Core Course 6 (Metabolism) 4C/50hrs	Days
Citric Acid Cycle	3 classes x 2
Core Course 7 (Cell Biology) 4C/50hrs	
(1) Introduction to Cell Biology (2) Tools of Cell Biology	4 classes x 2
Skill enhancement course (SEC)	
1. Tools and techniques in Biochemistry/Protein purification techniques	

1. Lehninger, Nelson & Cox, Biochemistry 6th Edition (core course 1)
2. Lodish, Molecular and Cell Biology 7th Edition (core course 7)
3. Alberts, Molecular Biology of the Cell

10 years qs.

- Most eukaryotic cells and many bacteria, which live under aerobic conditions and oxidize their organic fuels to carbon dioxide and water.

- pyruvate produced by glycolysis is further oxidized to H_2O and CO_2.

- This aerobic phase of catabolism is called **respiration**

- Cellular respiration occurs in three major stages:

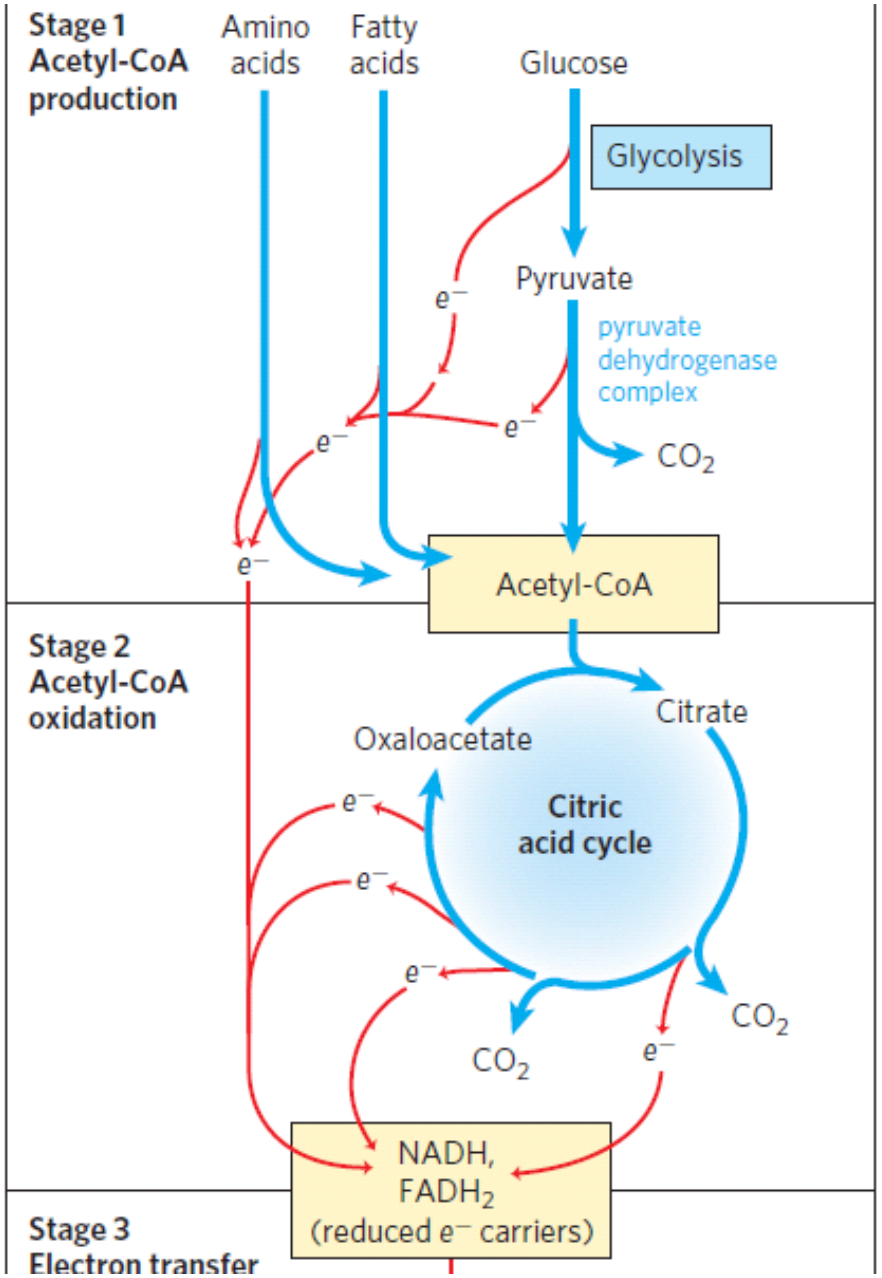
- In the first, organic fuel molecules—glucose, fatty acids, and some amino acids—are oxidized to yield two-carbon fragments in the form of the acetyl group of acetyl-coenzyme A (acetyl-CoA).

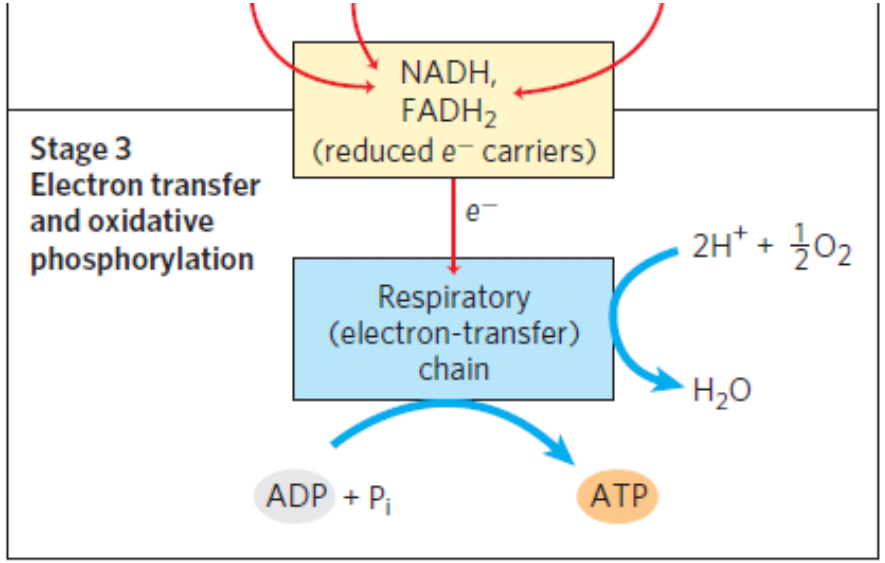
- In the second stage, the acetyl groups are fed into the citric acid cycle, which enzymatically oxidizes them to CO_2; the energy released is conserved in the reduced electron carriers NADH and $FADH_2$.

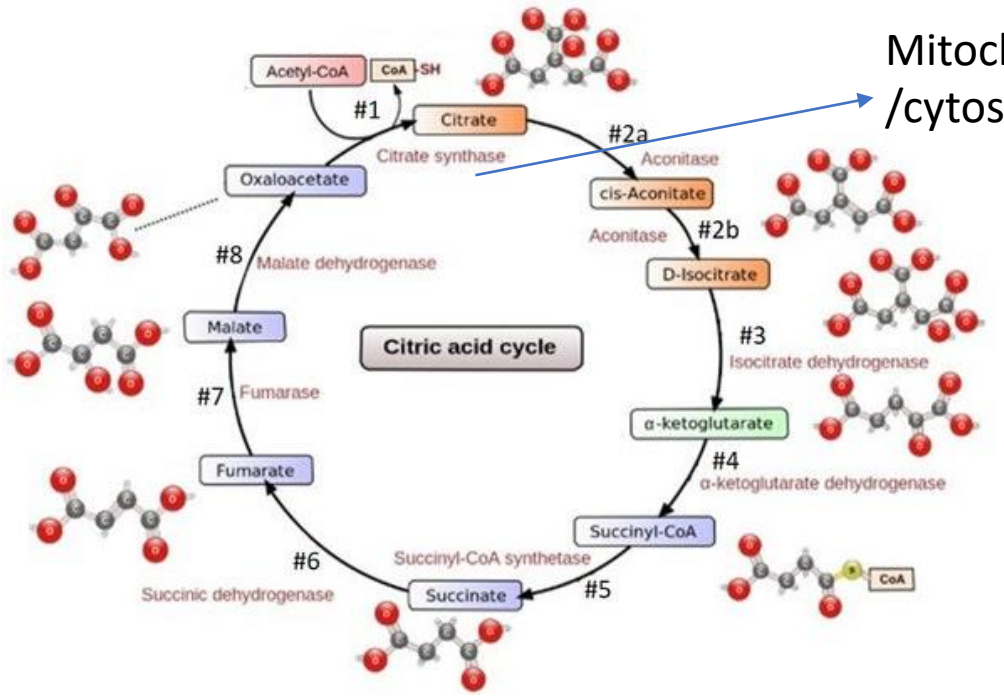
- In the third stage of respiration, these reduced coenzymes are themselves oxidized, giving up protons (H^+) and electrons.

