The Citric Acid Cycle

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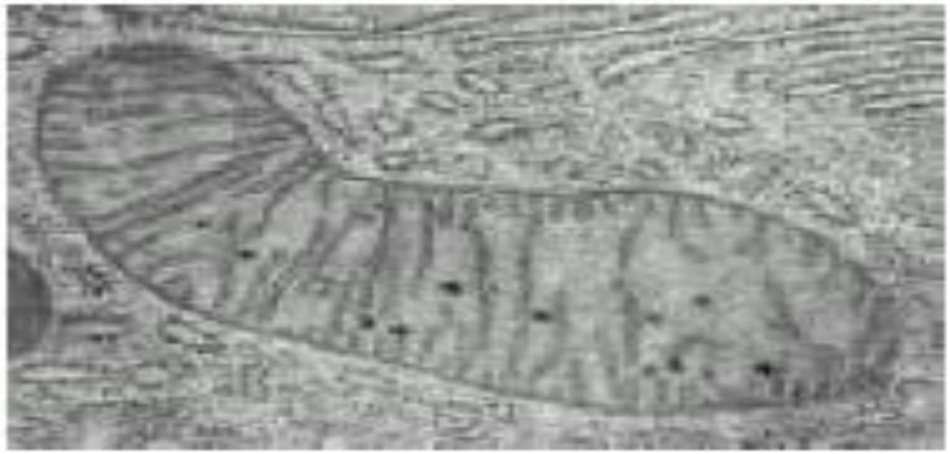
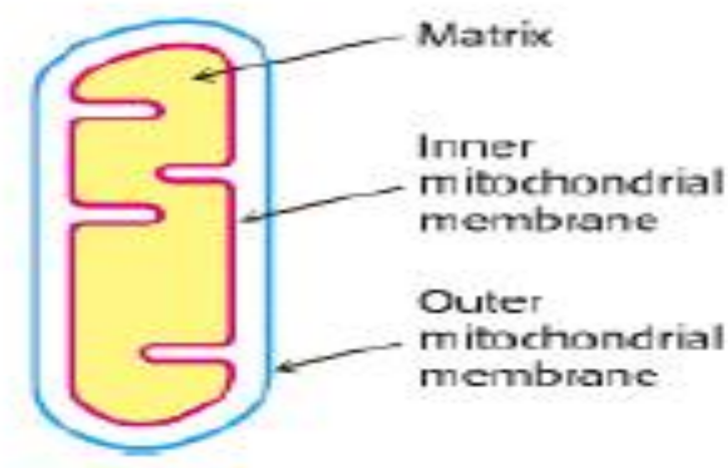
Introduction

- The aerobic processing of glucose starts with the complete oxidation of glucose derivatives to carbon dioxide.
- This oxidation takes place in the *citric acid cycle,* a series of reactions also known as the *tricarboxylic acid (TCA) cycle* or the *Krebs cycle.*
- The citric acid cycle is the *final common pathway for the oxidation of fuel molecules* amino acids, fatty acids, and carbohydrates.
- Most fuel molecules enter the cycle as acetyl coenzyme A

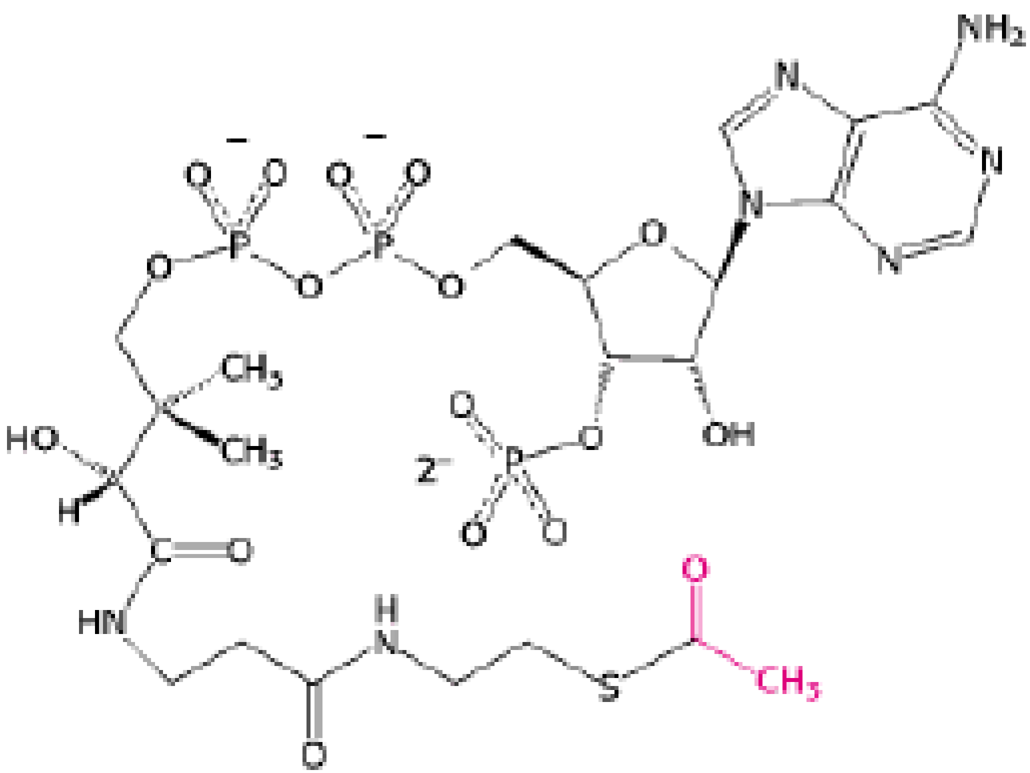
- Under aerobic conditions, the pyruvate generated from glucose is oxidatively decarboxylated to form acetyl CoA
- In eukaryotes, the reactions of the citric acid cycle take place inside mitochondria, in contrast with those of glycolysis, which take place in the cytosol

Mitochondrion





Acetyl Coenzyme A (Acetyl CoA)



Acetyl coenzyme A (Acetyl CoA)

