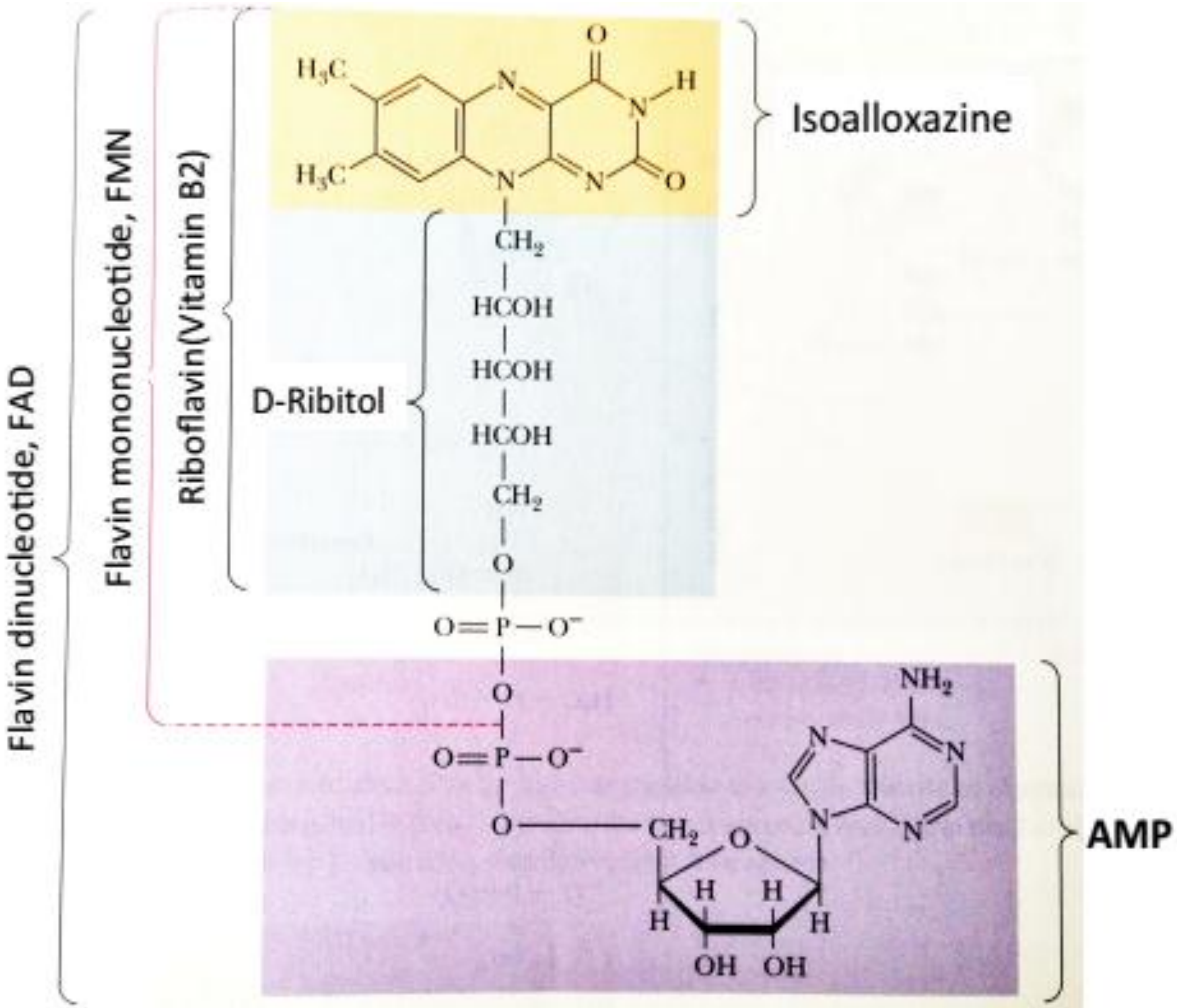




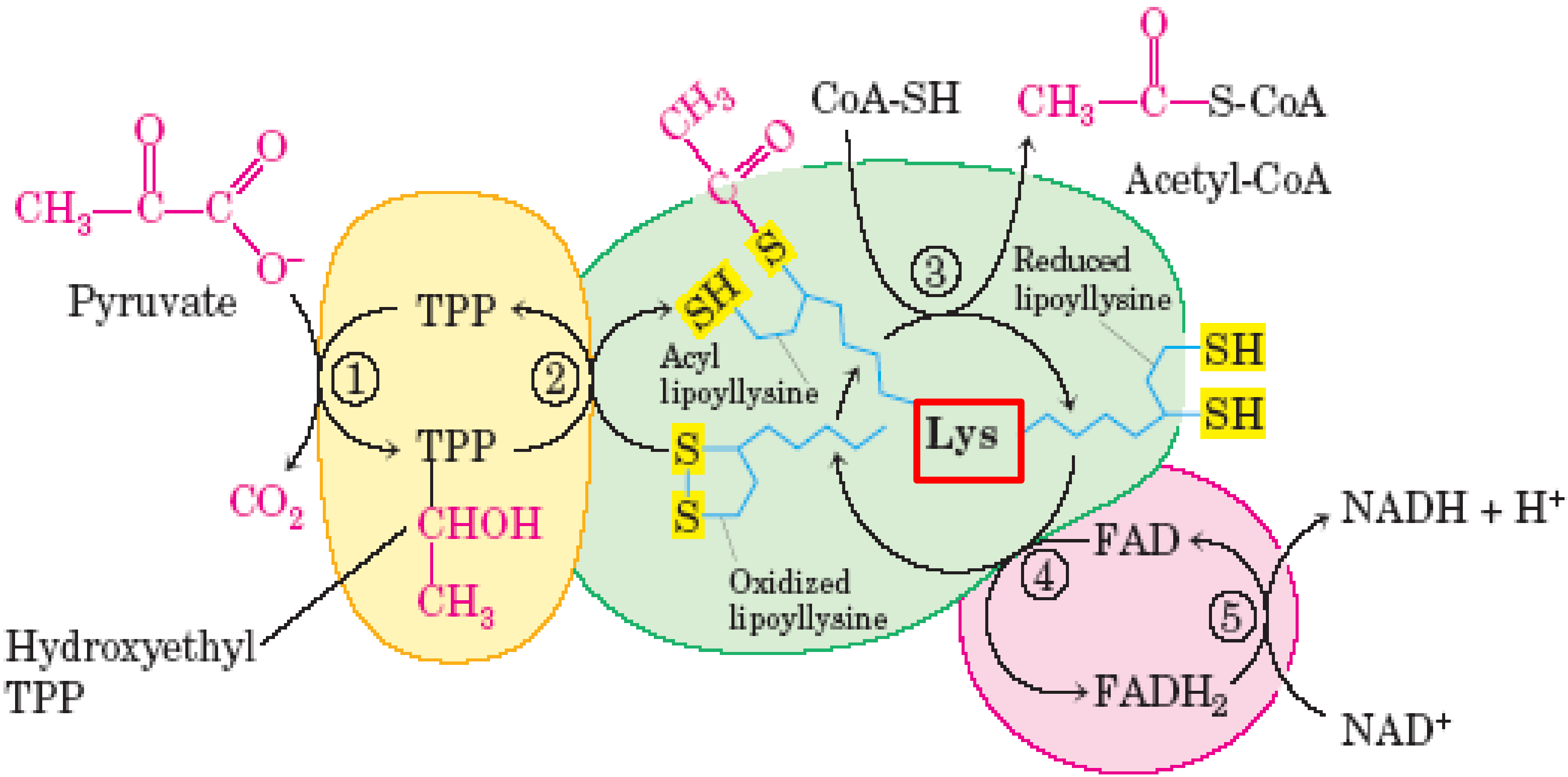


Lipoic acid（硫辛酸）

Dihydrolipoyl dehydrogenase (E3)二氢硫辛酰脱氢酶



The favin coenzymes—FAD/FADH$_2$



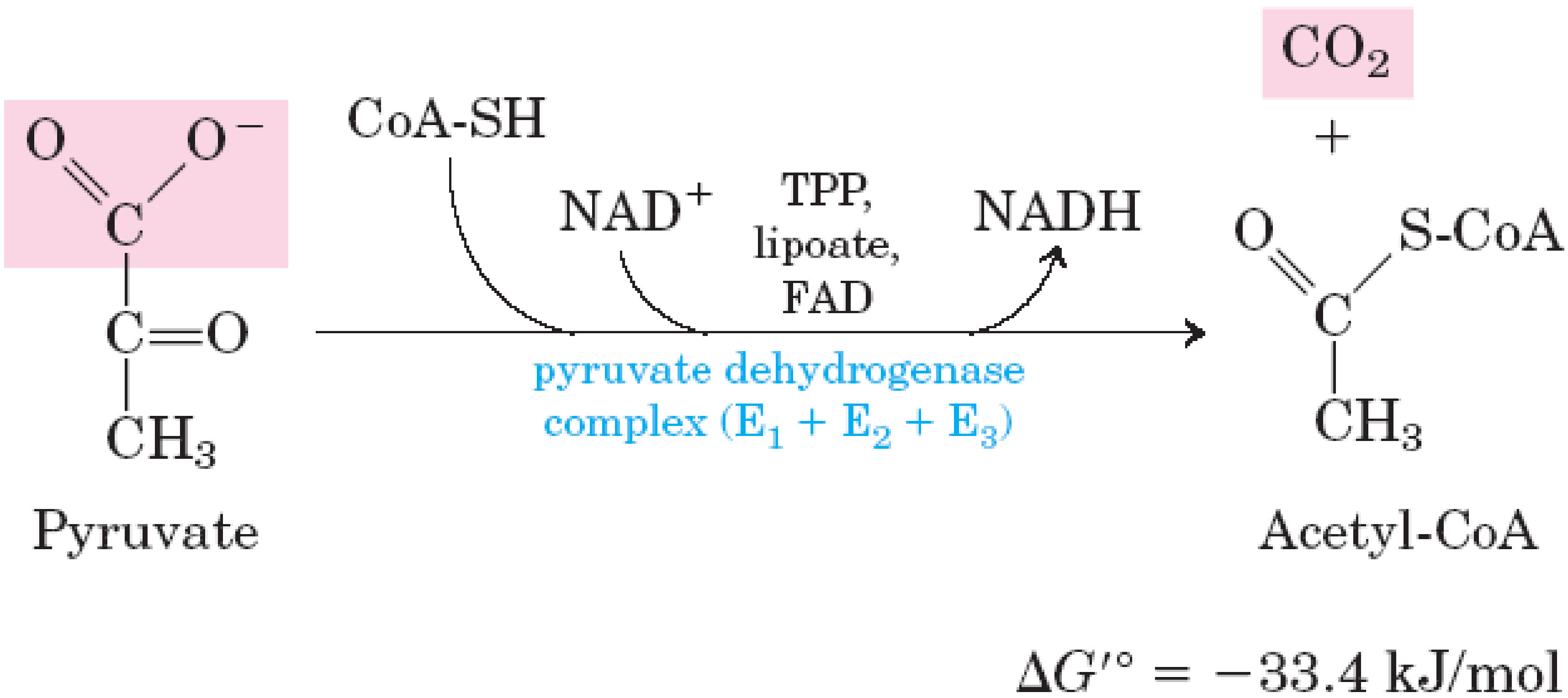
Pyruvate dehydrogenase, E_1

Dihydrolipoyl transacetylase, E_2

二氢硫辛酰转乙酰基酶

Dihydrolipoyl dehydrogenase, E_3

二氢硫辛酰脱氢酶


CoA-SH
NAD+
TPP, lipoate, FAD
NADH
CO2
pyruvate dehydrogenase complex (E1 + E2 + E3)
Pyruvate
Acetyl-CoA
ΔG'° = −33.4 kJ/mol

3. Reactions of TCA

central metabolic pathway中心代谢途径

a) generates NADH and $FADH_2$

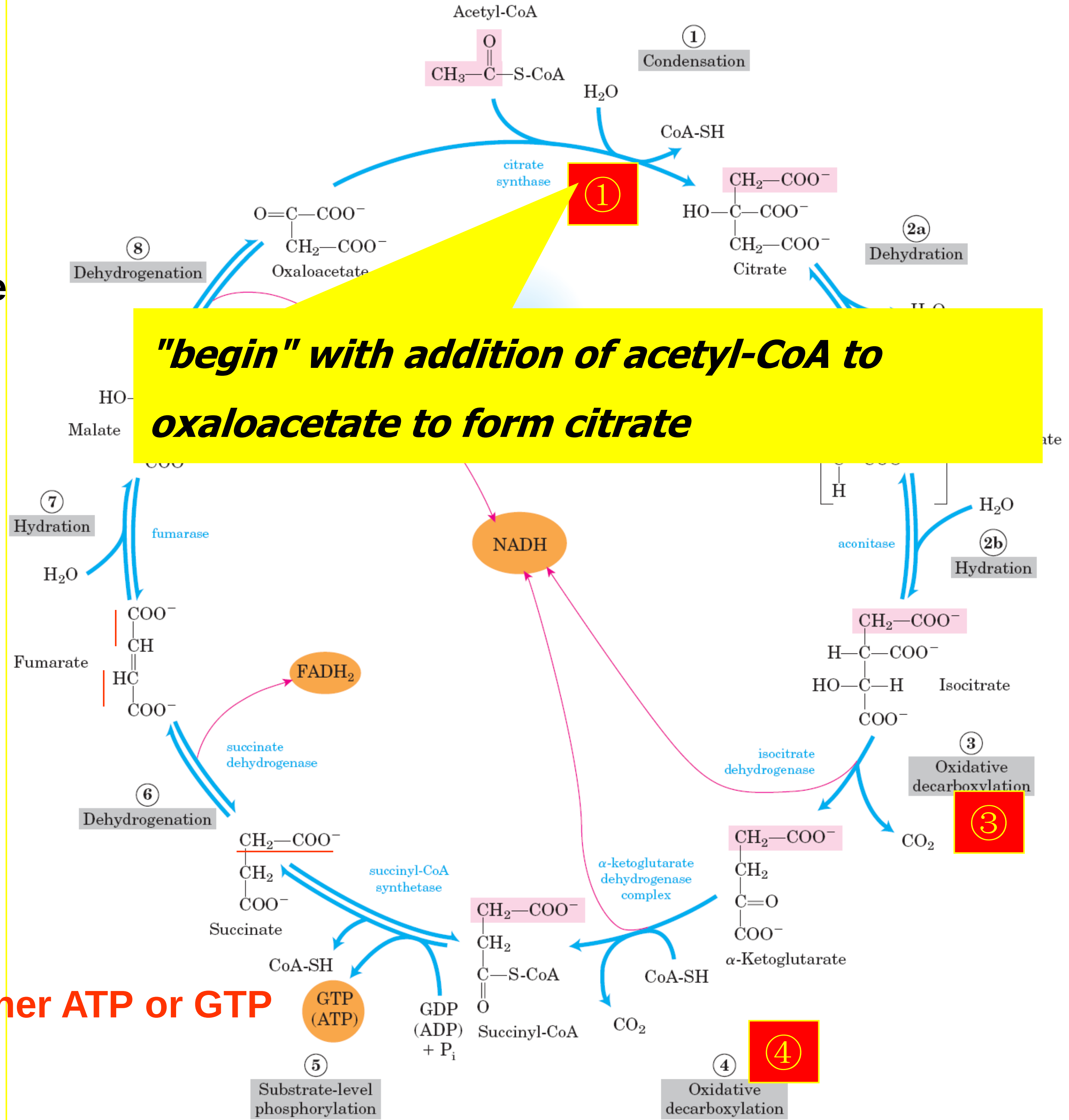
b) produces GTP via substrate-level phosphorylation

c) Many metabolic processes use intermediates of the TCA in their pathways.

Eight Steps

Steps1,3,and 4 are essentially irreversible