- The electrons are transferred to O_2—the final electron acceptor—via a chain of electron-carrying molecules known as the respiratory chain.

- In the course of electron transfer, the large amount of energy released is conserved in the form of ATP, by a process called oxidative phosphorylation .


Stage 1
Acetyl-CoA
production
Amino
acids
Fatty
acids
Glucose
Glycolysis
Pyruvate
pyruvate
dehydrogenase
complex
e^-
CO_2
Acetyl-CoA
Stage 2
Acetyl-CoA
oxidation
Citrate
Oxaloacetate
Citric
acid cycle
CO_2
CO_2
NADH,
$FADH_2$
(reduced e^- carriers)
Stage 3
Electron transfer


NADH,
$FADH_2$
(reduced e^- carriers)
Stage 3
Electron transfer
and oxidative
phosphorylation
e^-
$2H^+ + \frac{1}{2}O_2$
Respiratory
(electron-transfer)
chain
H_2O
ADP + P_i
ATP

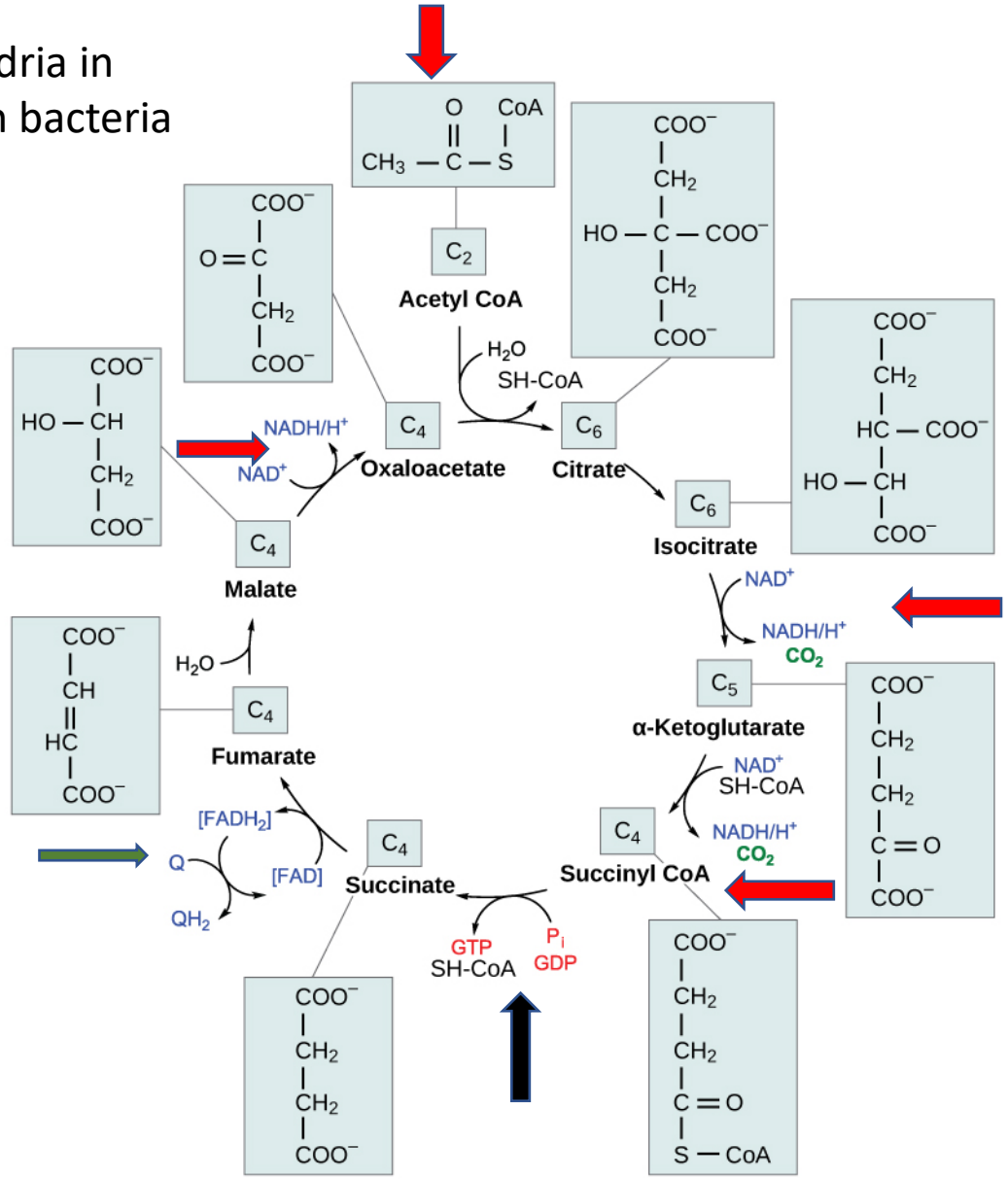


Mitochondria in /cytosol in bacteria

TCA/Citric acid cycle

1.Glucose → $CO_2 + H_2O$ (oxidation)

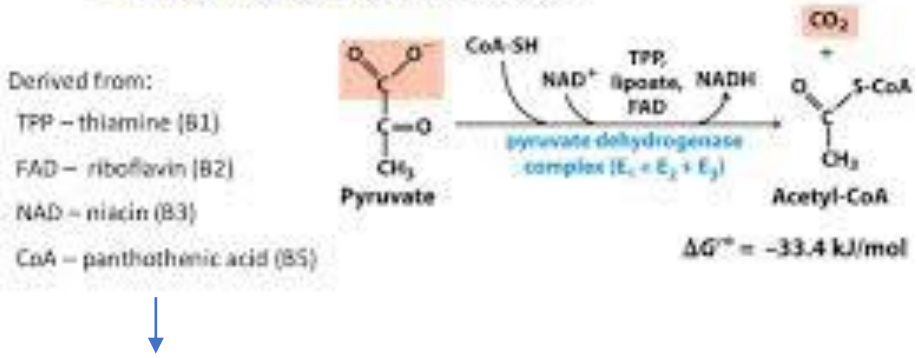
- ✓ Glycolysis (pyruvate)
- ✓ Fermentation (reduction of pyruvate to lactate/ethanol in absence of oxygen
- ✓ TCA cycle (aerobic oxidation of pyruvate to $CO_2 + H_2O$ & respiration)



1. Claisen condensation, 2. Dehydration/rehydration, 3. Oxidative decarboxylation, 4. Oxidative decarboxylation, 5. Substrate-level phosphorylation, 6. Dehydrogenation, 7. Hydration, 8. Dehydraogenation.
2. 1NADH = 2.5 ATP, FADH2 = 1.5 ATP