An Overview of the Citric Acid Cycle

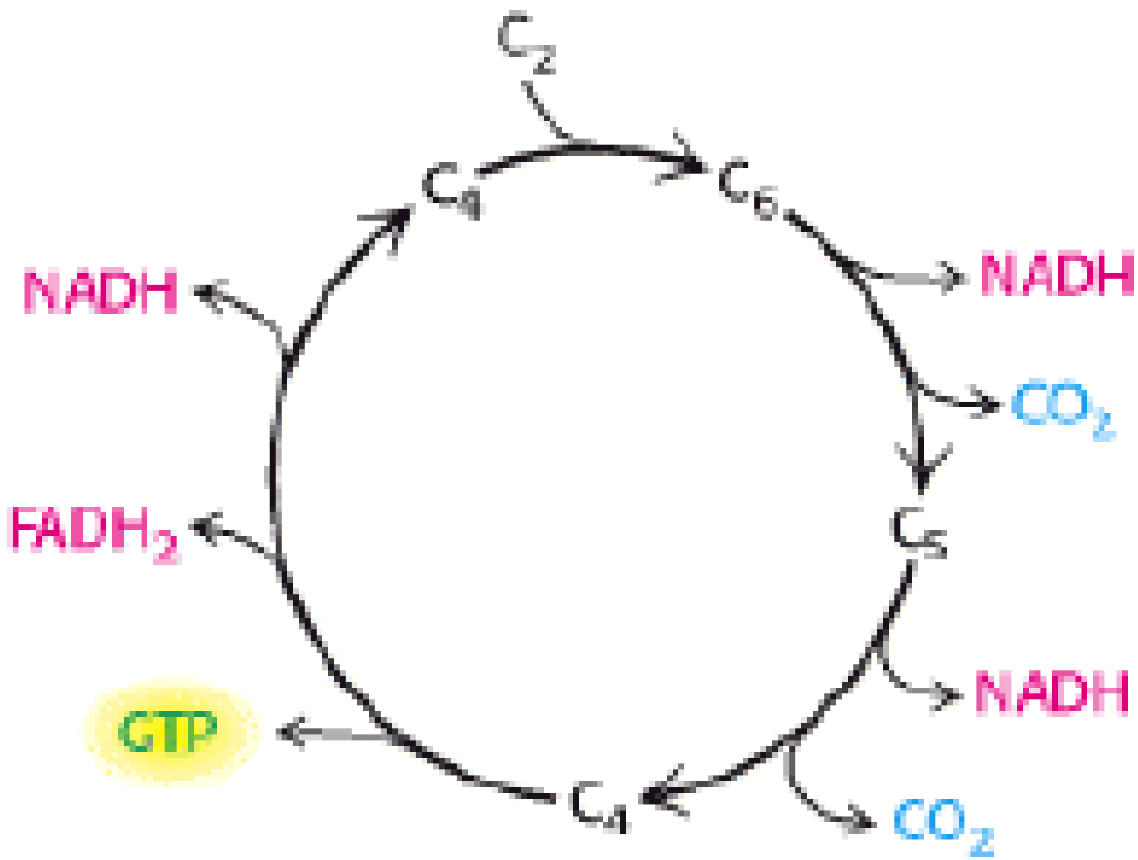
- The citric acid cycle is the central metabolic hub of the cell.
- It is the gateway to the aerobic metabolism of any molecule that can be transformed into an acetyl group or dicarboxylic acid
- The cycle is also an important source of precursors, not only for the storage forms of fuels, but also for the building blocks of many other molecules such as amino acids, nucleotide bases, cholesterol, and porphyrin (the organic component of heme).

What is the function of the citric acid cycle in transforming fuel molecules into ATP?

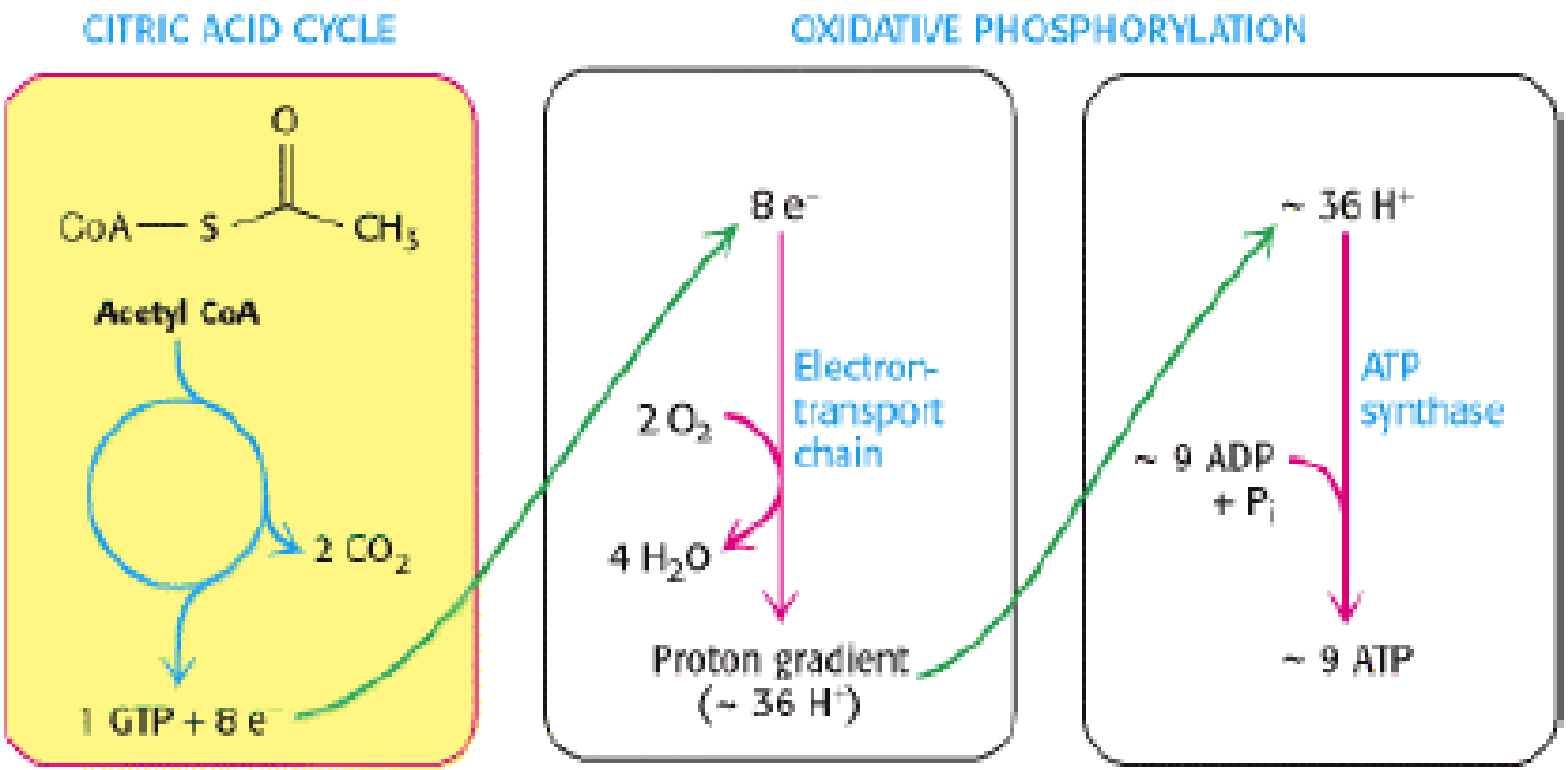
- Recall that fuel molecules are carbon compounds that are capable of being oxidized of losing electrons
- The citric acid cycle includes a series of oxidation-reduction reactions that result in the oxidation of an acetyl group to two molecules of carbon dioxide.
- The citric acid cycle, in conjunction with oxidative phosphorylation, provides the vast majority of energy used by aerobic cells in human beings, greater than 95%.