Four oxidation steps -- NADH and $FADH_2$.



Step5 may be either ATP or GTP

The Citric Acid Cycle Has Eight Steps

Phase 1: Introduction and Loss of Two Carbon Atoms

Step 1: Introduction of Two Carbon Atoms as **Acetyl-CoA**

Step 2: Isomerization（异构化） of **Citrate**（柠檬酸）

Step 3: Generation of CO_2 by an NAD^+ Linked Dehydrogenase

Step 4: Generation of a Second CO_2 by a Multienzyme Complex

Phase 2: Regeneration of Oxaloacetate（草酰乙酸）

Step 5: A Substrate-Level Phosphorylation

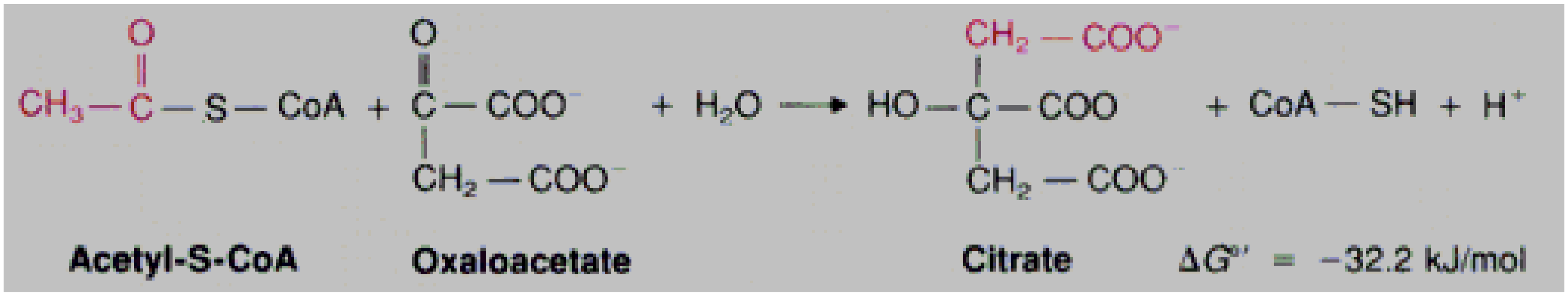
Step 6: A Flavin-Dependent Dehydrogenase

Step 7: Hydration 水合作用of a Carbon-Carbon Double Bond

Step 8: A Dehydrogenation that Regenerates Oxaloacetate

Phase 1: Introduction and Loss of Two Carbon Atoms

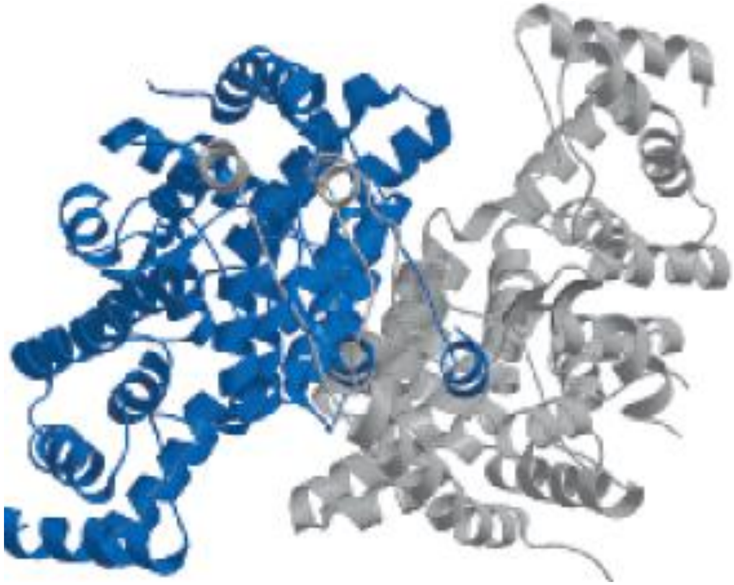
Step 1: Introduction of Two Carbon Atoms as Acetyl-CoA