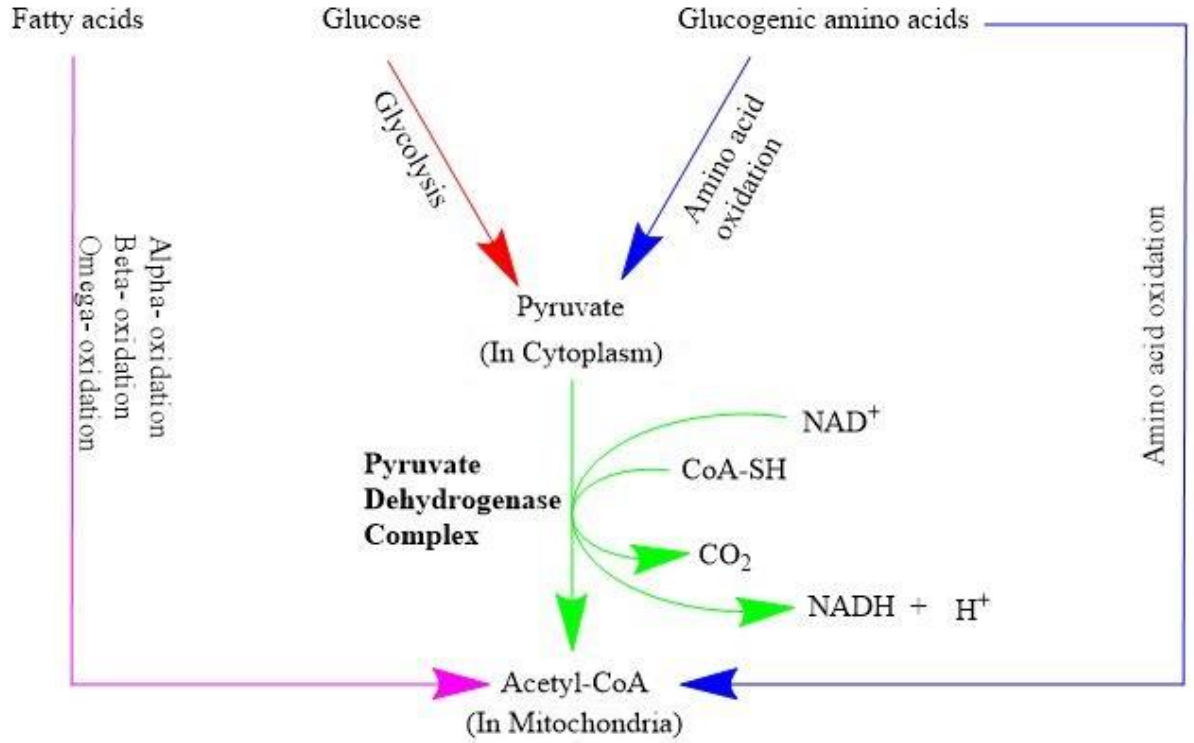
Conversion of Pyruvate to Acetyl-CoA

- Net Reaction:
  - *Oxidative decarboxylation* of pyruvate
  - First carbons of glucose to be fully oxidized (remember: 2 pyr/glc)
- Catalyzed by PDH
  - Requires 5 coenzymes
  - TPP, lipoate, and FAD are prosthetic groups
  - NAD^+ and CoA-SH are co-substrates

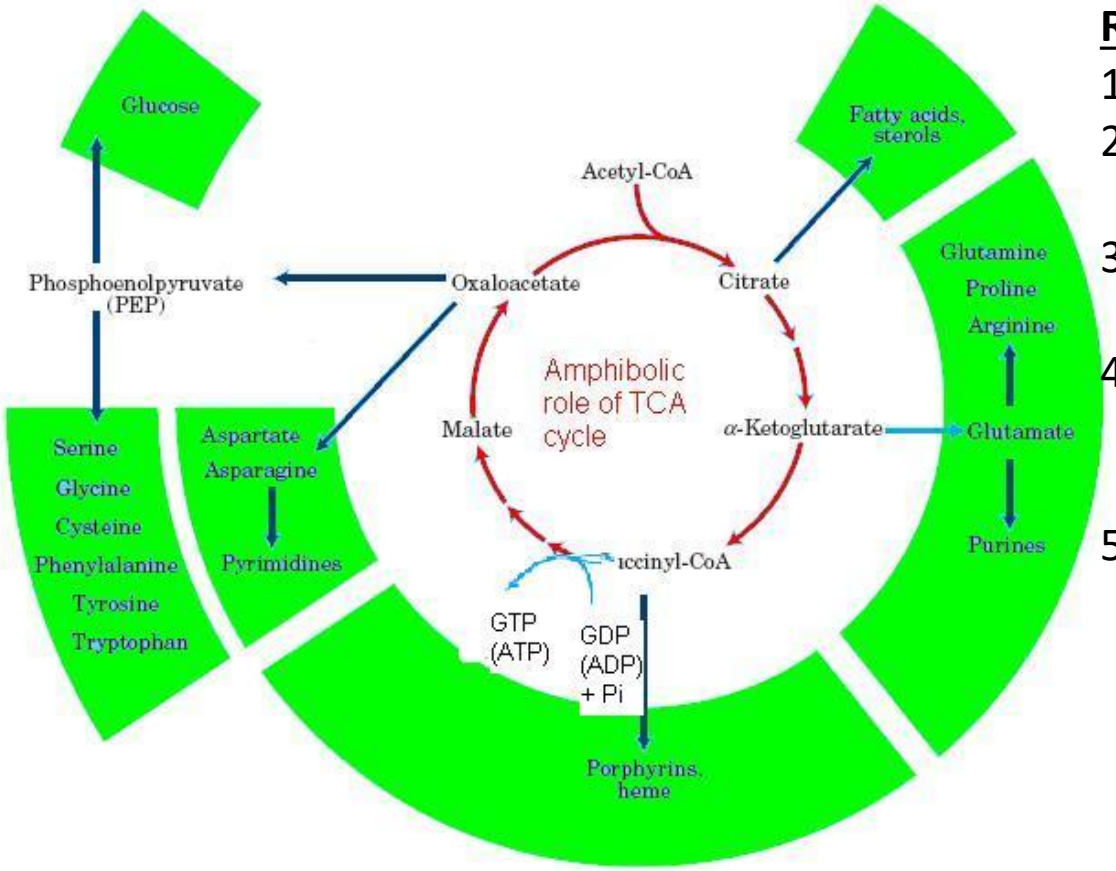


1. Coenzymes/prosthetic groups (electron carriers)
2. Coenzyme A (CoA-SH) : thiol group is acyl group carrier
3. Lipoate: 2 thiol groups
4. E1: pyruvate dehydrogenase (TPP-bound), E2: dihydrolipoyl transacetylase (lipoyl), E3:dihydrolipoyl dehydrogenase (FAD & NAD)

1. Conversion of pyruvate to acetyl groups and entry of these groups into the cycle
2. Cyclic reactions & the enzymes
3. Intermediates are used as precursors for other reactions.



TCA cycle is an amphibolic pathway: both catabolic and anabolic because it involves oxidative catabolism and provides precursors for biosynthetic processes. Anapleorotic reactions replace them by forming oxaloacetate (eg1. pyruvate to oxaloacetate/malate by PC/malic enzyme; eg.2 PEP to oxaloacetate by PEP carboxylase)



Regulation of TCA cycle:

1. Rate of conversion of pyruvate to acetyl CoA
2. Flux through citrate synthase, isocitrate dehydrogenase, alpha-keto-glutarate dehydrogenase
3. Concentrations of the stimulatory substrates (NAD+, ADP) and inhibitory by products (NADH, ATP)
4. Allosteric inhibition of acetyl CoA by high metabolic energies (ATP, NADH, fatty acids) and stimulated by reduced energy supply (AMP, NAD+, CoA)
5. Complexes of consecutive enzymes in a pathway allow substrate channeling between them.

Production of Acetyl-CoA

- In aerobic organisms, glucose and other sugars, fatty acids, and most amino acids are ultimately oxidized to CO_2 and H_2O via the citric acid cycle and the respiratory chain.

- Before entering the citric acid cycle, the carbon skeletons of sugars and fatty acids are degraded to the acetyl group of acetyl-CoA, the form in which the cycle accepts most of its fuel input.