- It is highly efficient because a limited number of molecules can generate large amounts of NADH and FADH2
- The four-carbon molecule, oxaloacetate, that initiates the first step in the citric acid cycle is regenerated at the end of one passage through the cycle.
- The oxaloacetate acts catalytically: it participates in the oxidation of the acetyl group but is itself regenerated.
- Thus, one molecule of oxaloacetate is capable of participating in the oxidation of many acetyl molecules.

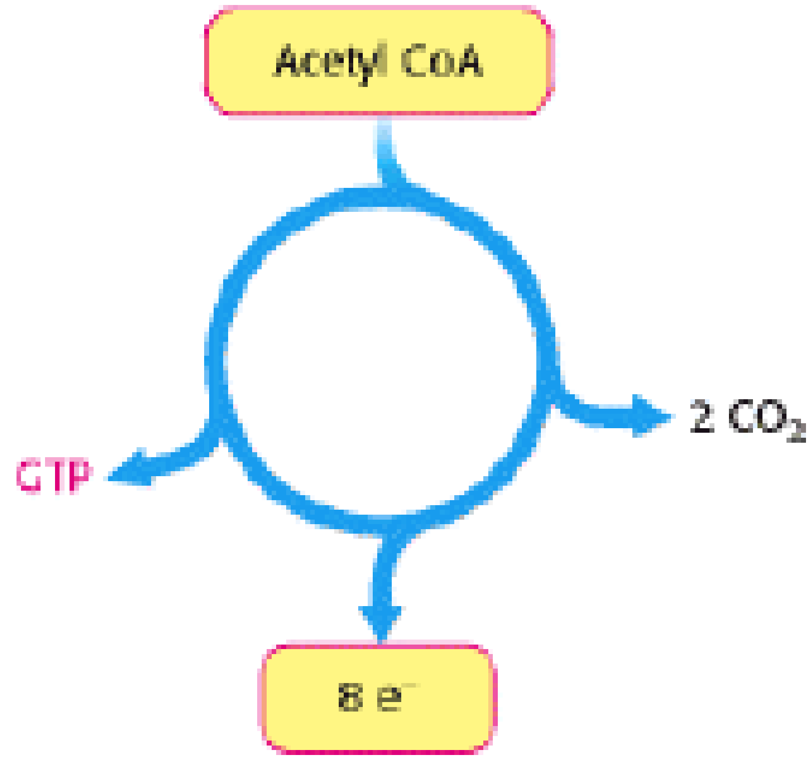
Overview of the Citric Acid Cycle



Cellular Respiration



Roundabouts, or traffic circles, function as hubs to facilitate traffic flow

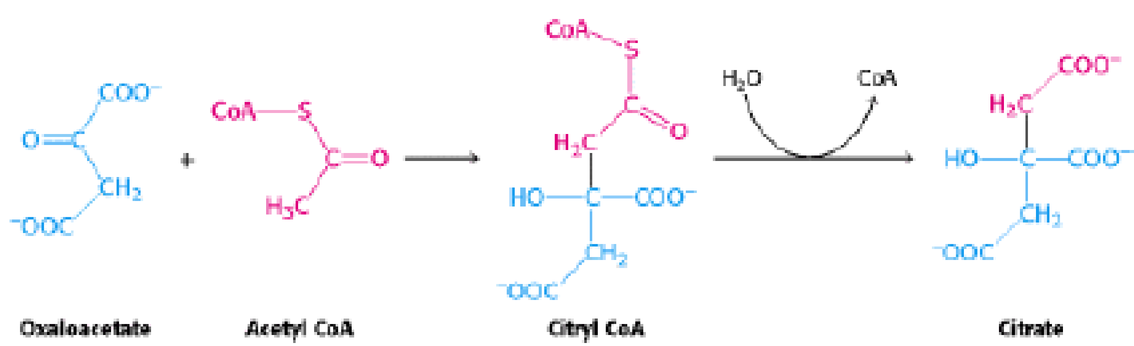


The Citric Acid Cycle Oxidizes Two-Carbon Units

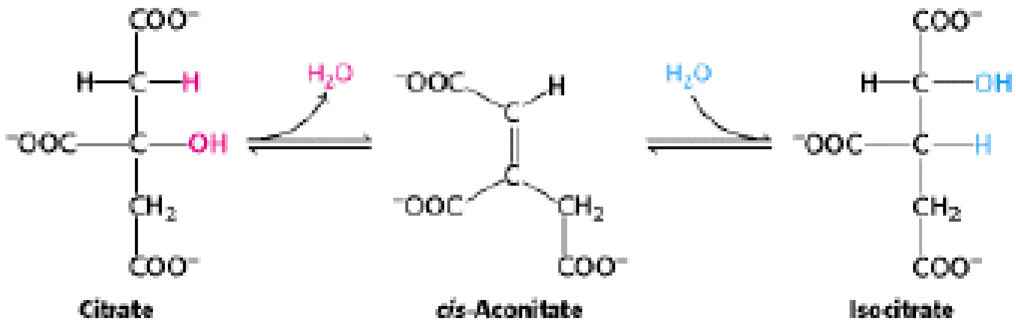
- *Acetyl CoA* is the fuel for the citric acid cycle.
- This important molecule is formed from the breakdown of glycogen (the storage form of glucose), fats, and many amino acids.
- The *Acetyl CoA* that supplies the cycle with acetyl residues is mainly derived from *β-oxidation* of fatty acids and from the *pyruvate dehydrogenase* reaction.
- In eight steps, it is oxidizes acetyl residues to carbon dioxide

1. Citrate Synthase Forms Citrate from Oxaloacetate and Acetyl Coenzyme A

- The citric acid cycle begins with the condensation of a four-carbon unit, oxaloacetate, and a two-carbon unit, the acetyl group of acetyl CoA.
- Oxaloacetate reacts with acetyl CoA and H2O to yield citrate and CoA.



2. Citrate Is Isomerized into Isocitrate

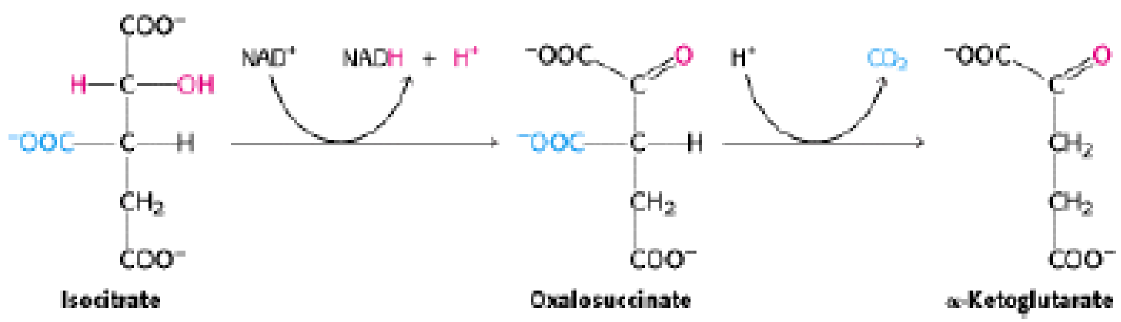


- Citrate is isomerized into isocitrate to enable the six-carbon unit to undergo oxidative decarboxylation
- The isomerization of citrate is accomplished by a *dehydration* step followed by a *hydration* step
- The result is an interchange of a hydrogen atom and a hydroxyl group. The enzyme catalyzing both steps is called *aconitase* because cis-*aconitate* is an intermediate.