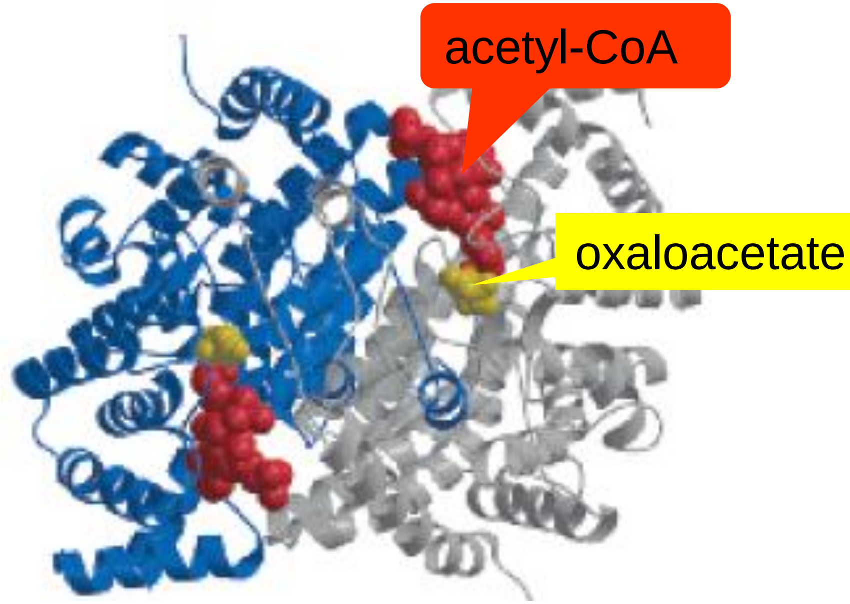


柠檬酸**合酶** citrate synthase

TCA 限速酶

The large, negative standard free-energy change of the citrate synthase reaction is essential to the operation of the cycle

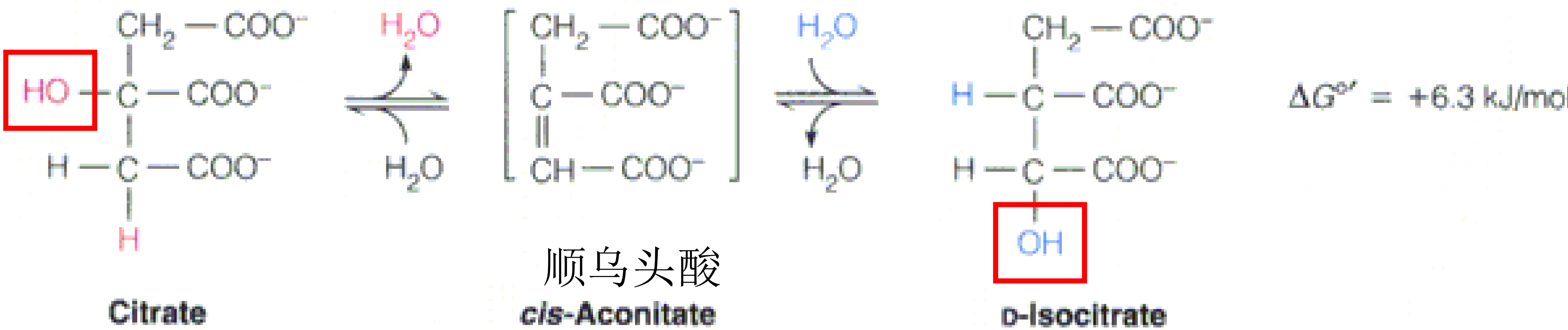




Structure of citrate synthase（二聚体）

Each subunit undergoes a conformational change on binding oxaloacetate creating a binding site for acetyl-CoA.

Step 2: Isomerization of Citrate



顺乌头酸

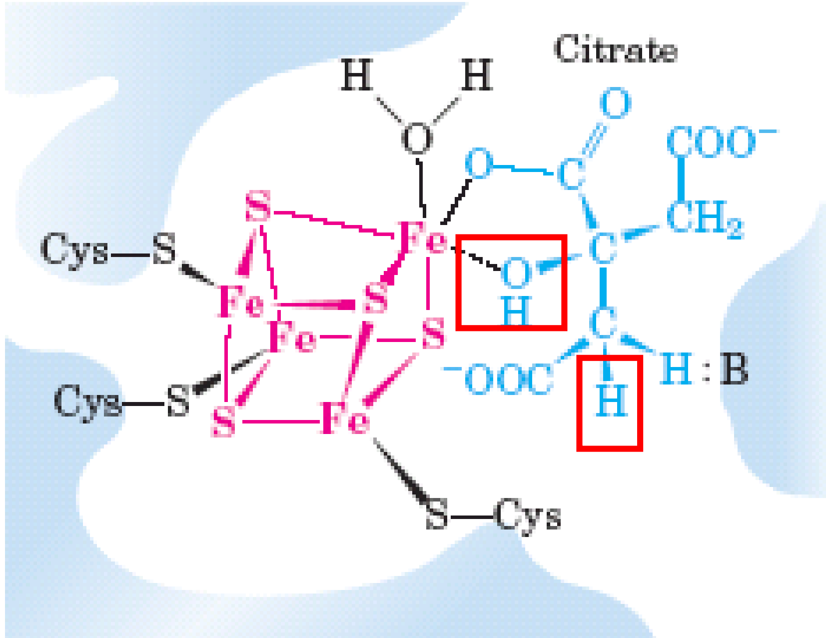
柠檬酸

异柠檬酸

叔醇化合物

仲醇化合物
（可氧化）

Aconitase 乌头酸酶

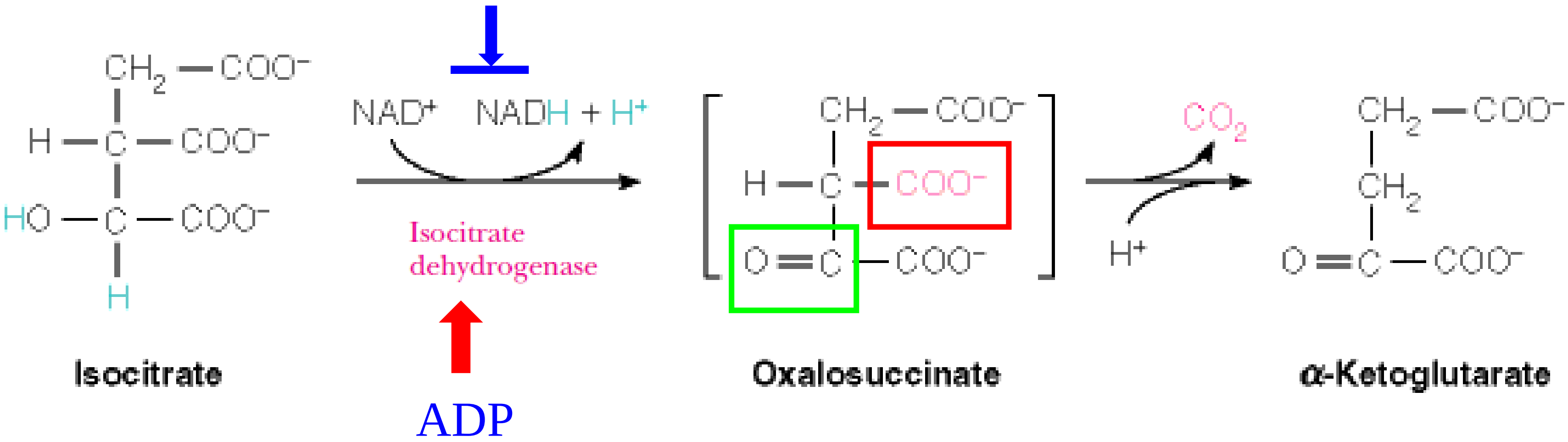


Aconitase
乌头酸酶
（iron-sulfur center）

- Three Cys residues bind **three iron** atoms
- The **fourth iron** is bound to one of the carboxyl groups of citrate and -OH group of citrate.
- **A basic residue (:B)** helps to position the **citrate** in the active site.

Step 3: Generation of CO_2 by an NAD$^+$ Linked Dehydrogenase

ATP，NADH inhibit the activity of isocitrate dehydrogenase

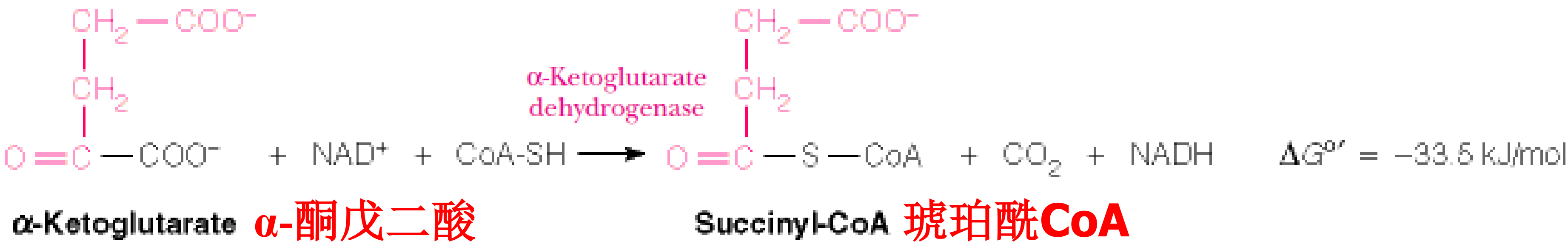


异柠檬酸　　草酰琥珀酸　　α-酮戊二酸

Isocitrate dehydrogenase （IDH）异柠檬酸脱氢酶

酮酸的形成可促进临近C-C键的断裂，有利于脱羧作用的进行

Step 4 Generation of a Second CO_2 by a Multienzyme Complex



α-酮戊二酸 琥珀酰CoA

1. α −KG dehydrogenase (E1)

