Krebs Cycle (KC)

- Also known as TCA cycle, or citric acid cycle
- Reactions of KC occur in mitochondrial matrix
- Common final degradative pathway for breakdown of monomers of CHO, fat and protein to CO_2 and H_20
  - Electrons removed from acetyl groups & attached to NAD^+ & FAD
  - Small amount of ATP produced from substrate level phosphorylation
- KC also provides intermediates for anabolic functions (eg. Gluconeogenesis
- Anaerobic metabolism (glycolysis & fermentation) only releases

  7% of the energy in glucose)
- Glycolysis is used for **rapid** ATP production :Rate of ATP formation in anaerobic is 100 times > ATP production by oxidative phosphorylation.

Some General Features of TCA Cycle

· The enzymes of TCA are found in the mitochondrial matrix.

· **Catabolic role** Amino acids, fats sugars enter the TCA cycle to produce energy.
· **Anabolic role** TCA cycle provides starting material for fats and amino acids. Note: carbohydrates cannot be synthesized from acetyl-CoA by humans. Pyruvate---Acetyl CoA is one way!

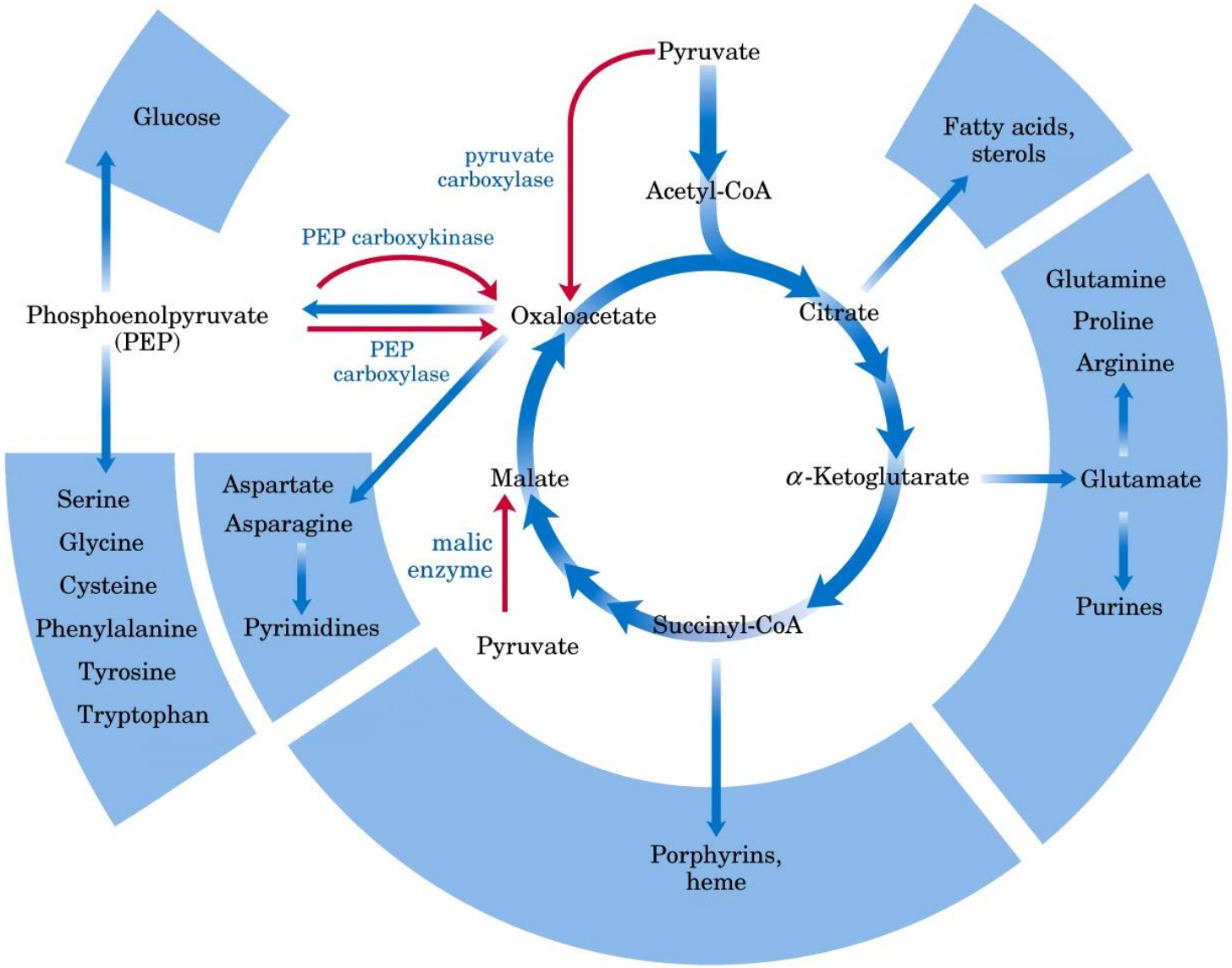
· In contrast to glycolysis, none of the intermediates are phosphorylated but all are either di- or tricarboxylic acids.