3. Isocitrate Is Oxidized and Decarboxylated to α-Ketoglutarate

- The oxidative decarboxylation of isocitrate is catalyzed by *isocitrate dehydrogenase*

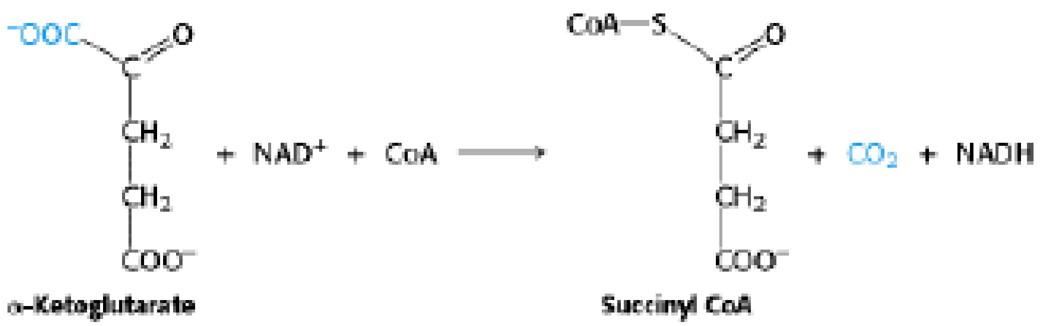
$$\text{Isocitrate} + NAD^+ \longrightarrow \alpha\text{-ketoglutarate} + CO_2 + NADH$$



- First of four oxidation-reduction reactions in the citric acid cycle. The intermediate in this reaction is oxalosuccinate, an unstable β-ketoacid.
- While bound to the enzyme, it loses CO2 to form a-ketoglutarate.
- The rate of formation of α-ketoglutarate is important in determining the overall rate of the cycle
- This oxidation generates the first high-transfer-potential electron carrier NADH in the cycle

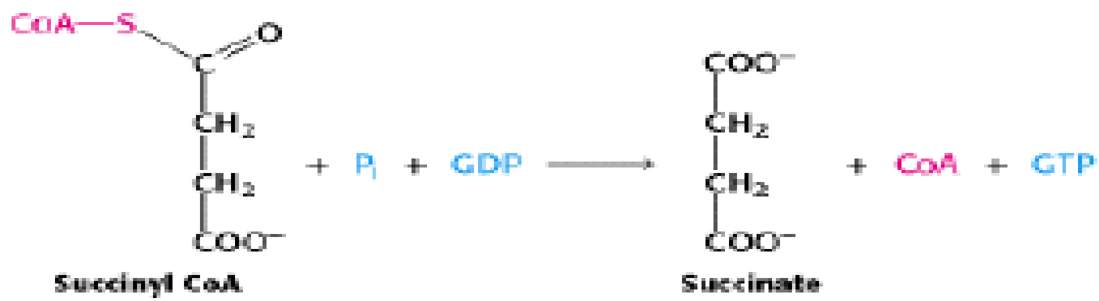
4. Succinyl Coenzyme A Is Formed by the Oxidative Decarboxylation of α-Ketoglutarate

- The conversion of isocitrate into α-ketoglutarate is followed by a second oxidative decarboxylation reaction
- Enzyme: 2-oxoglutarate dehydrogenase



$$\text{Pyruvate} + \text{CoA} + \text{NAD}^+ \longrightarrow \text{acetyl CoA} + CO_2 + \text{NADH}$$

5. A High Phosphoryl-Transfer Potential Compound Is Generated from Succinyl Coenzyme A

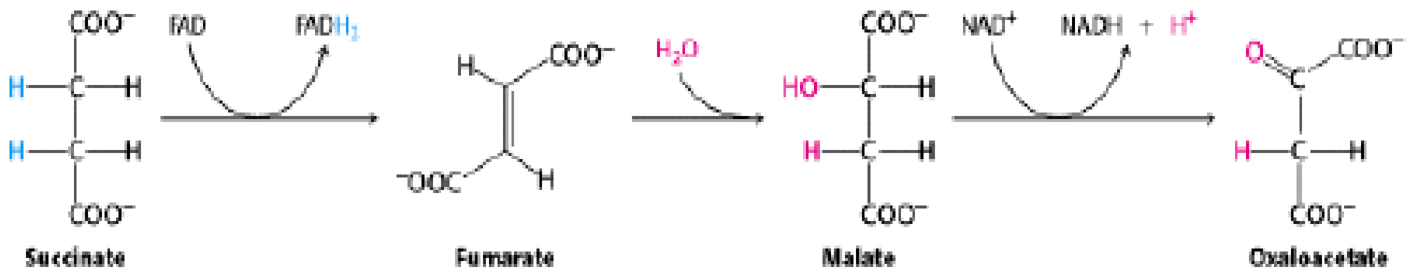


$$\mathrm{GTP + ADP \rightleftharpoons GDP + ATP}$$

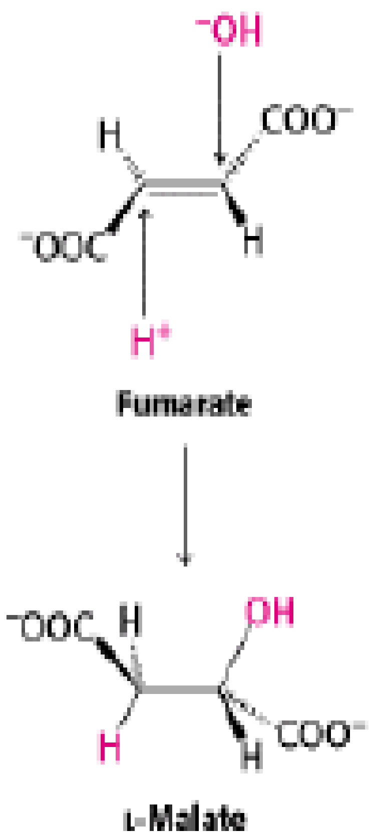
- This is the only step in the citric acid cycle that directly yields a compound with high phosphoryl transfer potential through a substrate-level phosphorylation.
- *The cleavage of the thioester bond of succinyl CoA is coupled to the phosphorylation of a purine nucleoside diphosphate, usually GDP.*
- This reaction is catalyzed by *succinyl CoA synthetase* (succinate thiokinase).

6. Oxaloacetate Is Regenerated by the Oxidation of Succinate

- Reactions of four-carbon compounds constitute the final stage of the citric acid cycle: the regeneration of oxaloacetate.