2. Dihydrolipoamide transacetylase (E2)二氢硫辛酰转琥珀酰酶

3. Dihydrolipoamide dehydrogenase (E3)二氢硫辛酰脱氢酶

five coenzymes

1. Thiamine Pyrophosphate 焦磷酸硫胺素, 辅羧酶(TPP)

2. Lipoic Acid- lipoamide 硫辛酰胺

3. FAD

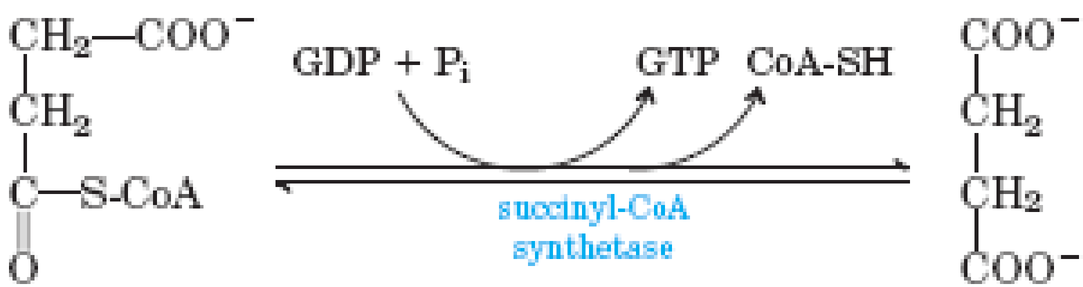
4. NAD^+

5. CoASH

Phase 2: Regeneration of Oxaloacetate（草酰乙酸）

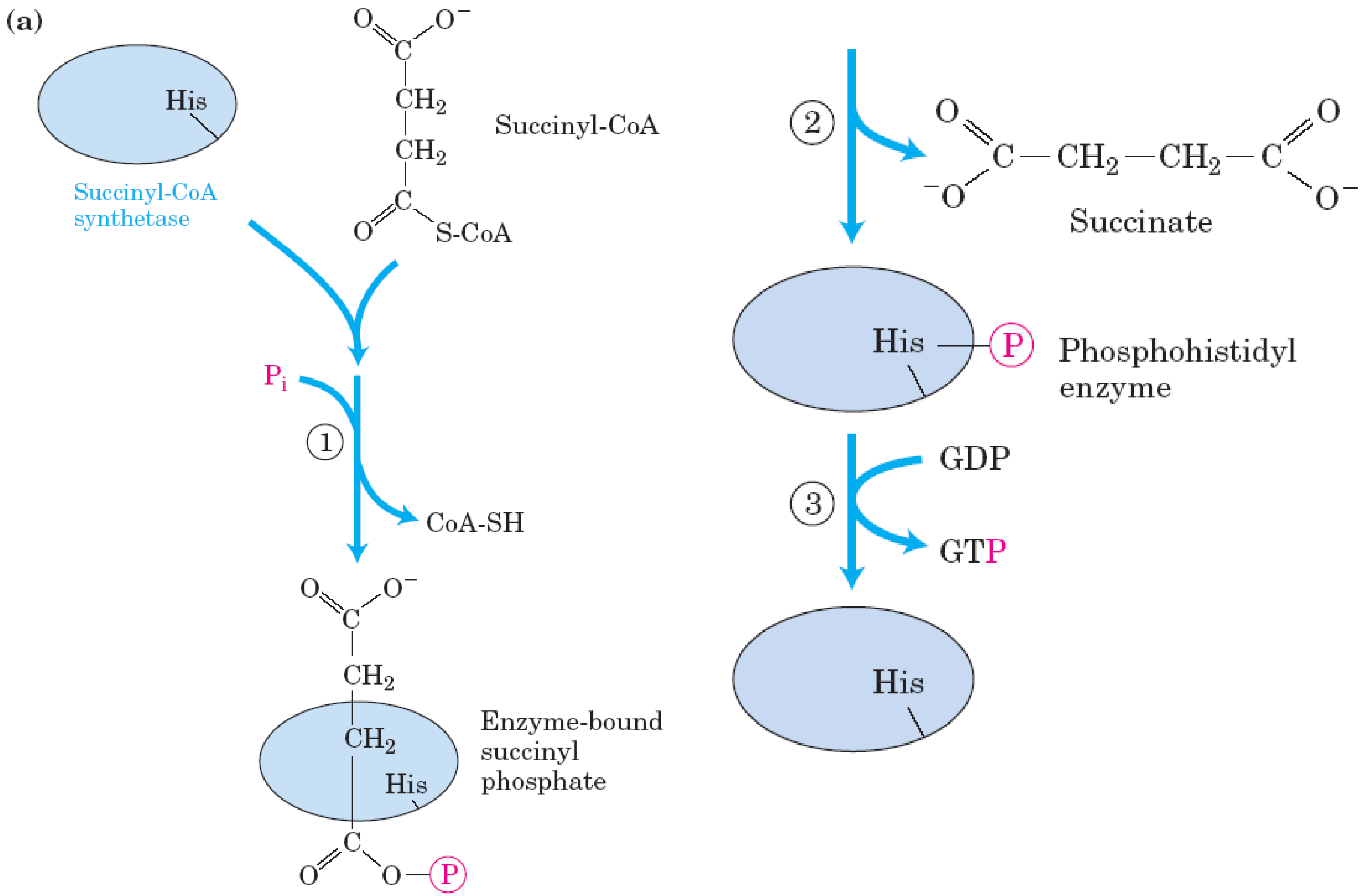
Step 5: Conversion of Succinyl-CoA to Succinate

- **琥珀酰CoA合成酶– GTP（animal）or ATP（plant & microorganism）**
- 底物水平磷酸化



Succinyl-CoA **琥珀酰CoA** Succinate **琥珀酸**

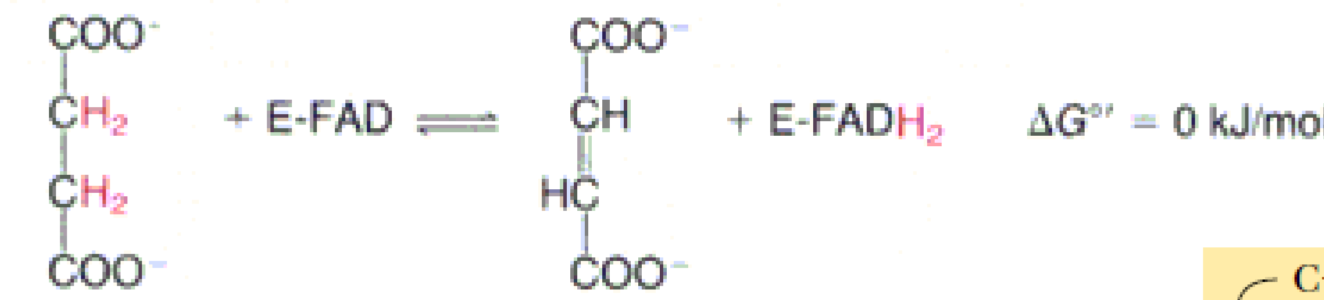
Different from **synthase** $\Delta G'^{\circ} = -2.9\ \text{kJ/mol}$



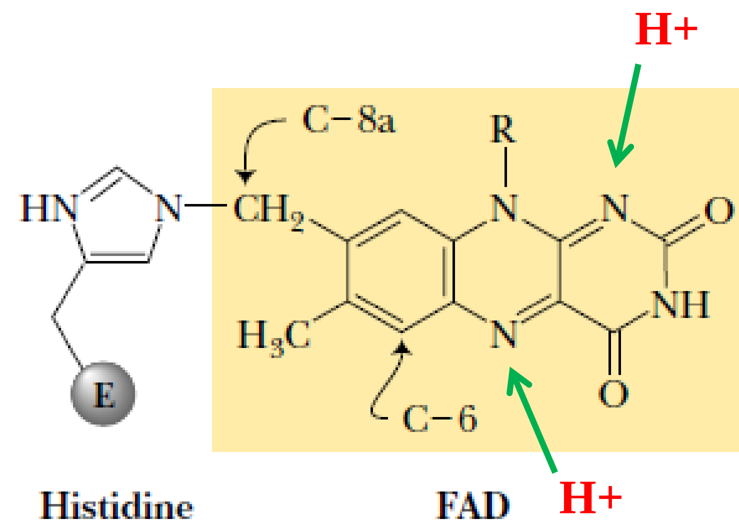
(a)
His
Succinyl-CoA synthetase
Succinyl-CoA
S-CoA
P_i
1
CoA-SH
Enzyme-bound succinyl phosphate
2
Succinate
His
P
Phosphohistidyl enzyme
3
GDP
GTP
His