- Pyruvate derived from glucose and other sugars by glycolysis, is oxidized to acetyl-CoA and CO_2 by the **pyruvate dehydrogenase (PDH) complex**, a cluster of located in the mitochondria of eukaryotic cells and in the cytosol of bacteria.

Pyruvate Is Oxidized to Acetyl-CoA and CO2

- The overall reaction catalyzed by the pyruvate dehydrogenase complex is an **oxidative decarboxylation**, an irreversible oxidation process in which the carboxyl group is removed from pyruvate as a molecule of CO_2 and the two remaining carbons become the acetyl group of acetyl-CoA.
- The NADH formed in this reaction gives up a hydride ion (:H2) to the respiratory chain, which carries the two electrons to oxygen.
- The transfer of electrons from NADH to oxygen ultimately generates 2.5 molecules of ATP per pair of electrons.

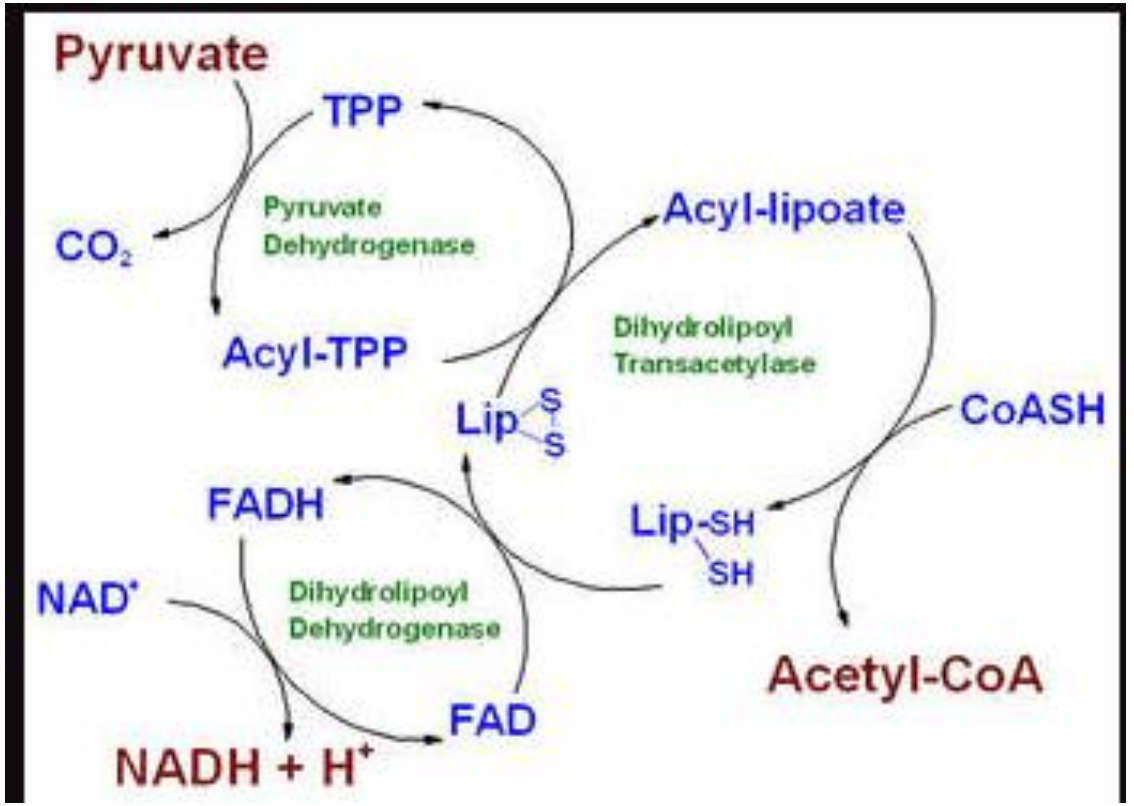
The Pyruvate Dehydrogenase Complex Requires Five Coenzymes

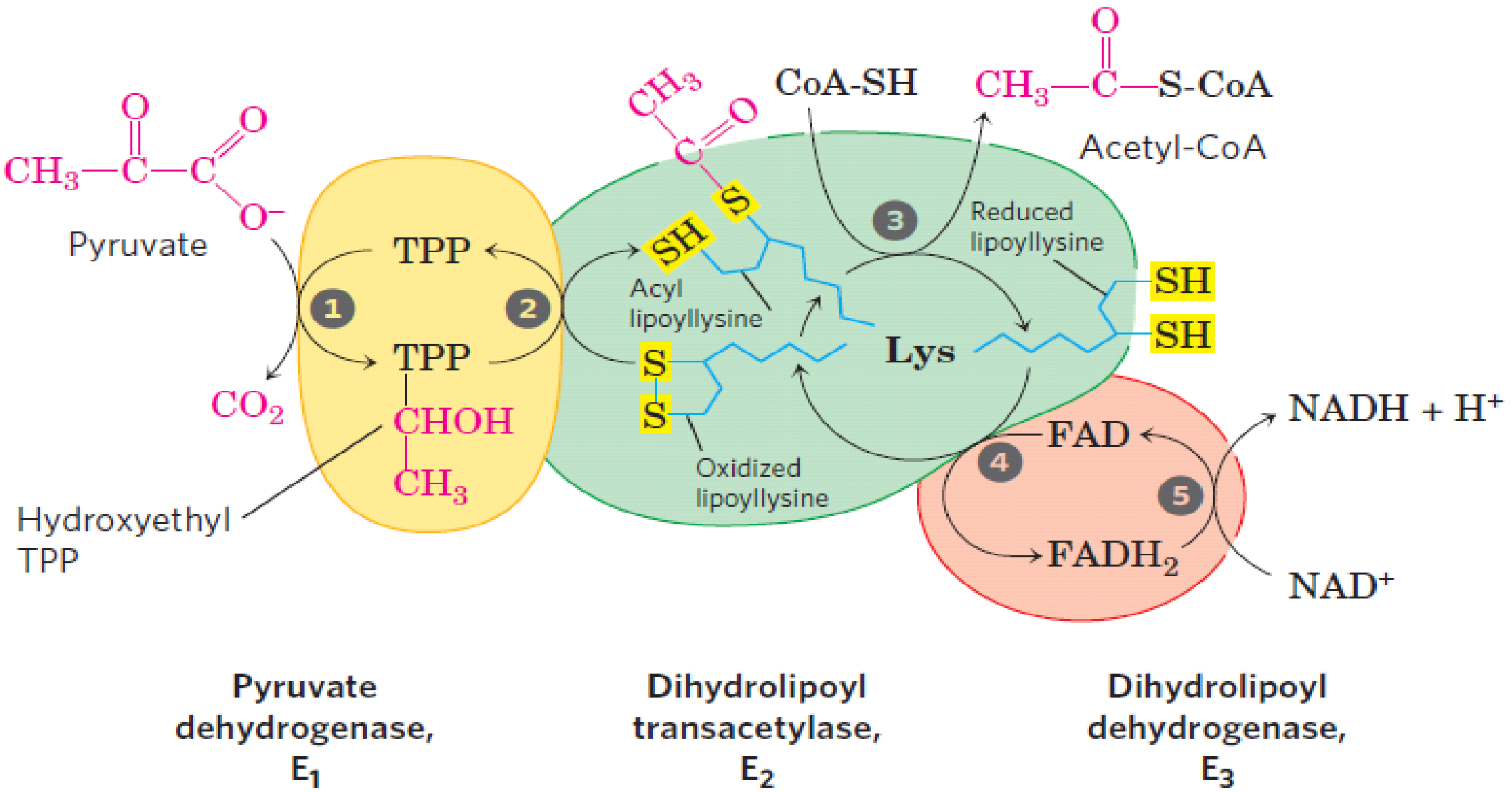
- The combined dehydrogenation and decarboxylation of pyruvate to the acetyl group of acetyl-CoA requires the sequential action of three different enzymes and five different coenzymes or prosthetic groups—
- Thiamine pyrophosphate (TPP)
- Flavin adenine dinucleotide (FAD)
- Coenzyme A (CoA, or CoA-SH -the thiol group)
- Nicotinamide adenine dinucleotide (NAD)
- Lipoate.