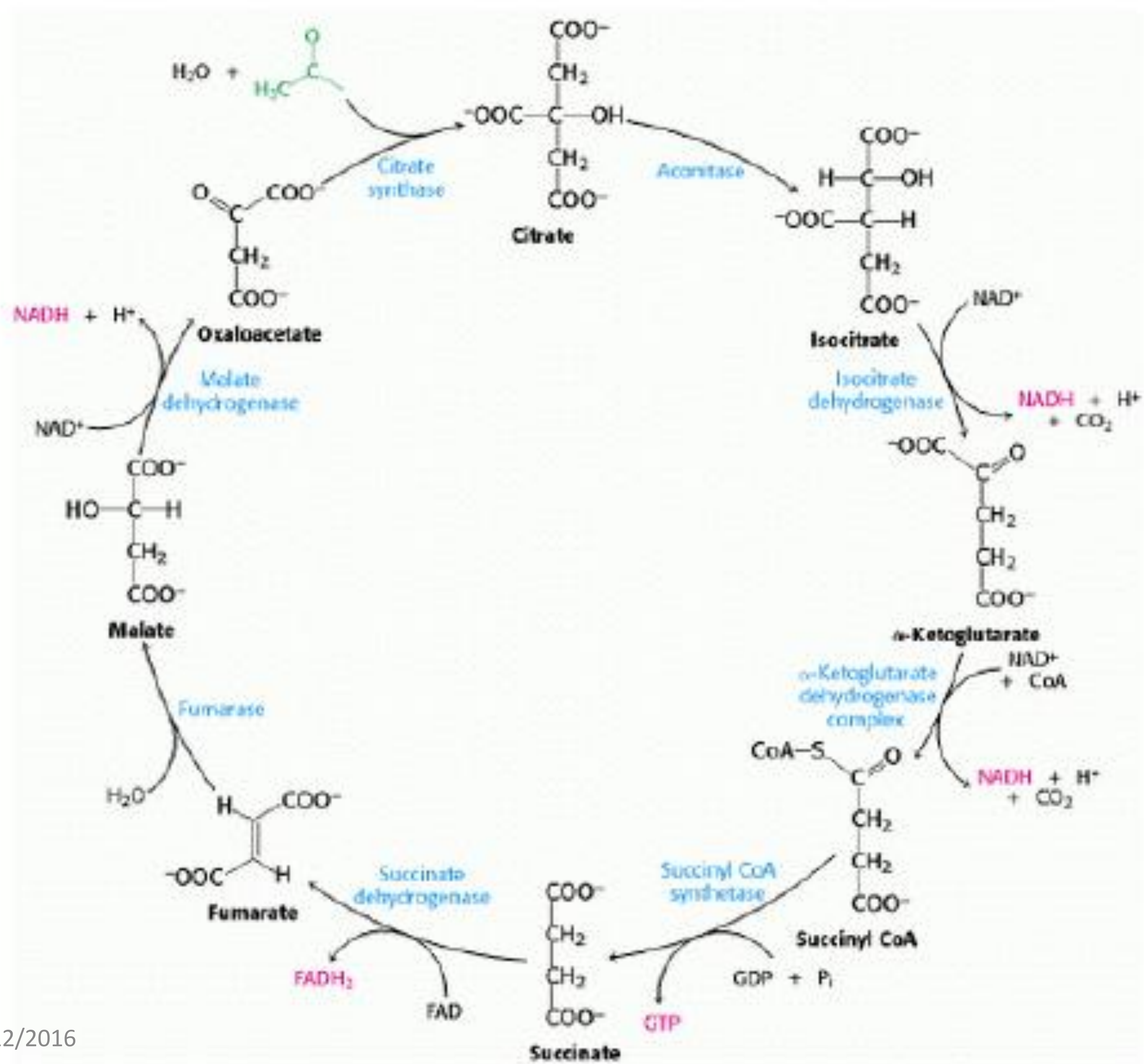


- A methylene group (CH2) is converted into a carbonyl group (C = O) in three steps: an oxidation, a hydration, and a second oxidation reaction.
- Not only is oxaloacetate thereby regenerated for another round of the cycle, but also more energy is extracted in the form of FADH2 and NADH. Succinate is oxidized to fumarate by *succinate dehydrogenase.*
- The hydrogen acceptor is FAD rather than NAD+, which is used in the other three oxidation reactions in the cycle.
- FAD is the hydrogen acceptor in this reaction because the free-energy change is insufficient to reduce NAD+.
- FAD is nearly always the electron acceptor in oxidations that remove two hydrogen *atoms* from a substrate