高能酰基磷酸

Step 6: Oxidation of Succinate to Fumarate



Succinate 琥珀酸　　Fumarate 延胡索酸



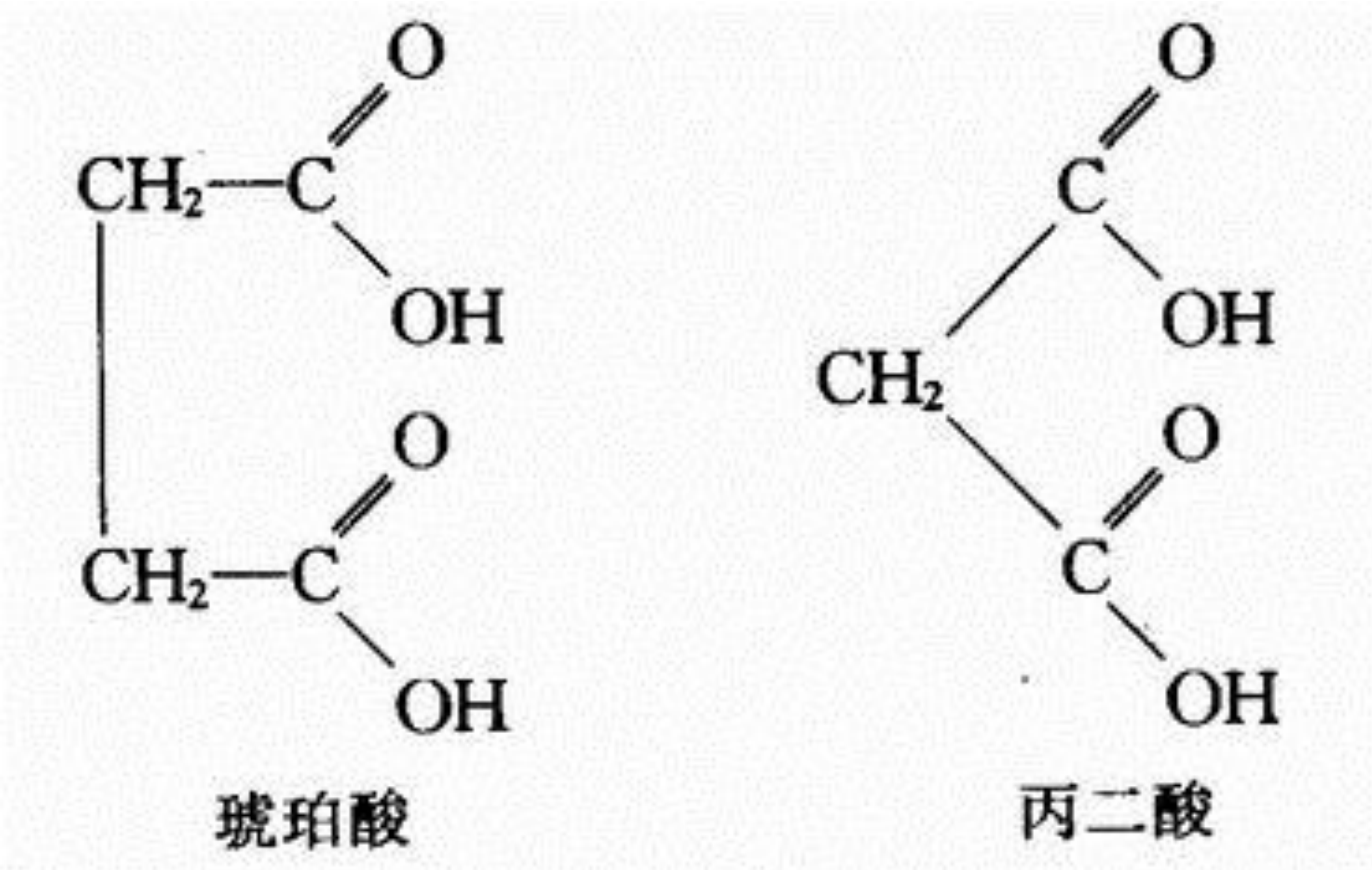
琥珀酸脱氢酶

- **succinate dehydrogenase-**

--A Flavin-Dependent Dehydrogenase

- **Only enzyme of TCA is membrane-bound**

Malonate (丙二酸) competitive inhibitor



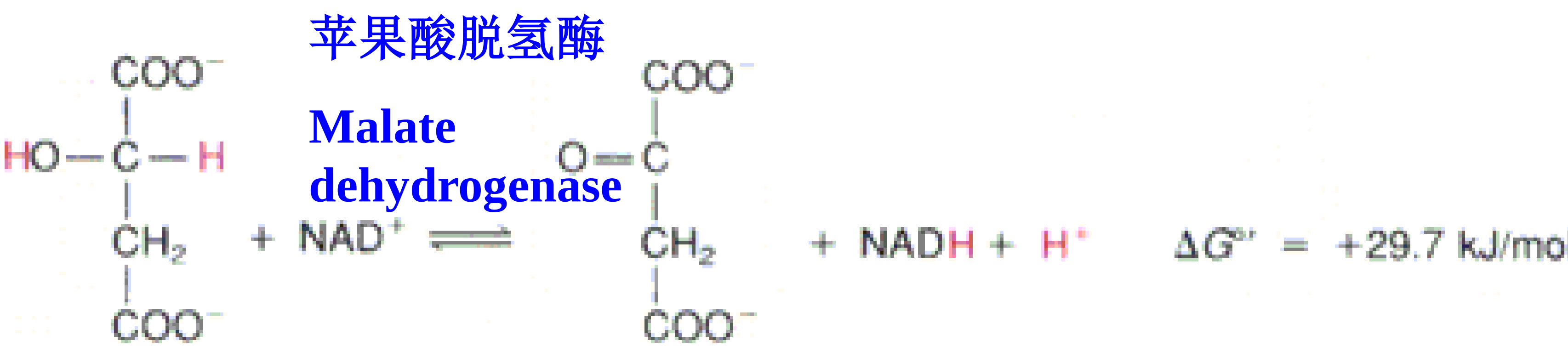
Step 7: Hydration of Fumarate to Malate

highly stereospecific

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

Fumarate 延胡索酸 L-Malate L-苹果酸

Step 8: Oxidation of Malate to Oxaloacetate

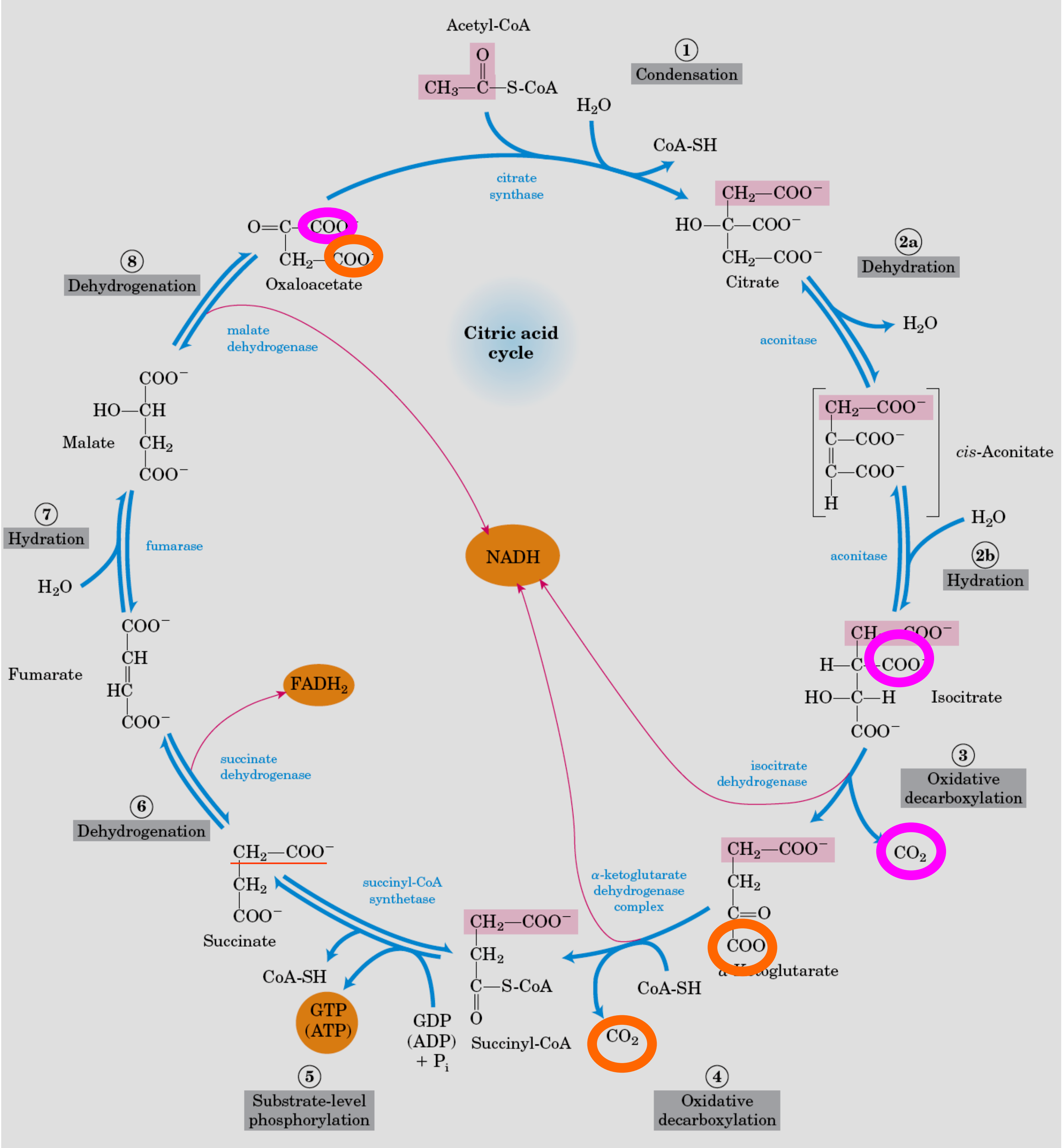


L-Malate L-苹果酸 Oxaloacetate 草酰乙酸

Reaction	Enzyme	$\Delta G^{\circ\prime}$ (kJ/mol)
1. Acetyl-CoA + oxaloacetate + H_2O $\longrightarrow$ citrate + CoA-SH + H^+	Citrate synthase	−32.2
2a. Citrate $\rightleftharpoons$ *cis*-aconitate + H_2O	Aconitase	+6.3
2b. *cis*-Aconitate + H_2O $\rightleftharpoons$ isocitrate	Aconitase	
3. Isocitrate + NAD^+ $\rightleftharpoons$ α-ketoglutarate + CO_2 + NADH	Isocitrate dehydrogenase	−20.9
4. α-Ketoglutarate + NAD^+ + CoA-SH $\rightleftharpoons$ succinyl-CoA + CO_2 + NADH	α-Ketoglutarate dehydrogenase complex	−33.5
5. Succinyl-CoA + P_i + GDP $\rightleftharpoons$ succinate + GTP + CoA-SH	Succinyl-CoA synthetase	−2.9
6. Succinate + FAD (enzyme-bound) $\rightleftharpoons$ fumarate + $FADH_2$ (enzyme-bound)	Succinate dehydrogenase	0
7. Fumarate + H_2O $\rightleftharpoons$ L-malate	Fumarase	−3.8
8. L-Malate + NAD^+ $\rightleftharpoons$ oxaloacetate + NADH + H^+	Malate dehydrogenase	+29.7
	Net	−57.3