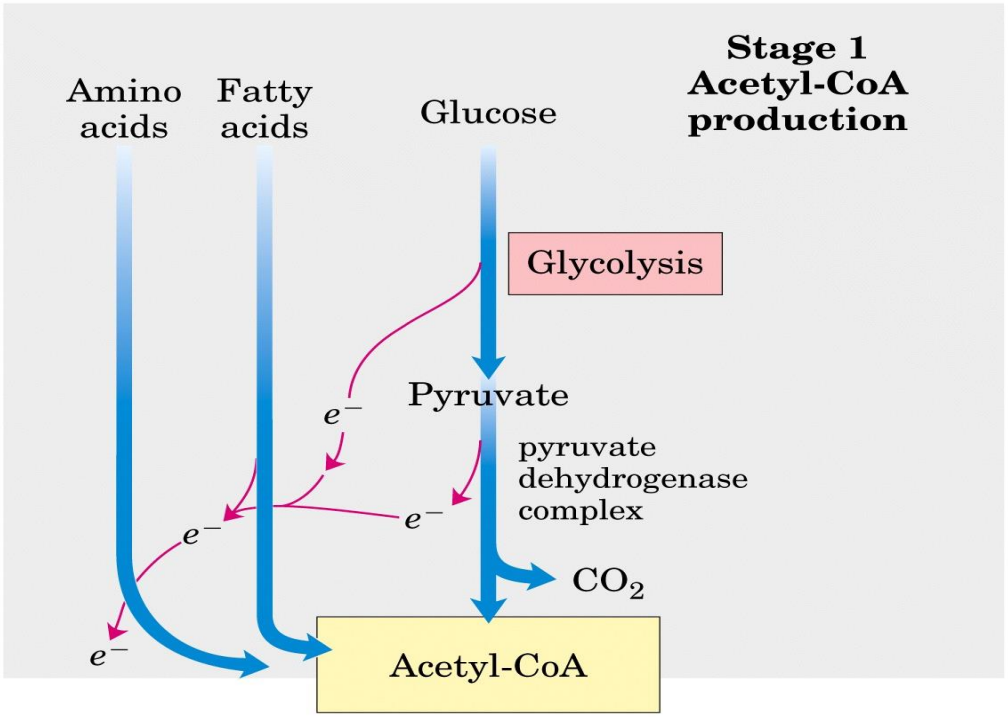
Amphibolic Nature of TCA Cycle:

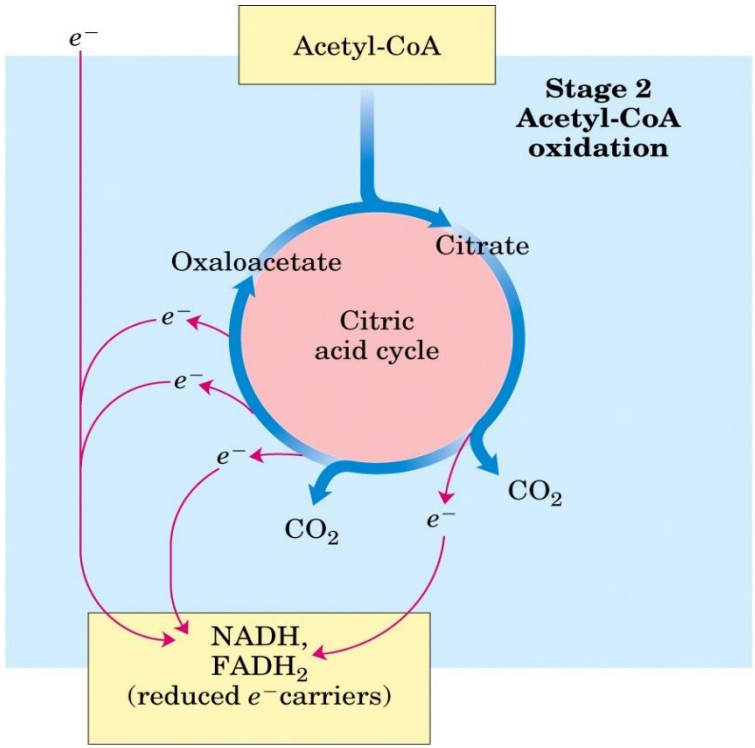
Some compounds feed in and some are removed for other uses