- Four different vitamins required in human nutrition are vital components of this system: thiamine (in TPP), riboflavin (in FAD), niacin (in NAD), and pantothenate (in CoA)

The PDH Complex Consists of Three Distinct Enzymes

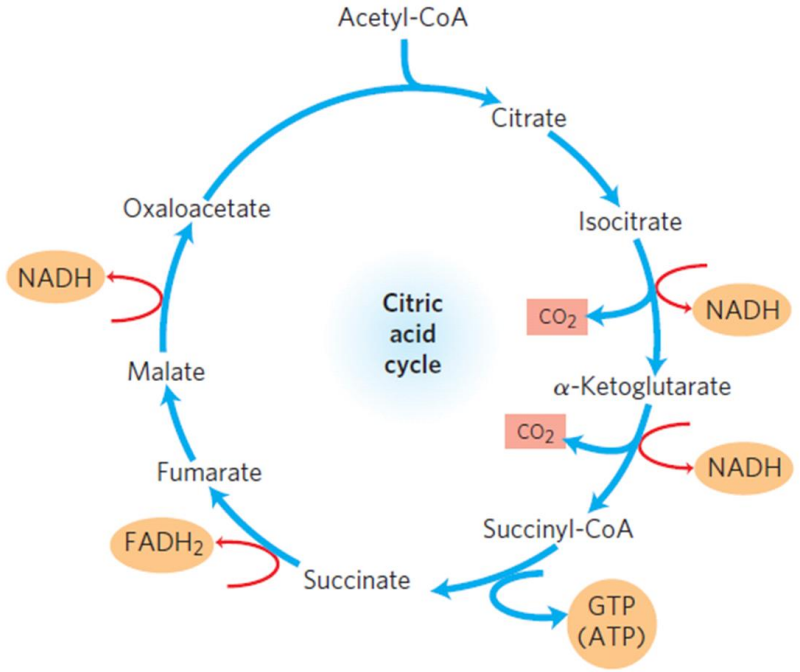
- Pyruvate dehydrogenase (E1)
- Dihydrolipoyl transacetylase (E2)
- Dihydrolipoyl dehydrogenase (E3)




Pyruvate
TPP
TPP
CHOH
CH3
CO2
Hydroxyethyl TPP
1
2
CH3
C
O
S
SH
Acyl lipoyllysine
S
S
Oxidized lipoyllysine
Lys
CoA-SH
3
Reduced lipoyllysine
SH
SH
CH3—C—S-CoA
Acetyl-CoA
FAD
4
5
FADH2
NADH + H+
NAD+
Pyruvate dehydrogenase, E1
Dihydrolipoyl transacetylase, E2
Dihydrolipoyl dehydrogenase, E3

Citric acid cycle

- the conversion of pyruvate to acetyl groups, then the entry of those groups into the **citric acid cycle**, also called the **tricarboxylic acid (TCA) cycle** or the **Krebs cycle** (after its discoverer, Hans Krebs).



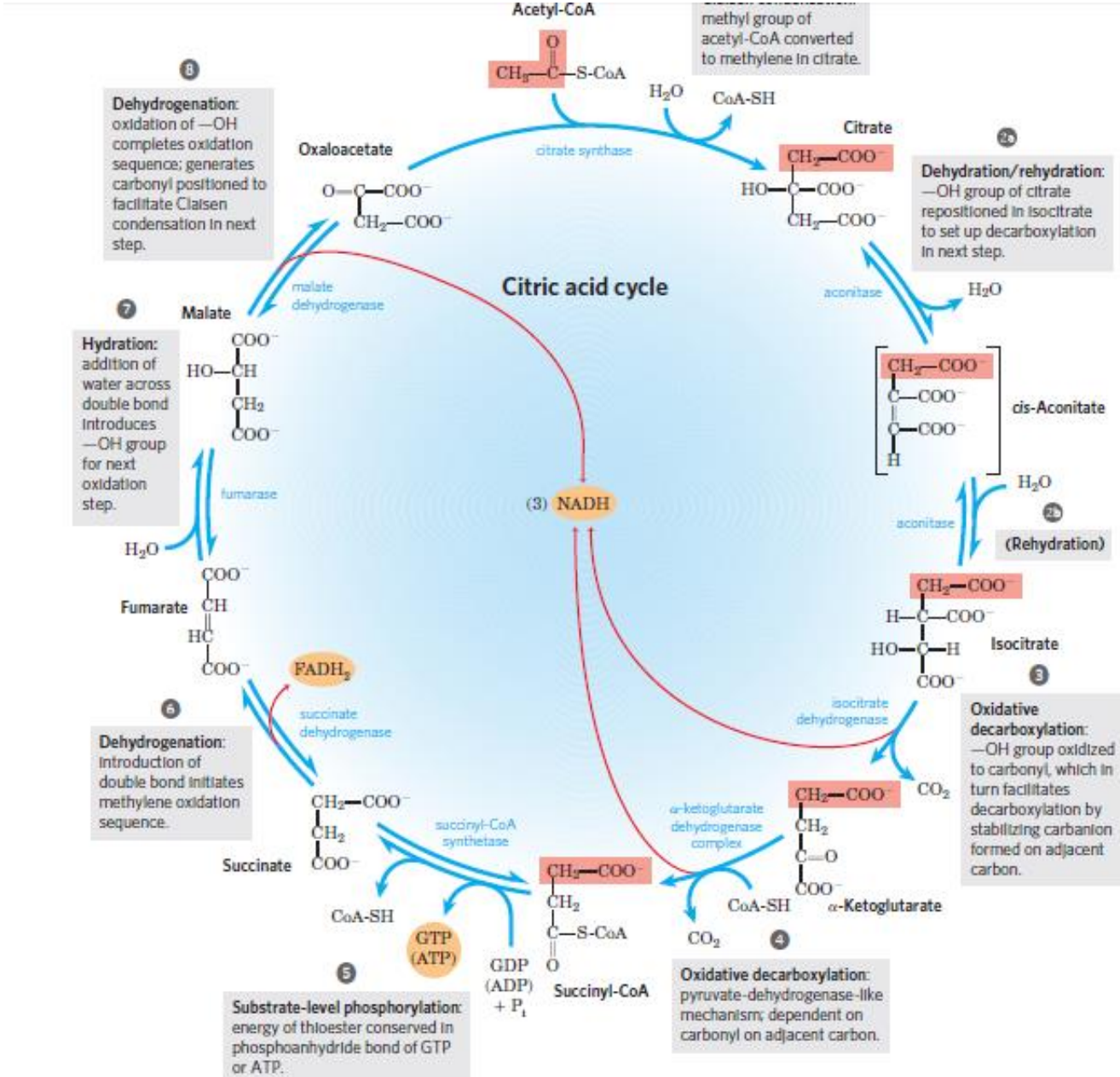
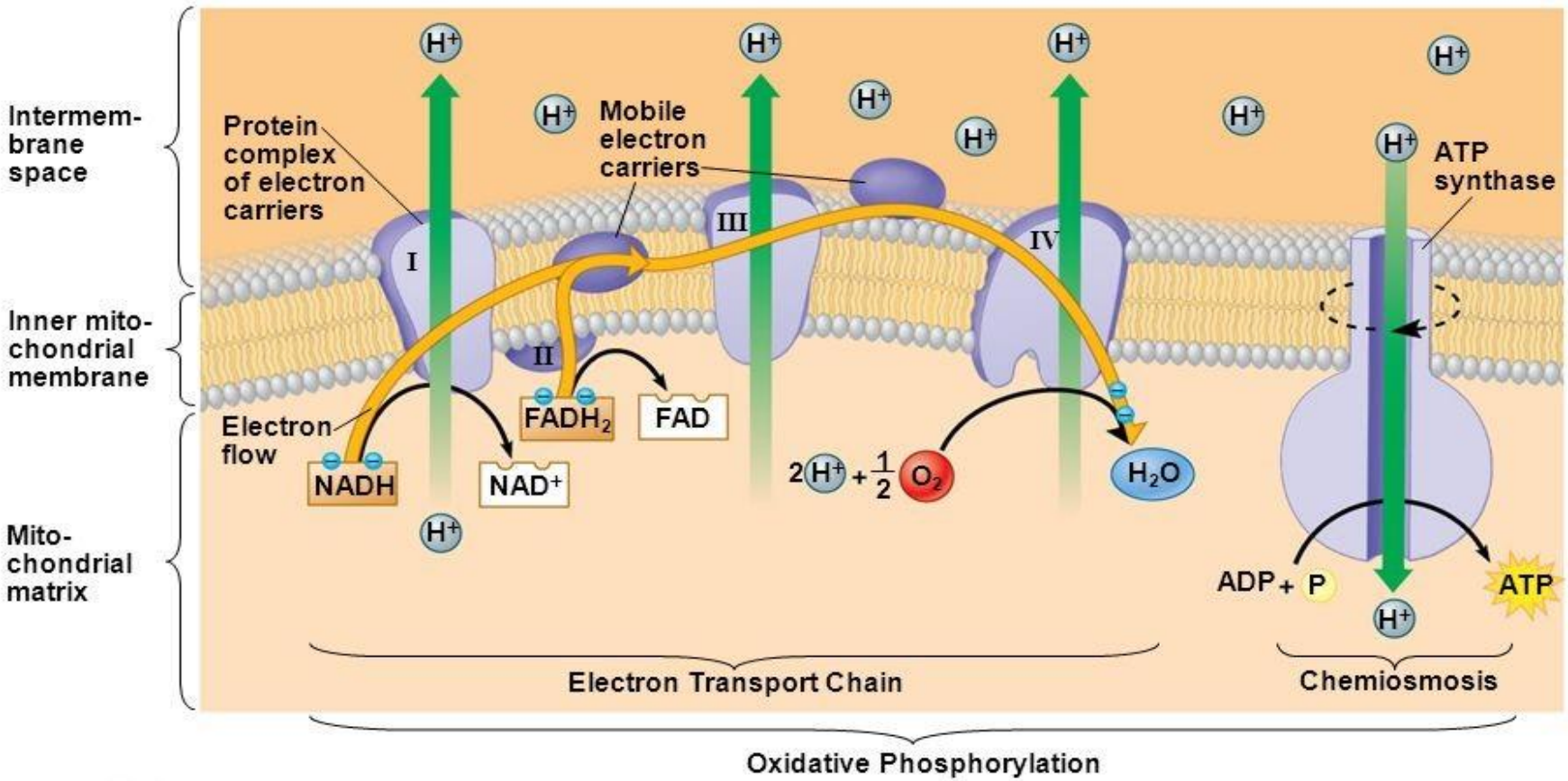