Note:

- **A two-carbon acetyl entered the cycle by combining with oxaloacetate.**
- **2C atoms emerged from the cycle as CO_2 from the oxidation of isocitrate and α-ketoglutarate.**
- **2C atoms appearing as CO_2 are not the same 2C that entered in the form of the acetyl group**


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

The Energy of Oxidations in the Cycle

For each acetyl-CoA oxidized by the citric acid cycle

3 NADH

1 FADH2

1 ATP or GTP

$$\text{Acetyl-CoA} + 3\ NAD^+ + [FAD] + ADP + P_i + 2\ H_2O \rightleftharpoons 2\ CO_2 + 3\ NADH + 3\ H^+ + [FADH_2] + ATP + CoASH$$

10 ATP

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

30-32ATP

The TCA cycle provides intermediates for biosynthesis besides generating energy

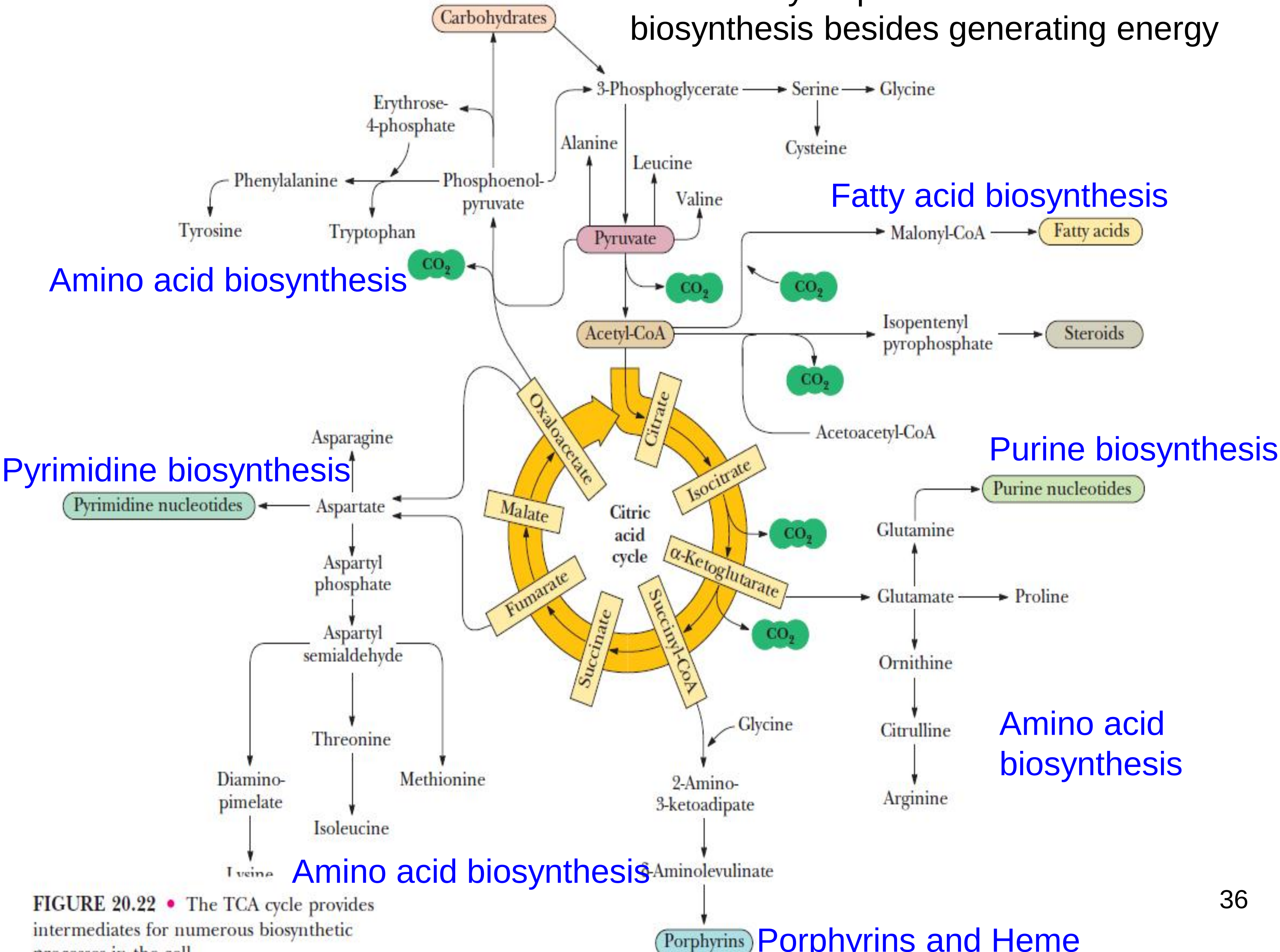
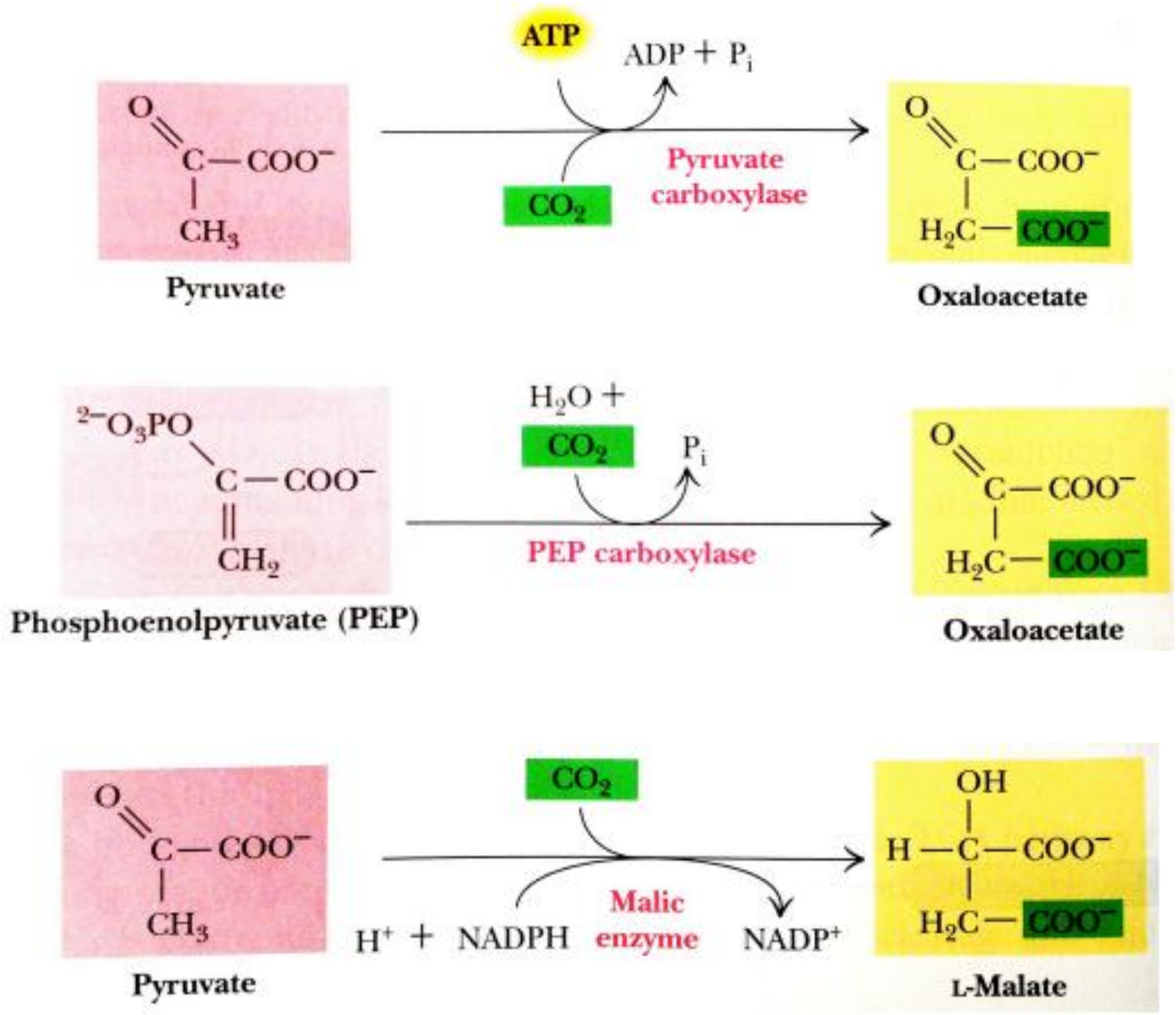


FIGURE 20.22 • The TCA cycle provides intermediates for numerous biosynthetic processes in the cell.