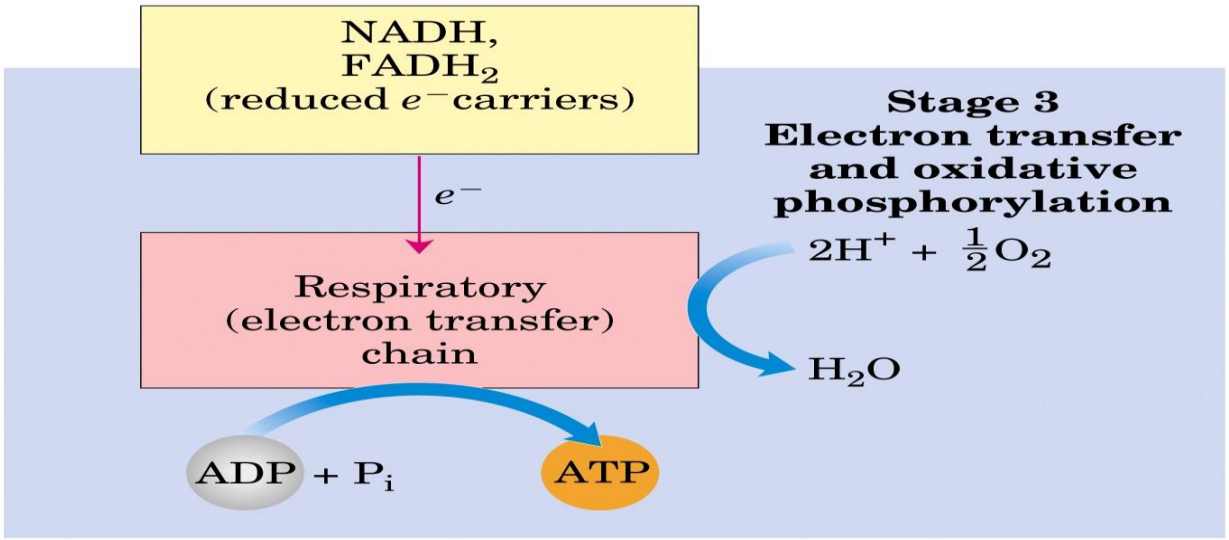
Anabolic

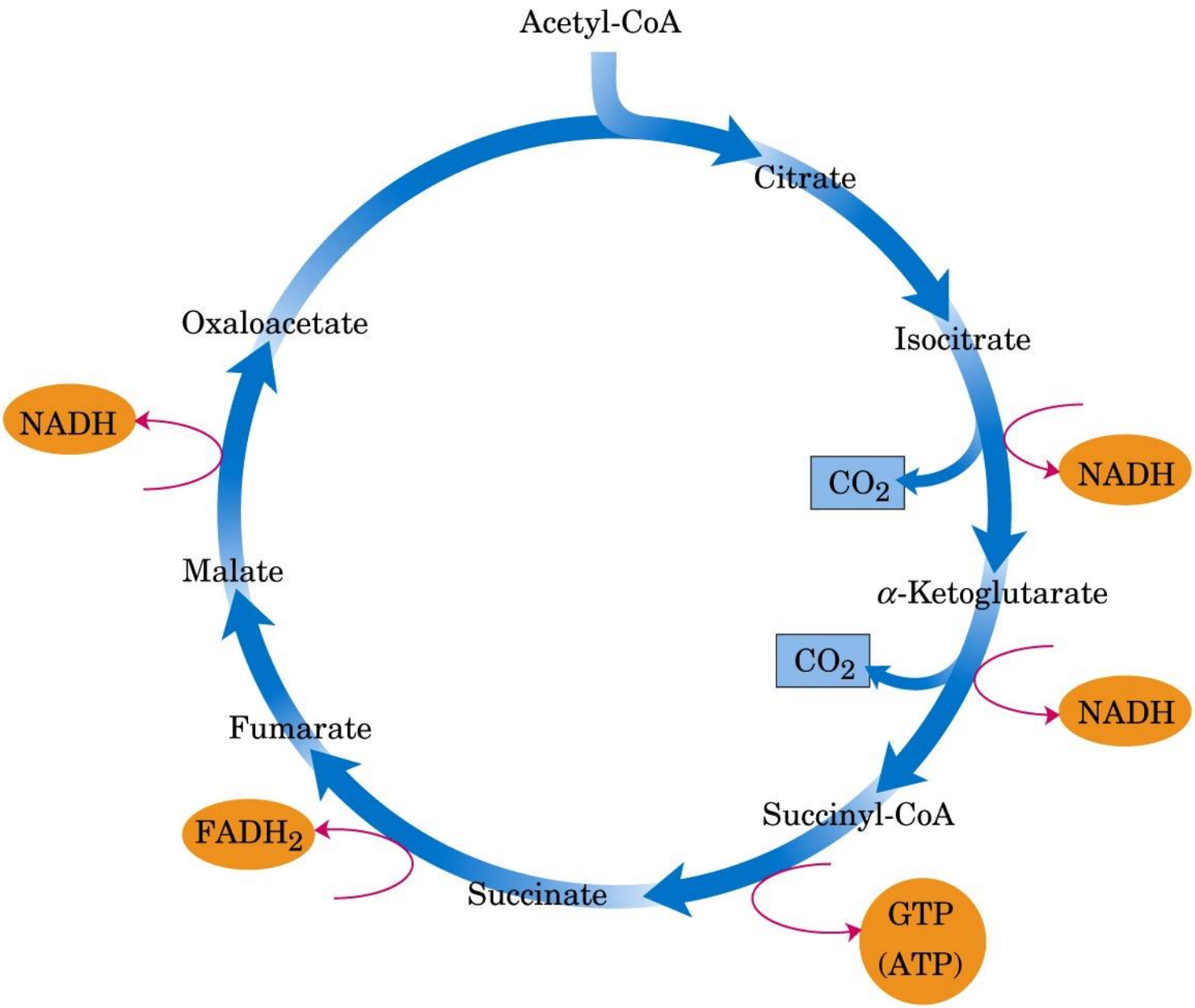
Catabolic




Stage 1
Acetyl-CoA
production
Amino
acids
Fatty
acids
Glucose
Glycolysis
Pyruvate
pyruvate
dehydrogenase
complex
e^-
e^-
e^-
e^-
e^-
CO_2
Acetyl-CoA


Acetyl-CoA
Stage 2
Acetyl-CoA
oxidation
Oxaloacetate
Citrate
Citric
acid cycle
e^-
e^-
e^-
e^-
e^-
CO_2
CO_2
NADH,
$FADH_2$
(reduced e^-carriers)