Citric acid cycle
Acetyl-CoA
methyl group of acetyl-CoA converted to methylene in citrate.
Oxaloacetate
citrate synthase
Citrate
Dehydration/rehydration: —OH group of citrate repositioned in isocitrate to set up decarboxylation in next step.
aconitase
cis-Aconitate
(Rehydration)
Isocitrate
Oxidative decarboxylation: —OH group oxidized to carbonyl, which in turn facilitates decarboxylation by stabilizing carbanion formed on adjacent carbon.
isocitrate dehydrogenase
α-ketoglutarate dehydrogenase complex
α-Ketoglutarate
Oxidative decarboxylation: pyruvate-dehydrogenase-like mechanism; dependent on carbonyl on adjacent carbon.
Succinyl-CoA
succinyl-CoA synthetase
Substrate-level phosphorylation: energy of thioester conserved in phosphoanhydride bond of GTP or ATP.
Succinate
succinate dehydrogenase
Dehydrogenation: introduction of double bond initiates methylene oxidation sequence.
Fumarate
fumarase
Hydration: addition of water across double bond introduces —OH group for next oxidation step.
Malate
malate dehydrogenase
Dehydrogenation: oxidation of —OH completes oxidation sequence; generates carbonyl positioned to facilitate Claisen condensation in next step.
(3) NADH
CoA-SH
H2O
CO2
GTP (ATP)
GDP (ADP) + Pi
FADH2

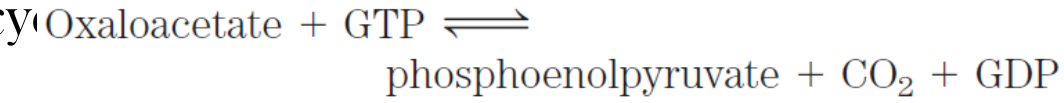
Figure 6.10



The Glyoxylate cycle

- Vertebrates cannot convert fatty acids or the acetate derived from them to carbohydrates.
- Conversion of phosphoenolpyruvate to pyruvate and pyruvate to acetyl-CoA are exergonic and irreversible.
- If a cell cannot convert acetate into PEP, acetate cannot serve as the starting material for the gluconeogenic pathway, which leads from phosphoenolpyruvate to glucose.
- Without this capacity, a cell or organism is unable to convert fuels or metabolites that are degraded to acetate (fatty acids and certain amino acids) into carbohydrates.

- Phosphoenolpyruvate can be synthesized from oxaloacetate in the reversible reaction catalyzed by PEP carboxykinase:



- In the citric acid cycle, there is no *net conversion* of acetate to oxaloacetate; in vertebrates, for every two carbons that enter the cy

- In many organisms other than vertebrates, the glyoxylate cycle serves as a mechanism for converting acetate to carbohydrate.

The Glyoxylate Cycle Produces Four-Carbon Compounds from Acetate

- In plants, certain invertebrates, and some microorganisms (including *E. coli and yeast)* acetate can serve both as an energy-rich fuel and as a source of PEP for carbohydrate synthesis.

- In these organisms, the glyoxylate cycle catalyzes the net conversion of acetate to succinate or their four-carbon intermediates of the citric acid cycle:

$$2\ \text{Acetyl-CoA} + \text{NAD}^+ + 2\text{H}_2\text{O} \longrightarrow \text{succinate} + 2\text{CoA} + \text{NADH} + \text{H}^+$$

- The acetyl-CoA condenses with oxaloacetate to form citrate, and citrate is converted to isocitrate, exactly as in the citric acid cycle.

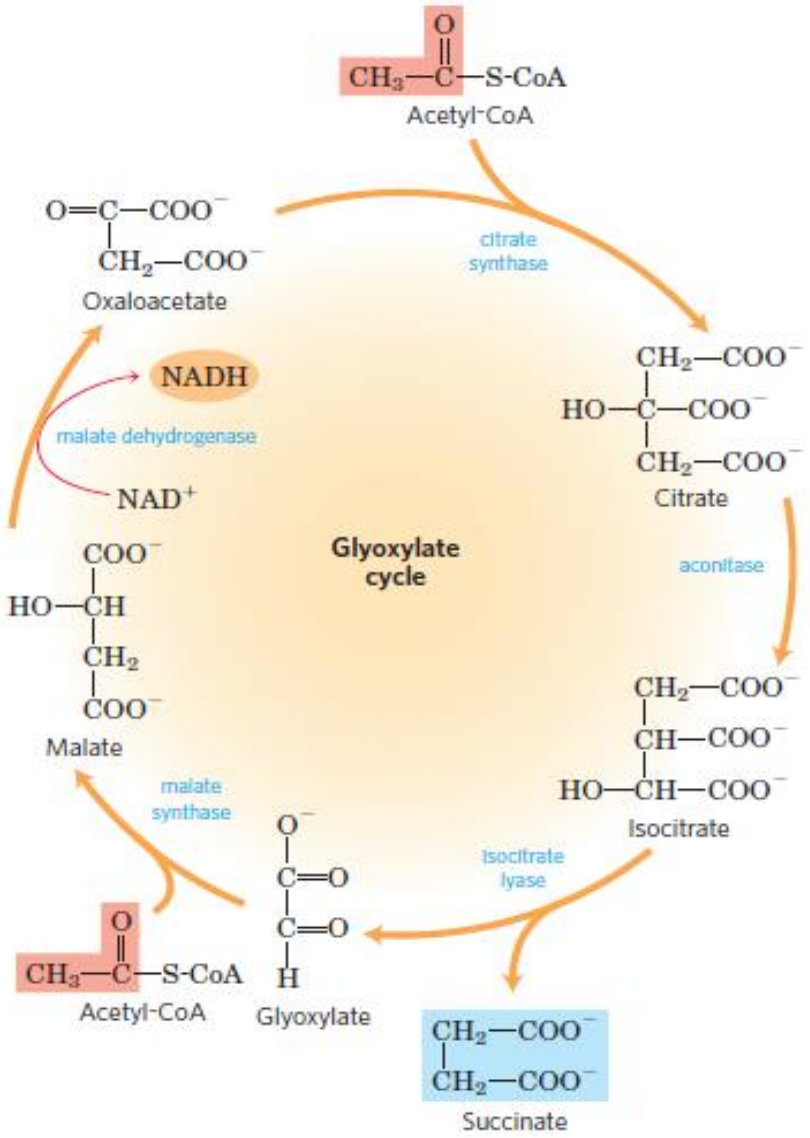
- The next step is not the breakdown of isocitrate by isocitrate dehydrogenase but the cleavage of isocitrate by isocitrate lyase, forming succinate and glyoxylate.