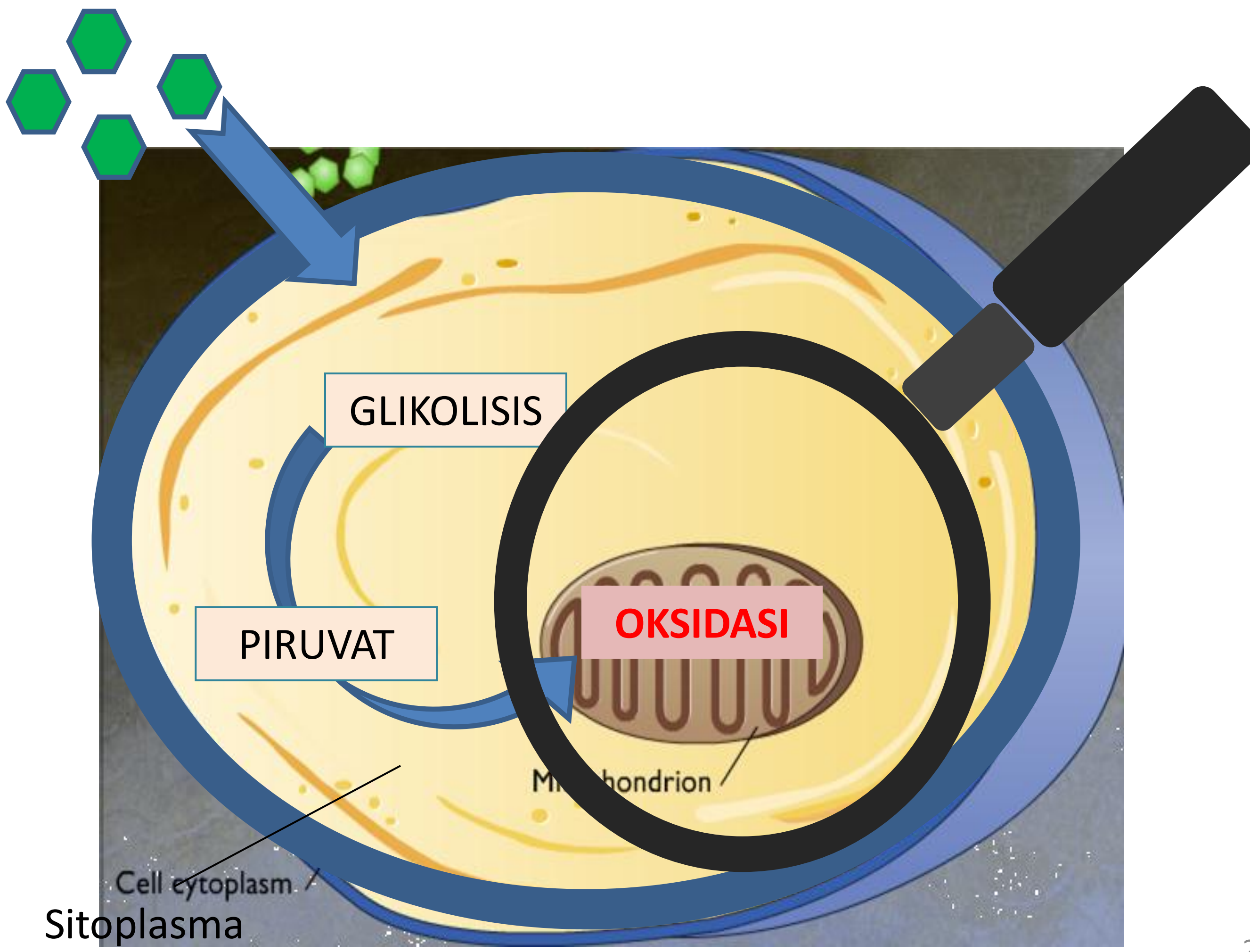


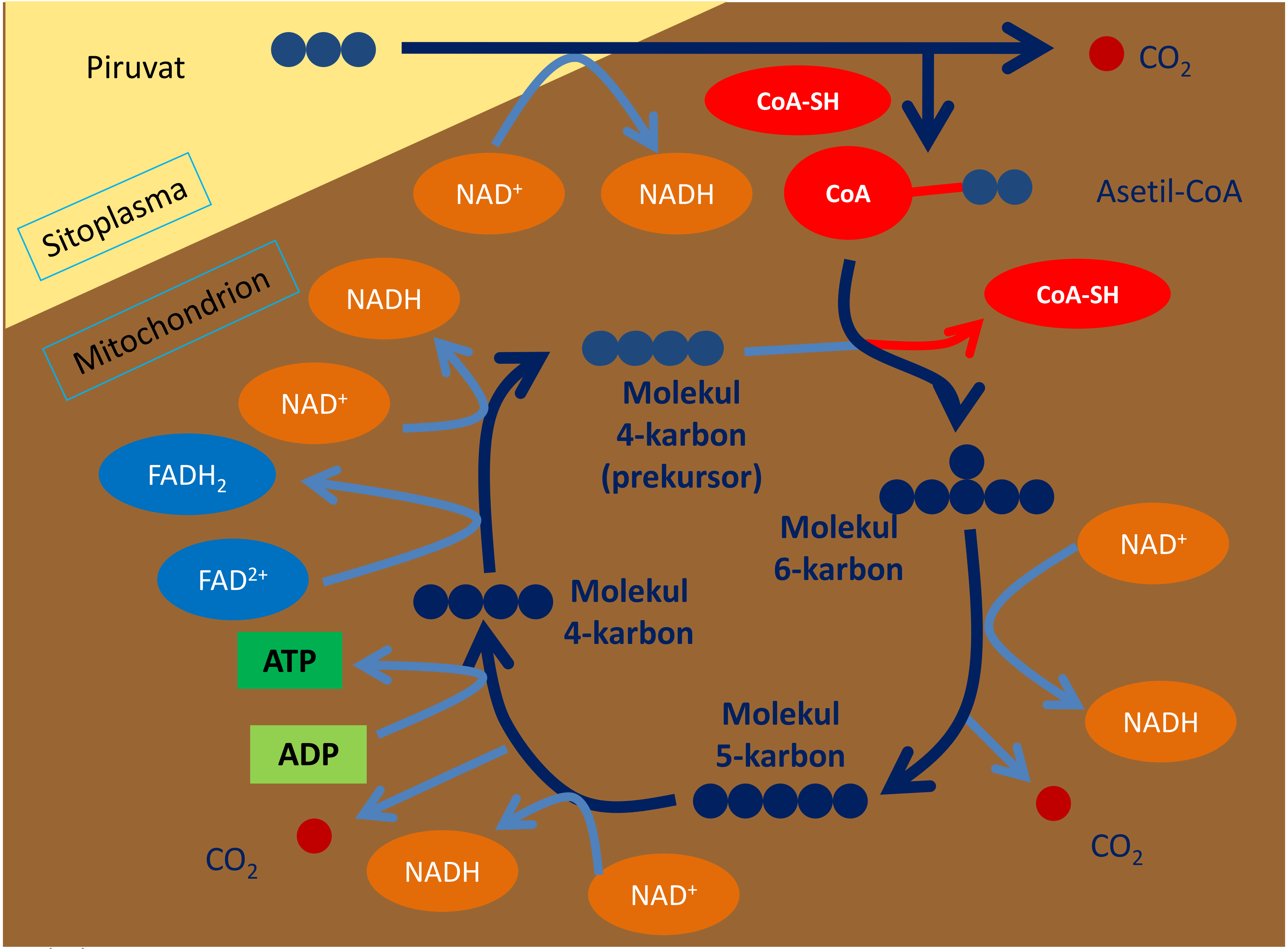
- The hydration of fumarate to form l-malate.
- *Fumarase* catalyzes a stereospecific trans addition of a hydrogen atom and a hydroxyl group.
- Finally, malate is oxidized to form oxaloacetate. This reaction is catalyzed by *malate dehydrogenase*, and NAD+ is again the hydrogen acceptor.

$$\text{Malate} + NAD^+ \rightleftharpoons \text{oxaloacetate} + NADH + H^+$$


Citrate
Citrate synthase
Aconitase
Isocitrate
Isocitrate dehydrogenase
NAD+
NADH + H+
+ CO2
α-Ketoglutarate
α-Ketoglutarate dehydrogenase complex
NAD+
+ CoA
NADH + H+
+ CO2
Succinyl CoA
Succinyl CoA synthetase
GDP + Pi
GTP
Succinate
Succinate dehydrogenase
FAD
FADH2
Fumarate
Fumarase
H2O
Malate
Malate dehydrogenase
NAD+
NADH + H+
Oxaloacetate

KREBS CYCLE


Molekul makanan
GLIKOLISIS
PIRUVAT
OKSIDASI
Cell cytoplasm
Sitoplasma


Piruvat
CO_2
CoA-SH
Sitoplasma
NAD^+
NADH
CoA
Asetil-CoA
Mitochondrion
NADH
CoA-SH
Molekul 4-karbon (prekursor)
NAD^+
$FADH_2$
Molekul 6-karbon
NAD^+
FAD^{2+}
Molekul 4-karbon
ATP
NADH
ADP
Molekul 5-karbon
CO_2
CO_2
NADH
NAD^+

Stoichiometry of the Citric Acid Cycle

- The net reaction of the citric acid cycle is:

$$\text{Acetyl CoA} + 3\,NAD^+ + FAD + GDP + P_i + 2\,H_2O \longrightarrow 2\,CO_2 + 3\,NADH + FADH_2 + GTP + 2\,H^+ + CoA$$

- The **net outcome** is that each rotation of the tricarboxylic acid cycle converts 1 acetyl residue and 2 molecules of H2O → 2 molecules of CO2.
- At the same time, 1 GTP, 3 NADH+H+ and 1 reduced ubiquinone (QH2) are produced

Let us recapitulate the reactions that give this stoichiometry:

1.