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

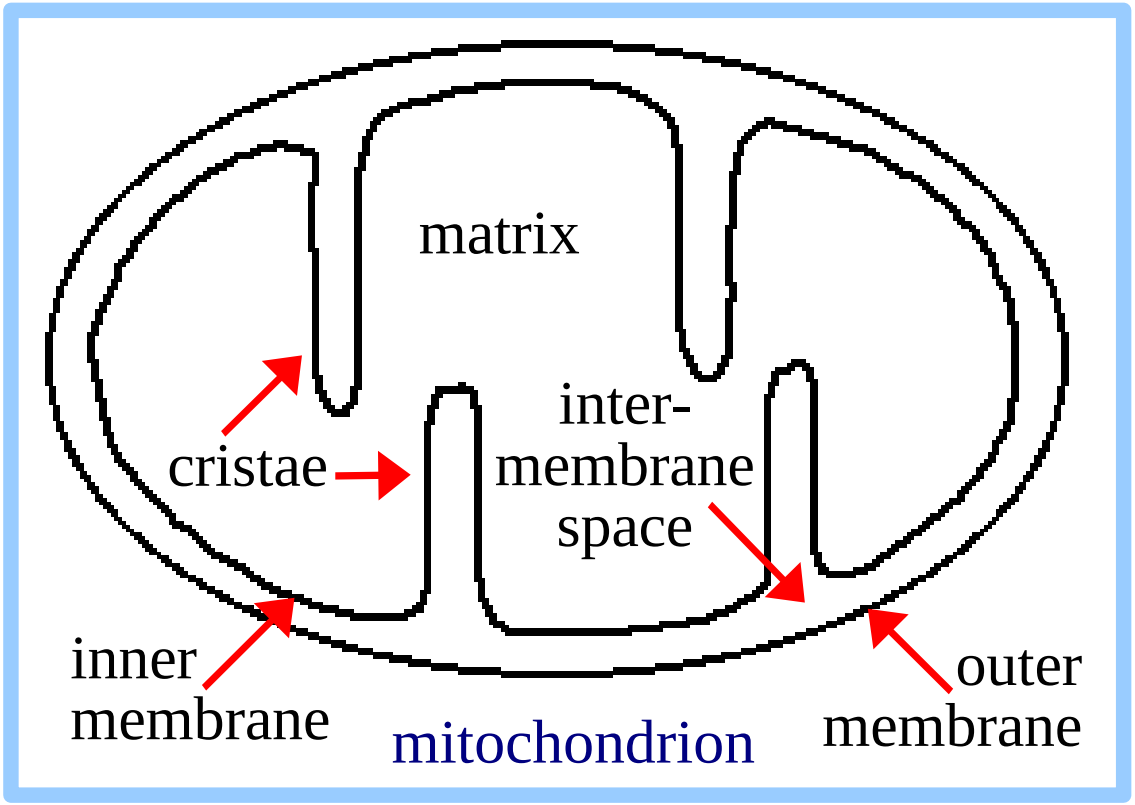

Acetyl-CoA
Citrate
Isocitrate
NADH
CO_2
α-Ketoglutarate
CO_2
NADH
Succinyl-CoA
GTP
(ATP)
Succinate
$FADH_2$
Fumarate
Malate
Oxaloacetate
NADH

Pyruvate transport and mitochondial structure:

The mitochondria enclosed by a double mb. All of the glycolytic enzymes are found in the cytosol of the cell. Charged molecules such as pyruvate must be transported in and out of the mithochondria.

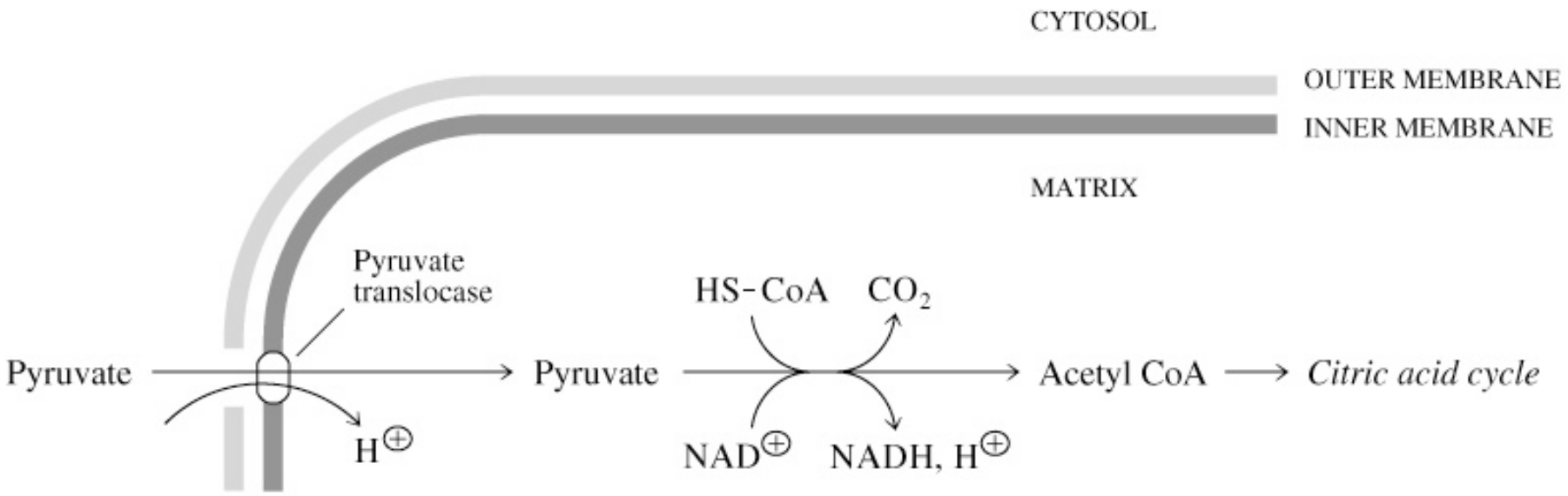
Small charged molecules with M.wt. < 10000 can freely diffuse through outer mitochondrial mb. through aqueous channels called porins.

A transport protein called pyruvate translocase specifically transports pyruvate through the inner mitochondrial mb. into the Mitochondrial matrix.