- The glyoxylate then condenses with a second molecule of acetyl-CoA to yield malate, in a reaction catalyzed by malate synthase.

- The malate is subsequently oxidized to oxaloacetate, which can condense with another molecule of acetyl-CoA to start another turn of the cycle.

- Each turn of the glyoxylate cycle consumes two molecules of acetyl-CoA and produces one molecule of succinate.

- The succinate may be converted through fumarate and malate into oxaloacetate, which can then be converted to phosphoenolpyruvate by PEP carboxykinase, and thus to glucose by gluconeogenesis.



The citrate synthase, aconitase, and malate dehydrogenase of the glyoxylate cycle are isozymes of the citric acid cycle enzymes; **isocitrate lyase and malate synthase** are unique to the glyoxylate cycle.

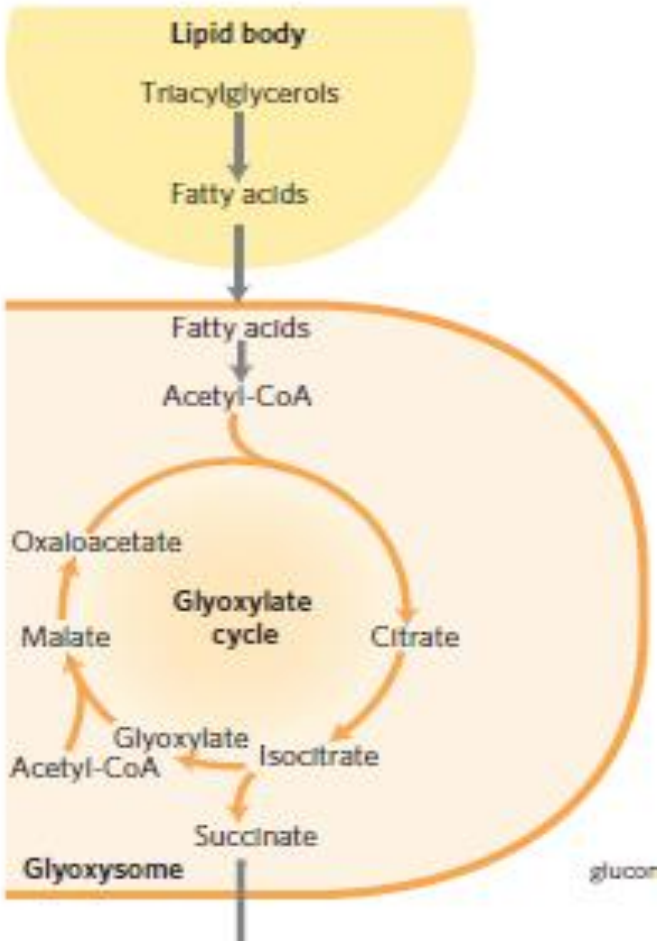
The glyoxylate cycle was elucidated by Hans Kornberg and Neil Madsen in the laboratory of Hans Krebs.

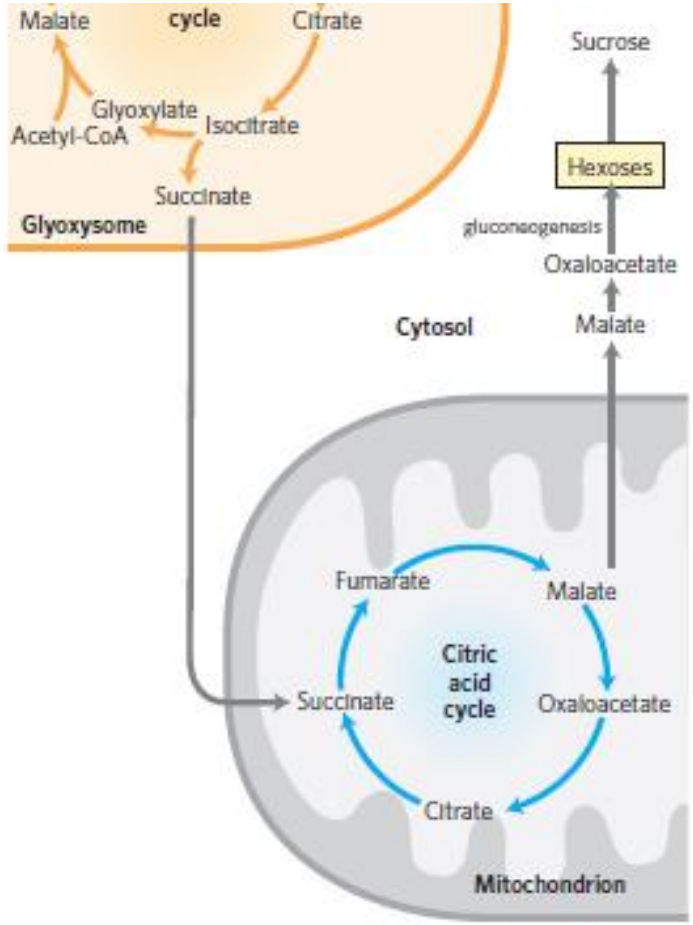
Vertebrates do not have the enzymes specific to the glyoxylate cycle (isocitrate lyase and malate synthase) and therefore cannot bring about the net synthesis of glucose from lipids.

- In plants, the enzymes of the glyoxylate cycle are sequestered in membrane-bounded organelles called **glyoxysome**s, which are specialized peroxisomes.
- Those enzymes common to the citric acid and glyoxylate cycles have two **isozymes**, one specific to mitochondria, the other to glyoxysomes.
- Glyoxysomes are not present in all plant tissues at all times.
- They develop in **lipid-rich seeds during germination**, before the developing plant acquires the ability to make glucose by photosynthesis.
- In addition to this, glyoxysomes contain all the enzymes needed for the degradation of the fatty acids stored in seed oils.
- Acetyl-CoA formed from lipid breakdown is converted to succinate via the glyoxylate cycle, and the succinate is exported to mitochondria, where citric acid cycle enzymes transform it to malate.
- A cytosolic isozyme of malate dehydrogenase oxidizes malate to oxaloacetate, a precursor for gluconeogenesis. Germinating seeds can therefore convert the carbon of stored lipids into glucose.