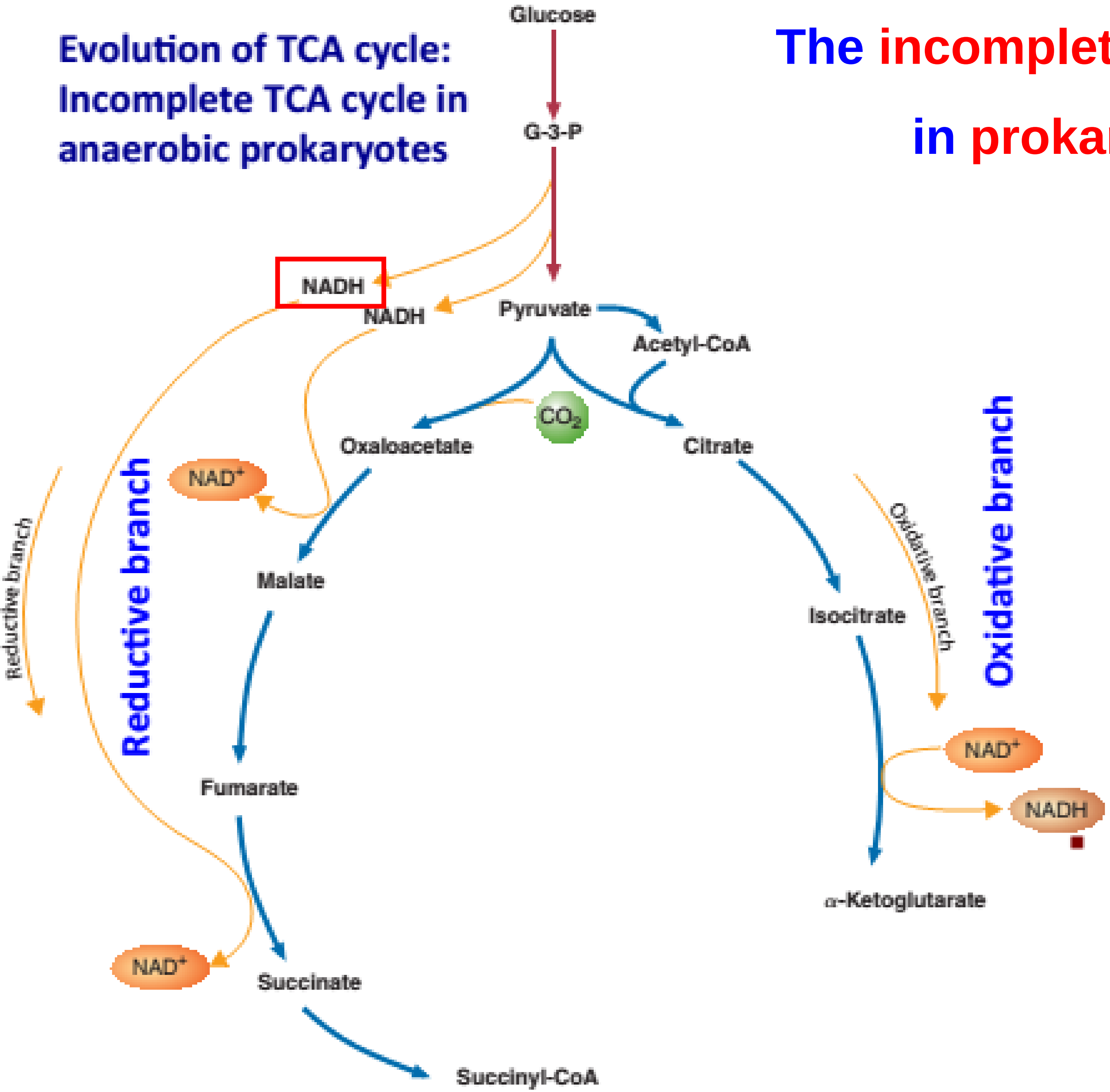
Anaplerotic reactions to fill up TCA

回补反应



The incomplete TCA Cycle in prokaryotes

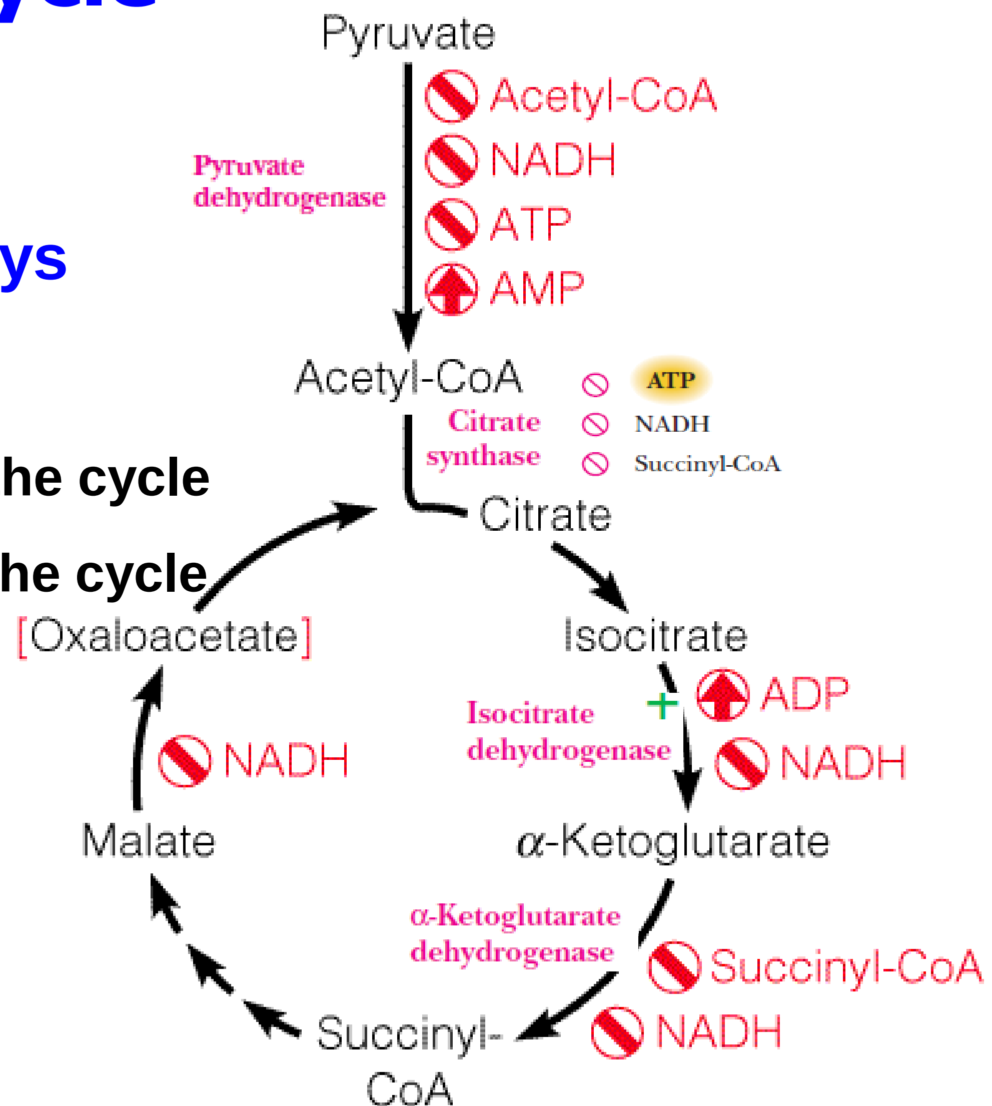
Evolution of TCA cycle: Incomplete TCA cycle in anaerobic prokaryotes



4. Regulation of Pyruvate Dehydrogenase and the Citric Acid Cycle

regulated in two primary ways

- **controlling the entry of fuel into the cycle**
- **controlling key reactions within the cycle**



4 .1 Controlling the **entry** of fuel into the cycle

➢**Pyruvate dehydrogenase** -- major regulatory point for entry of materials into TCA

□ **Allosteric Regulation（E2 & E3）**

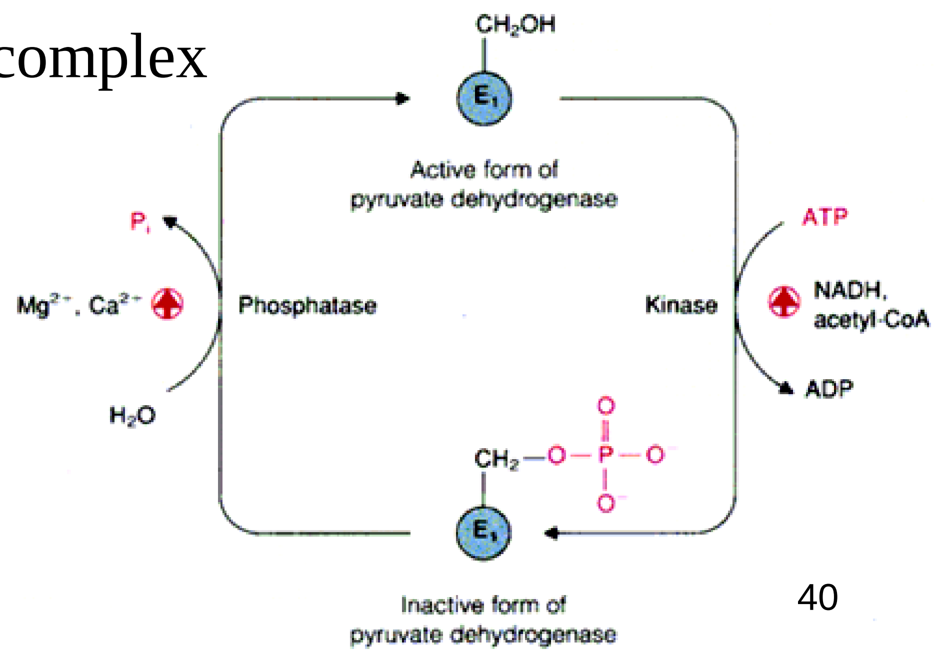
E2 - inhibited by **acetyl-CoA**, activated by **CoA-SH**