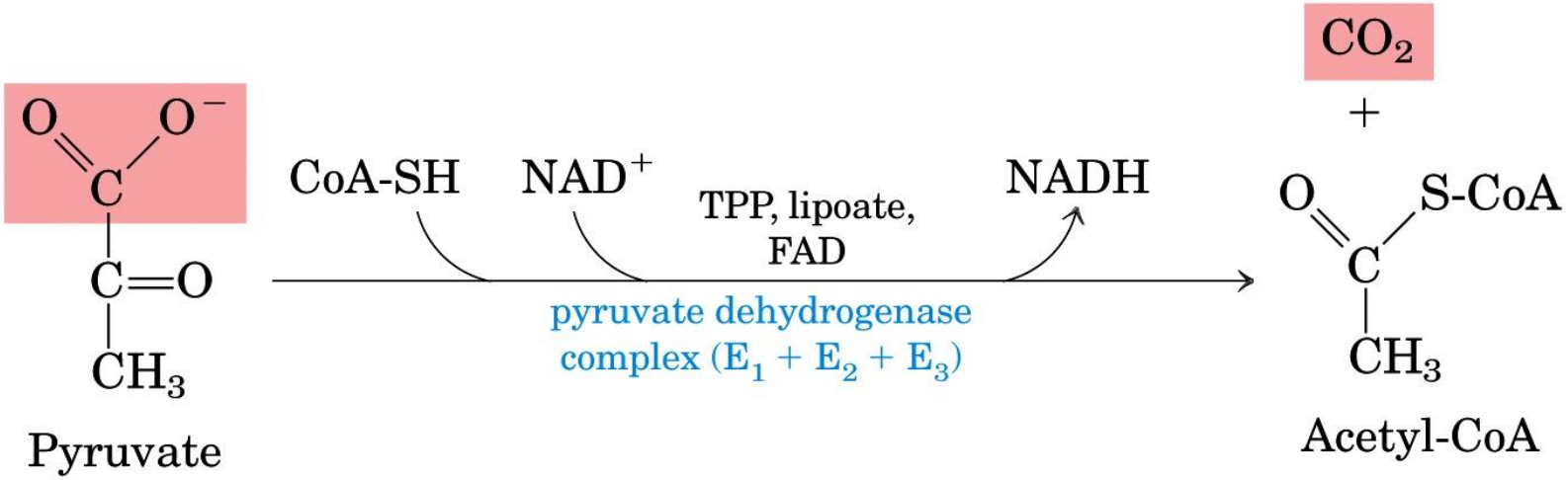
Entry of Pyruvate into the Mitochondrion

Pyruvate translocase transports pyruvate into the mitochondria in symport with H^+



Pyruvate → Acetyl CoA

- Pyruvate produced in cytosol and transported into mitochondria
- Cannot directly enter KC
  - First converted to acetyl CoA by pyruvate dehydrogenase complex
- Oxidative decarboxylation of pyruvate