The Citric Acid and Glyoxylate Cycles Are Coordinately Regulated

- In germinating seeds, the enzymatic transformations of dicarboxylic and tricarboxylic acids occur in three intracellular compartments: mitochondria, glyoxysomes, and the cytosol. There is a continuous interchange of metabolites among these compartments





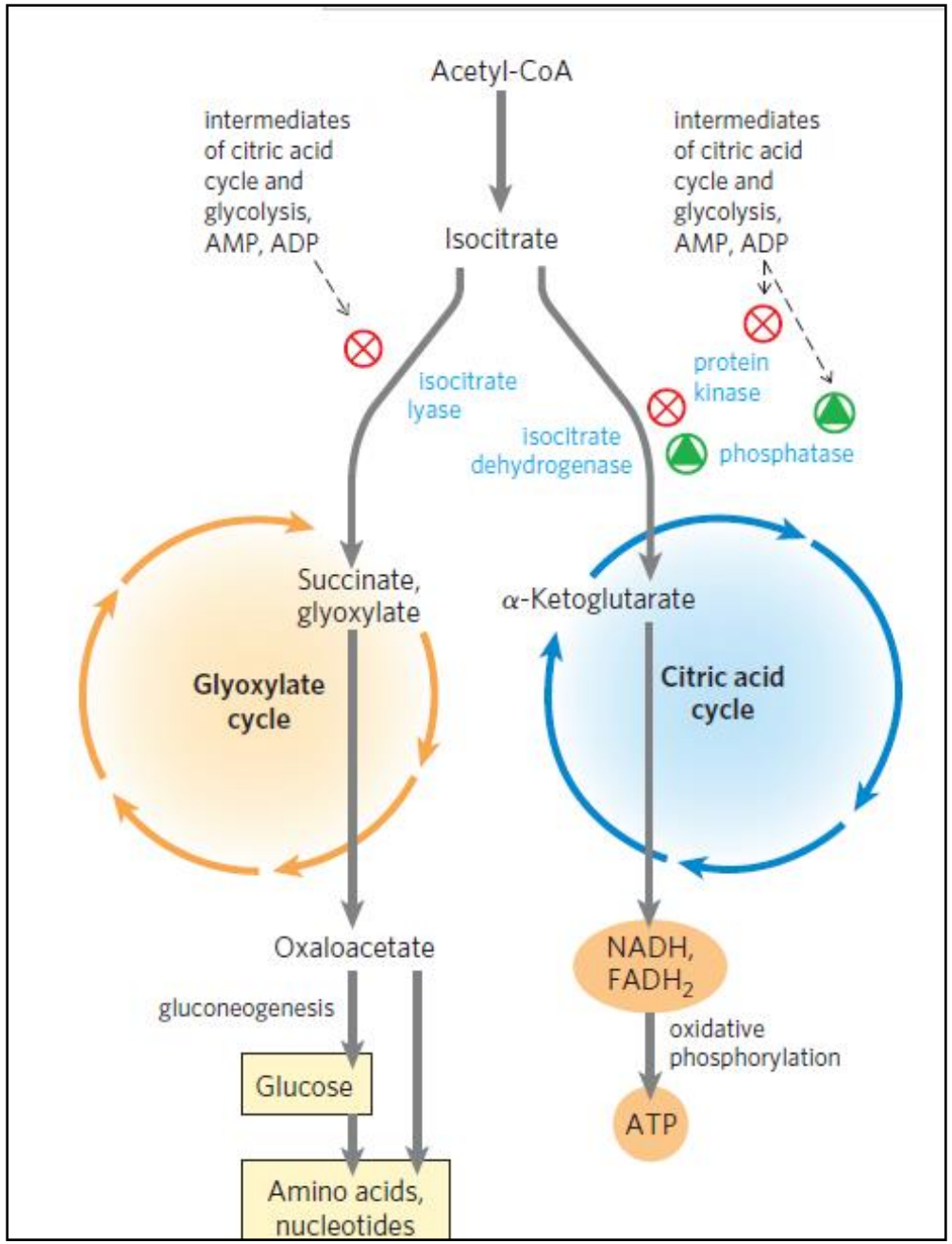
- The carbon skeleton of oxaloacetate from the citric acid cycle (in the mitochondrion) is carried to the glyoxysome in the form of aspartate.

- Aspartate is converted to oxaloacetate, which condenses with acetyl-CoA derived from fatty acid breakdown. The citrate thus formed is converted to isocitrate by aconitase, then split into glyoxylate and succinate by isocitrate lyase.

- The succinate returns to the mitochondrion, where it reenters the citric acid cycle and is transformed into malate, which enters the cytosol and is oxidized (by cytosolic malate dehydrogenase) to oxaloacetate.

- Oxaloacetate is converted via gluconeogenesis into hexoses and sucrose, which can be transported to the growing roots and shoot.

- Four distinct pathways participate in these conversions: fatty acid breakdown to acetyl-CoA (in glyoxysomes), the glyoxylate cycle (in glyoxysomes), the citric acid cycle (in mitochondria), and gluconeogenesis (in the cytosol).


Acetyl-CoA
intermediates of citric acid cycle and glycolysis, AMP, ADP
Isocitrate
intermediates of citric acid cycle and glycolysis, AMP, ADP
isocitrate lyase
protein kinase
isocitrate dehydrogenase
phosphatase
Succinate, glyoxylate
α-Ketoglutarate
Glyoxylate cycle
Citric acid cycle
Oxaloacetate
NADH, FADH2
gluconeogenesis
oxidative phosphorylation
Glucose
ATP
Amino acids, nucleotides

- The sharing of common intermediates requires that these pathways be coordinately regulated. **Isocitrate** is a crucial intermediate, at the **branch point** between the glyoxylate and citric acid cycles.

- **Isocitrate dehydrogenase** is regulated by covalent modification: a specific **protein kinase phosphorylates** and thereby **inactivates** the dehydrogenase.

- This inactivation shunts isocitrate to the glyoxylate cycle, where it begins the synthetic route toward glucose.

- A **phosphoprotein phosphatase removes the phosphoryl group** from isocitrate dehydrogenase, reactivating the enzyme and sending more isocitrate through the energy-yielding citric acid cycle.

- The regulatory protein kinase and phosphoprotein phosphatase are separate enzymatic activities of a single polypeptide.

- The phosphoprotein phosphatase that activates isocitrate dehydrogenase is stimulated by **intermediates of the citric acid cycle and glycolysis** and by indicators of **reduced cellular energy** supply.
- The same metabolites *inhibit the* protein kinase activity of the bifunctional polypeptide.
- Thus, the accumulation of intermediates of the central energy-yielding pathways - indicating energy depletion—results in the activation of isocitrate dehydrogenase.
- When the concentration of these regulators falls, signaling a sufficient flux through the energy-yielding citric acid cycle, isocitrate dehydrogenase is inactivated by the protein kinase.
- The same intermediates of glycolysis and the citric acid cycle that activate isocitrate dehydrogenase are allosteric inhibitors of isocitrate lyase.
- When energy yielding metabolism is sufficiently fast to keep the concentrations of glycolytic and citric acid cycle intermediates low, isocitrate dehydrogenase is inactivated, the inhibition of isocitrate lyase is relieved, and isocitrate flows into the glyoxylate pathway, to be used in the biosynthesis of carbohydrates, amino acids, and other cellular components.

- The glyoxylate cycle is active in the germinating seeds of some plants and in certain microorganisms that can live on acetate as the sole carbon source.

- In plants, the pathway takes place in glyoxysomes in seedlings. It involves several citric acid cycle enzymes and two additional enzymes: isocitrate lyase and malate synthase.

- In the glyoxylate cycle, the bypassing of the two decarboxylation steps of the citric acid cycle makes possible the *net formation of succinate,* oxaloacetate, and other cycle intermediates from acetyl-CoA. Oxaloacetate thus formed can be used to synthesize glucose via gluconeogenesis.

- Vertebrates lack the glyoxylate cycle and cannot synthesize glucose from acetate or the fatty acids that give rise to acetyl-CoA.

- The partitioning of isocitrate between the citric acid cycle and the glyoxylate cycle is controlled at the level of isocitrate dehydrogenase, which is regulated by reversible phosphorylation.