E3 - inhibited by **NADH**, activated by **NAD^+.**

ATP: allosteric inhibitor of the complex

AMP: activator

□ **Covalent Regulation (E1)**



4 .2 Key reactions **within the cycle**:

allosteric regulation & Covalent Regulation

- **isocitrate dehydrogenase**

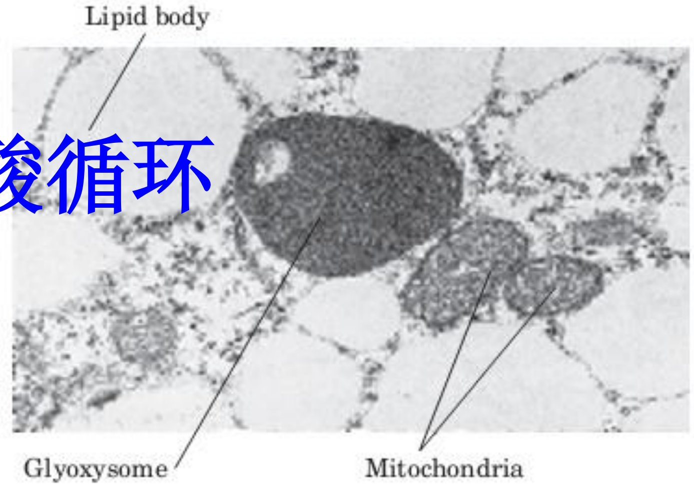
- Activated by ADP, inhibited by NADH

- Phosphorylation of one **serine** residue in the enzyme prevents binding of **isocitrate**

- **α-ketoglutarate dehydrogenase**

Inhibited by succinyl-CoA and NADH

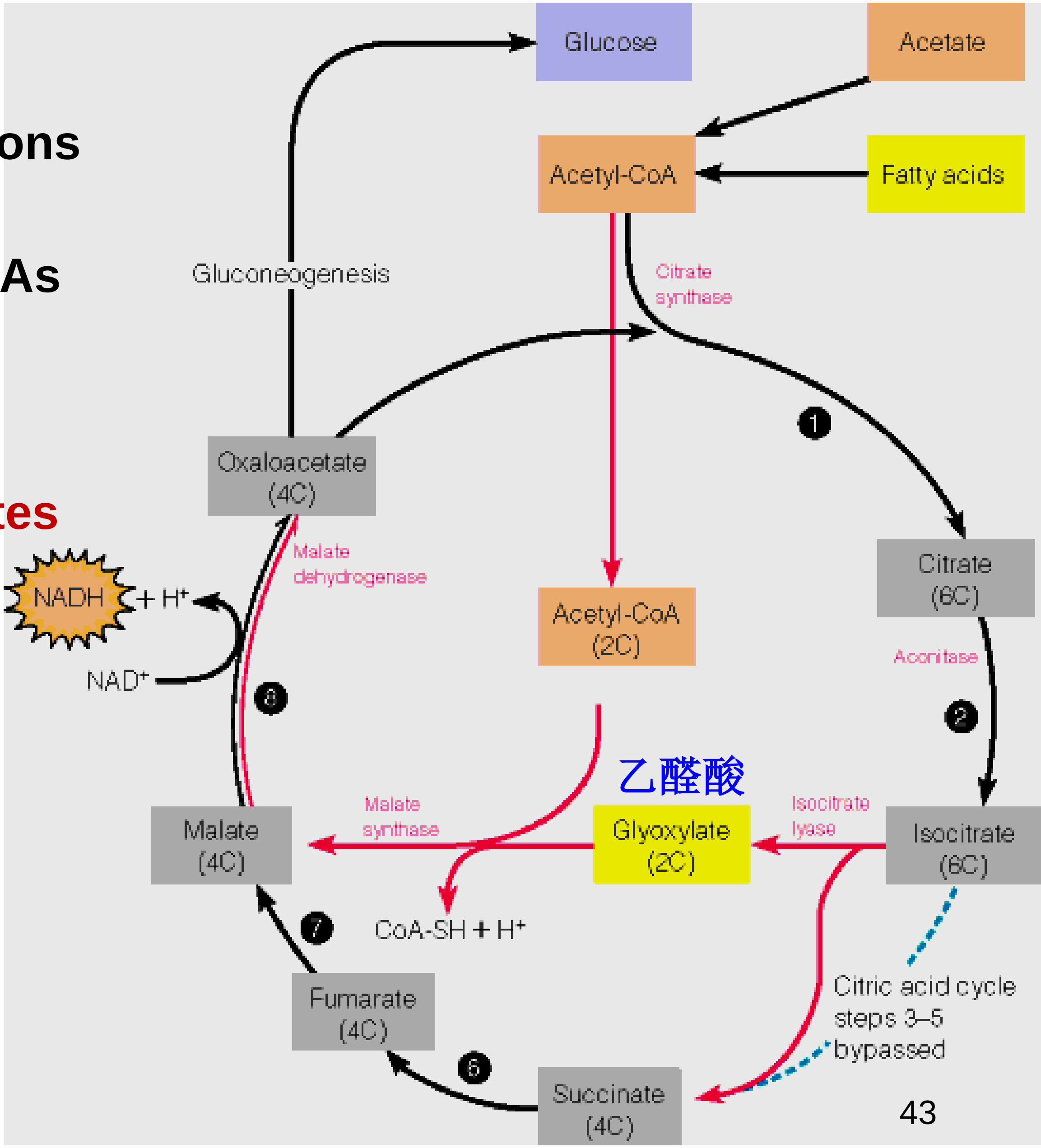
5 .Glyoxylate Cycle　乙醛酸循环

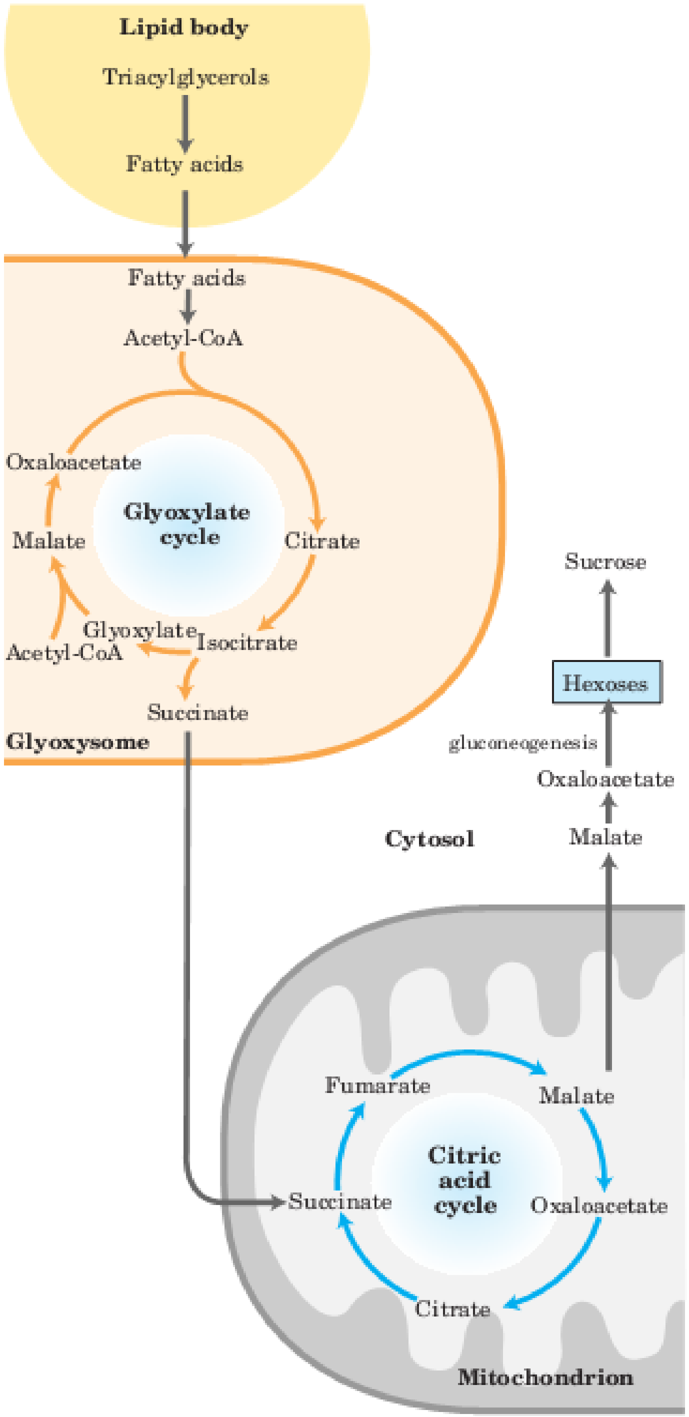


- **Plants**
- **some bacteria (including *E. coli* and yeast)**
- **converting acetate（乙酸）to carbohydrate.**
- **No decarboxylations（脱羧）steps**

  - **isocitrate lyase 异柠檬酸裂合酶**
  - **malate synthase 苹果酸合酶**

- **five steps**
- **Lack CO_2 liberating reactions**
- **Consumes two acetyl-CoAs and one oxaloacetate**
- **Produces two oxaloacetates**





- In <u>plants</u>, <u>bacteria</u> and <u>yeast</u> (not animals)
- Glyoxylate cycle：2C→G

➢In <u>animals</u>, acetyl CoA is <u>not</u> a carbon source for the <u>net</u> formation of glucose !!

Summary

- **Pyruvate produced in glycolysis （cytosol）is transported into mitochondria**
- **Pyruvate is converted to acetyl-coenzyme A by the pyruvate dehydrogenase complex**
- **Acetyl-CoA enters the TCA cycle and is oxidized to CO_2**
- **The electrons liberated from TCA are passed via NADH and $FADH_2$ through an membrane-associated electron-transport pathway to O_2, the final electron receptor and produce ATP**

思考题

1. Although oxygen does not participate directly in the citric acid cycle, the cycle operates only when O_2 is present. Why?

2. What is Glyoxylate Cycle？ What are the main enzymes？

3. Thiamine deficiency（硫胺素缺乏） individuals with a thiamine-deficient diet have relatively high levels of pyruvate in their blood. Explain this in biochemical terms.

4.什么是三羧酸循环？详述其具体过程。三羧酸循环的生理意义是什么？