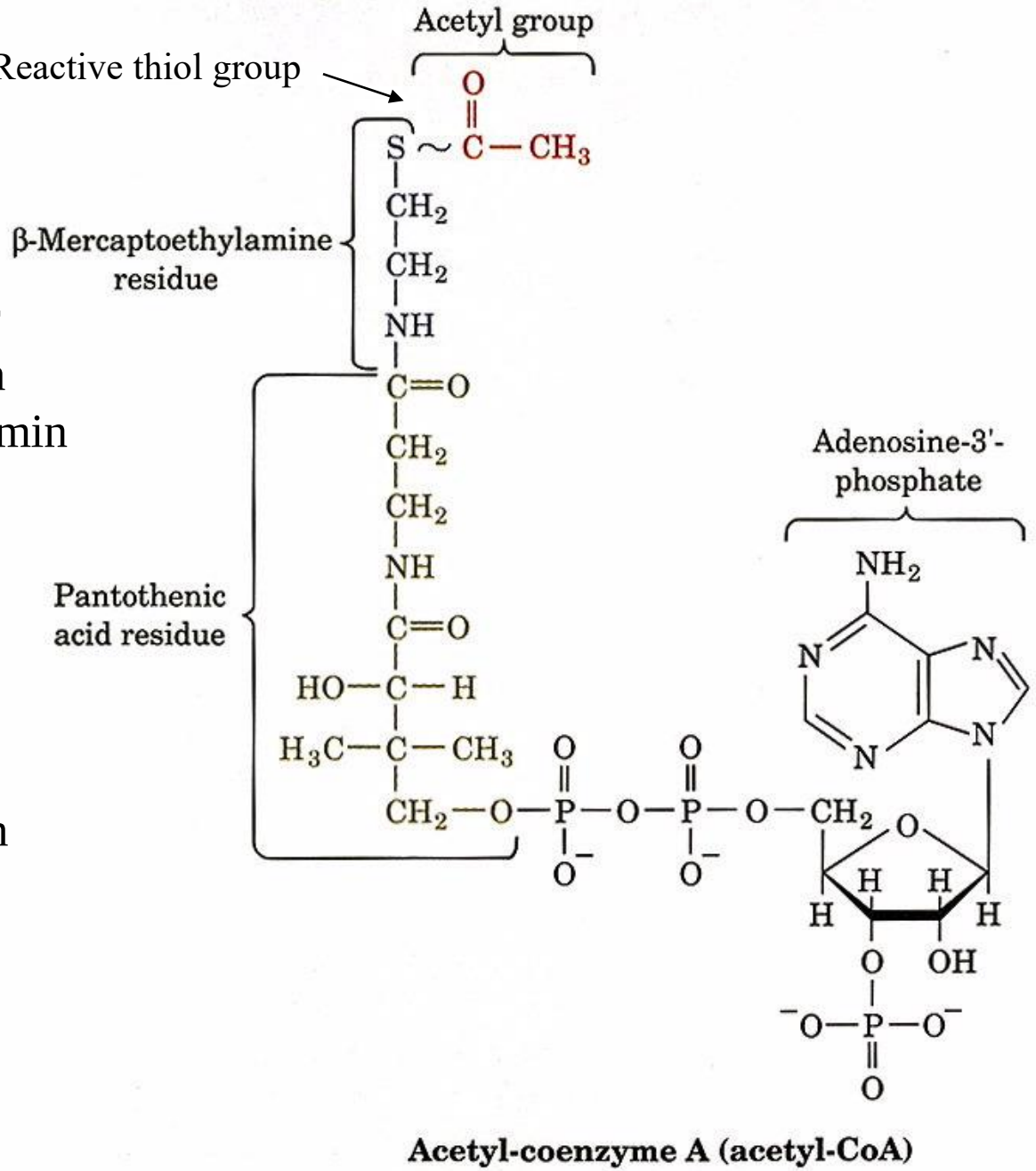


Coenzyme A

-Coenzyme: an organic cofactor required for the action of certain enzymes often containing a vitamin as a building block.

-"high energy" compound