- 2 carbon atoms enter the cycle in the condensation of an acetyl unit (from acetyl CoA) with oxaloacetate.
- 2 carbon atoms leave the cycle in the form of CO2 in the successive decarboxylations catalyzed by isocitrate dehydrogenase and a-ketoglutarate dehydrogenase.
- Interestingly, the results of isotope-labeling studies revealed that the two carbon atoms that enter each cycle are not the ones that leave.

2:

- 4 pairs of hydrogen atoms leave the cycle in four oxidation reactions.
- 2 molecules of NAD+ are reduced in the oxidative decarboxylations of isocitrate and a -ketoglutarate, one molecule of FAD is reduced in the oxidation of succinate, and 1 molecule of NAD+ is reduced in the oxidation of malate.

3.

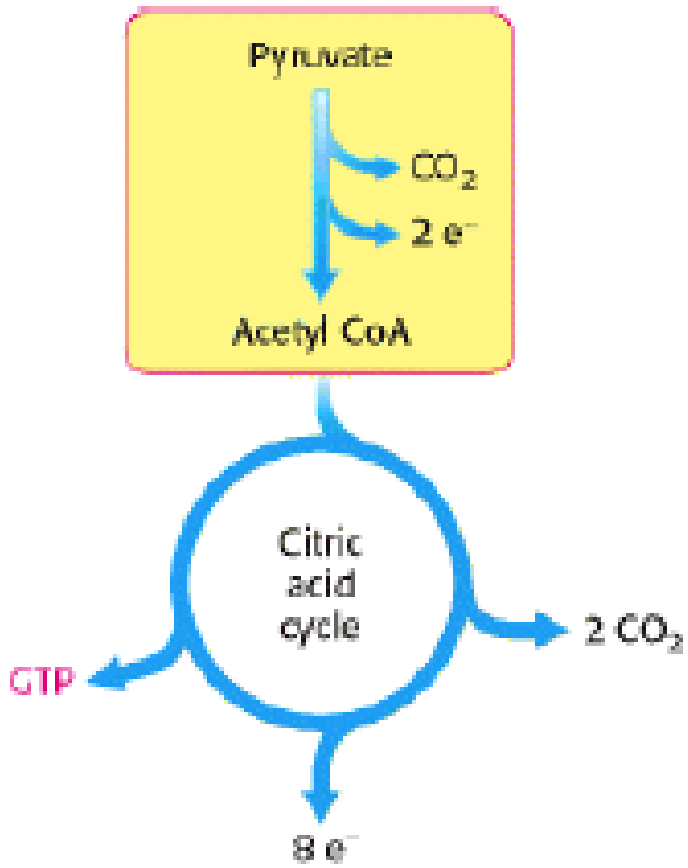
- One compound with high phosphoryl transfer potential, usually GTP, is generated from the cleavage of the thioester linkage in succinyl CoA

4.

- 2 molecules of water are consumed: one in the synthesis of citrate by the hydrolysis of citryl CoA and the other in the hydration of fumarate

- Recall that molecular oxygen does not participate directly in the citric acid cycle.
- However, the cycle operates only under aerobic conditions because NAD+ and FAD can be regenerated in the mitochondrion only by the transfer of electrons to molecular oxygen. *Glycolysis has both an aerobic and an anaerobic mode, whereas the citric acid cycle is strictly aerobic.*
- Glycolysis can proceed under anaerobic conditions because NAD+ is regenerated in the conversion of pyruvate into lactate.

The Link between Glycolysis and the Citric Acid Cycle.



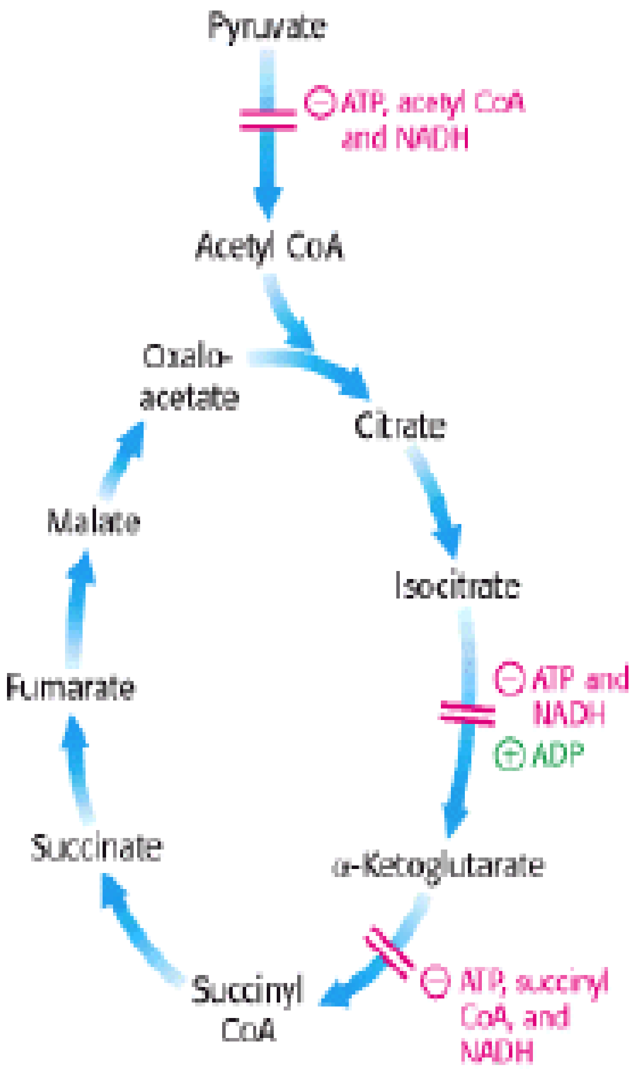
- Pyruvate produced by glycolysis is converted into acetyl CoA, the fuel of the citric acid cycle.

Citric acid cycle

Step	Reaction	Enzyme	Prosthetic group	Type*	DG° kcal mol-1	DG° kJ mol-1
1	Acetyl CoA + oxaloacetate + $H_2O \rightarrow$ citrate + CoA + H^+	Citrate synthase		a	-7.5	-31.4
2a	Citrate $\rightleftharpoons$ *cis*-aconitate + H_2O	Aconitase	Fe-S	b	+2.0	+8.4
2b	*cis*-Aconitate+ $H_2O \rightleftharpoons$ isocitrate	Aconitase	Fe-S	c	-0.5	-2.1
3	Isocitrate + $NAD^+ \rightleftharpoons$ α-ketoglutarate + CO_2 + NADH	Isocitrate dehydrogenase		d + e	-2.0	-8.4
4	α-Ketoglutarate + NAD^+ + CoA $\rightleftharpoons$ 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 $\rightleftharpoons$ succinate + GTP + CoA	Succinyl CoA synthetase		f	-0.8	-3.3
6	Succinate + FAD (enzyme-bound) $\rightleftharpoons$ fumarate + $FADH_2$ (enzyme-bound)	Succinate dehydrogenase	FAD, Fe-S	e	˜0	0
7	Fumarate + $H_2O \rightleftharpoons$ l-malate	Fumarase		c	-0.9	-3.8
8	l-Malate + $NAD^+ \rightleftharpoons$ 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.