- Hydrolysis of thioester bond more exergonic than ATP

-acetylCoA common breakdown product of CHO, FAs, AAs



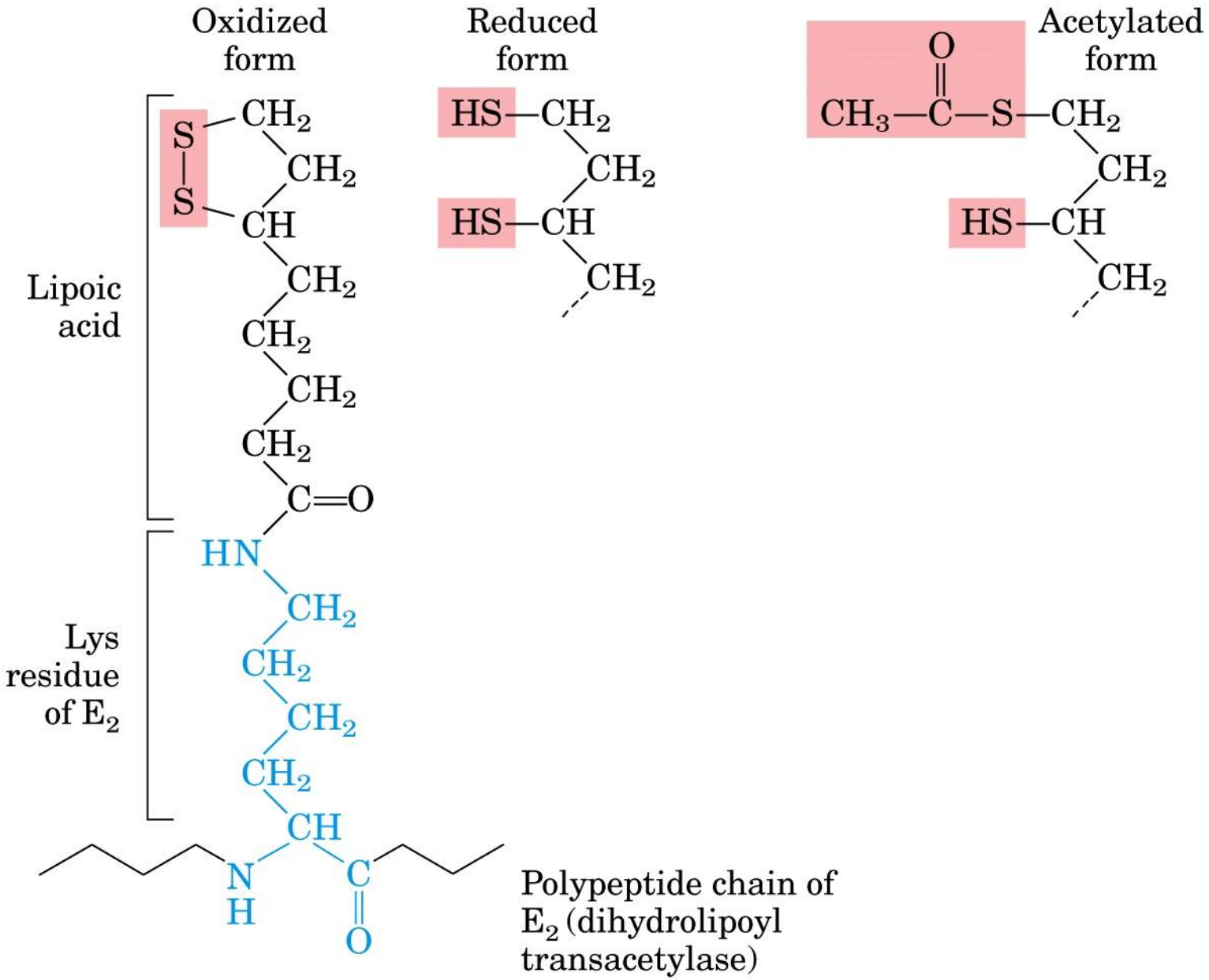
Acetyl-coenzyme A (acetyl-CoA)