Control of the Citric Acid Cycle

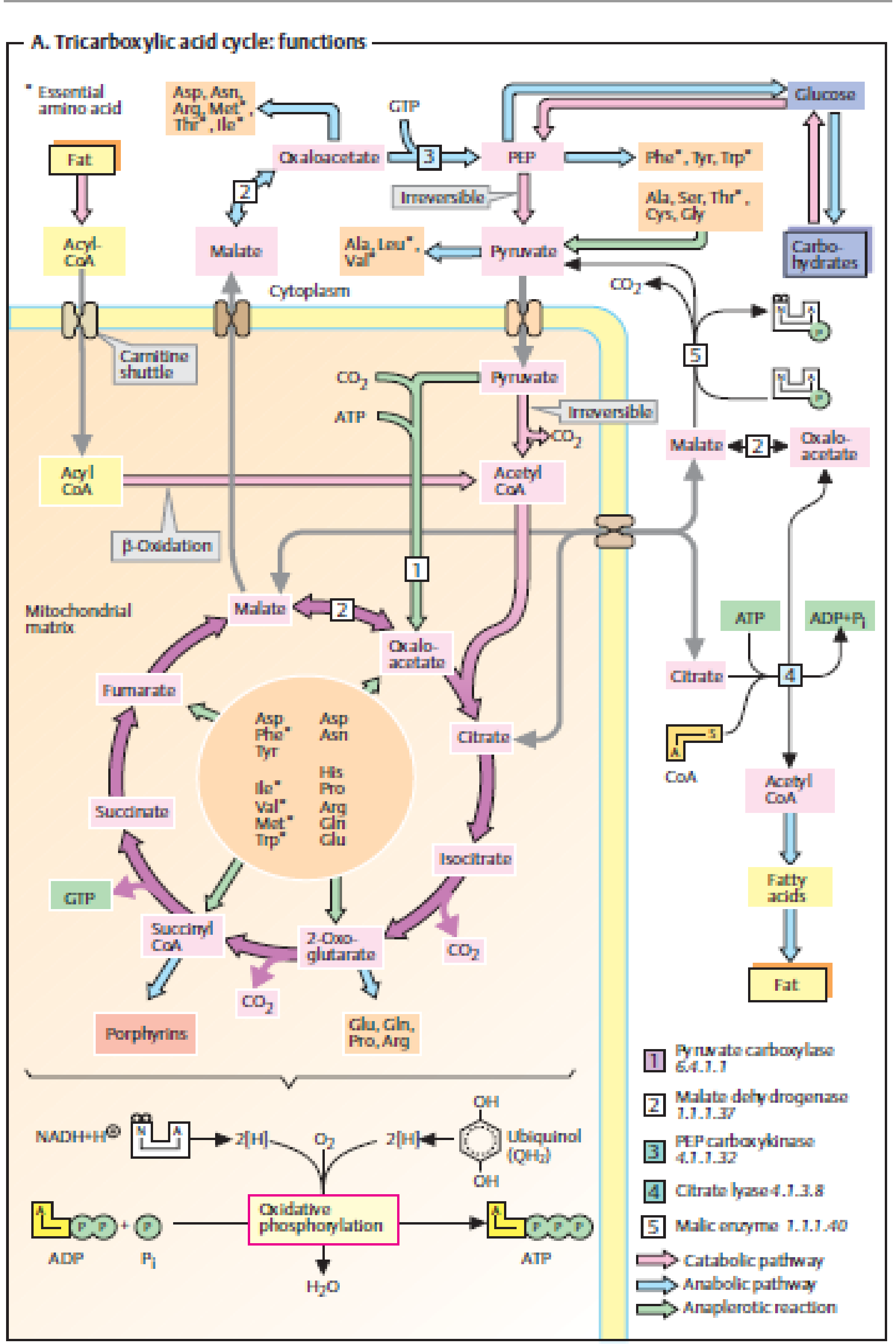


- The citric acid cycle is regulated primarily by the concentration of ATP and NADH
- The primary control points are:

1. the allosteric enzymes isocitrate dehydrogenase and
2. a-ketoglutarate dehydrogenase

Tricarboxylic acid cycle: functions

- "hub of intermediary metabolism."
- It has both catabolic and anabolic functions—it is **amphibolic**
- As a **catabolic pathway**, it initiates the **"terminal oxidation"** of energy substrates
- The reducing equivalents obtained in this way are then used for *oxidative phosphorylation*—i. e., to aerobically synthesize ATP
- The tricarboxylic acid cycle also supplies important **precursors for anabolic pathways.**
- Intermediates in the cycle are converted into:
  - ❑ Glucose (gluconeogenesis; precursors: oxaloacetate and malate)
  - ❑ Porphyrins (precursor: succinyl-CoA)
  - ❑ Amino acids (precursors: 2-oxoglutarate, oxaloacetate)
  - ❑ Fatty acids and isoprenoids (precursor: citrate)


A. Tricarboxylic acid cycle: functions
* Essential amino acid
Fat
Acyl-CoA
Asp, Asn, Arg, Met*, Thr*, Ile*
Oxaloacetate
GTP
PEP
Glucose
Phe*, Tyr, Trp*
Irreversible
Ala, Ser, Thr*, Cys, Gly
Malate
Ala, Leu*, Val*
Pyruvate
Carbo-hydrates
Cytoplasm
CO_2
Carnitine shuttle
Pyruvate
ATP
Irreversible
Malate
Oxalo-acetate
Acyl CoA
Acetyl CoA
β-Oxidation
Mitochondrial matrix
Malate
Oxalo-acetate
Fumarate
Citrate
Asp Phe* Tyr
Asp Asn
His Pro Arg Gln Glu
Ile* Val* Met* Trp*
Succinate
Isocitrate
GTP
Succinyl CoA
2-Oxo-glutarate
CO_2
Porphyrins
Glu, Gln, Pro, Arg
ATP
ADP+P_i
Citrate
CoA
Acetyl CoA
Fatty acids
Fat
NADH+H⊕
2[H]
O_2
OH
Ubiquinol (QH_2)
Oxidative phosphorylation
ADP
P_i
ATP
H_2O
1 Pyruvate carboxylase 6.4.1.1
2 Malate dehydrogenase 1.1.1.37
3 PEP carboxykinase 4.1.1.32
4 Citrate lyase 4.1.3.8
5 Malic enzyme 1.1.1.40
Catabolic pathway
Anabolic pathway
Anaplerotic reaction

TERIMA KASIH
SELAMAT BELAJAR...