Pyruvate Dehydrogenase Complex (PDH)

Enzyme	Abbreviated	Prosthetic group	Function
Pyruvate dehydrogenase	E1	Thiamine Pyrophosphate TPP*	Decaboxylation and aldehyde group transfer
Dihydrolipoyl transacetylase	E2	Lipoamide, Co-A	Carrier of hydrogens or acetyl group
Dihydrolipoyl dehydrogenase	E3	FAD**, NAD	Electron carriers

***TPP** is a derivative of thiamine (vitamin B1). Nutritional deficiency of thiamine leads to the disease **beriberi**. Beriberi affects especially the **brain**, because TPP is required for CHO metabolism, & the brain depends on glucose metabolism for energy.
Limb weakness, heart disease (green vegetables, fruit, fresh meat)

** **F**lavin **A**denine **D**inucleotide is derived from the vitamin riboflavin


Oxidized form
Reduced form
Acetylated form
Lipoic acid
Lys residue of E_2
Polypeptide chain of E_2 (dihydrolipoyl transacetylase)

Enzymes and Reactions of the Citric Acid Cycle

- Multi-step catalyst
  - Citrate synthase
  - Aconitase
  - Isocitrate Dehydrogenase
  - alpha-Ketoglutarate Dehydrogenase
  - Succinyl-CoA Synthetase
  - Succinate Dehydrogenase
  - Fumarase
  - Malate Dehydrogenase

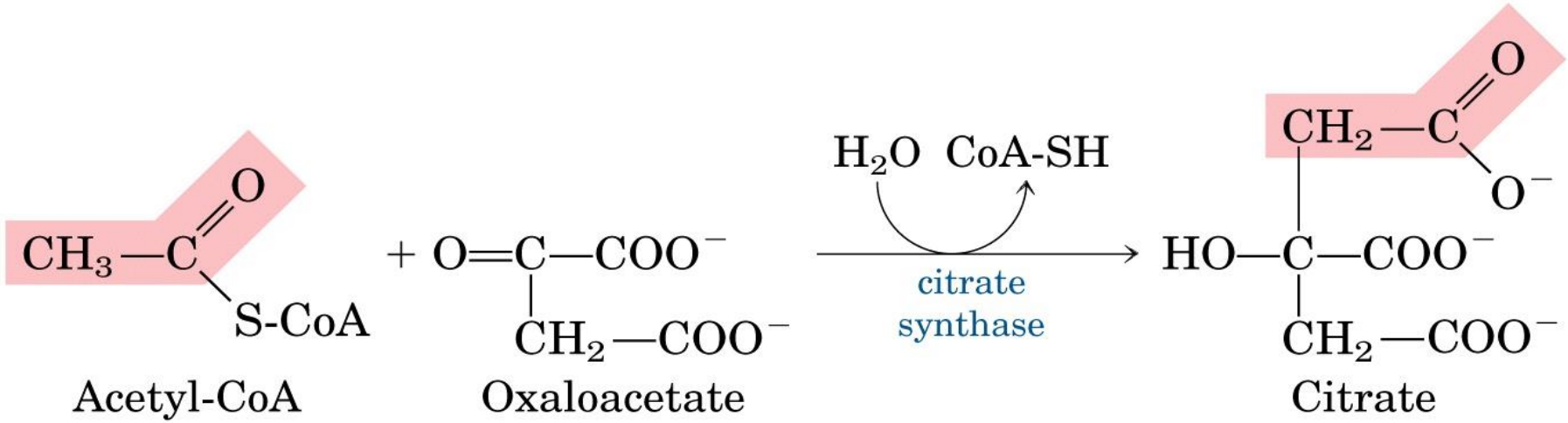
Formation of citrate

Oxaloacetate condenses with acetyl CoA to form Citrate

Non-equilibrium (irreversible) reaction catalysed by citrate synthase

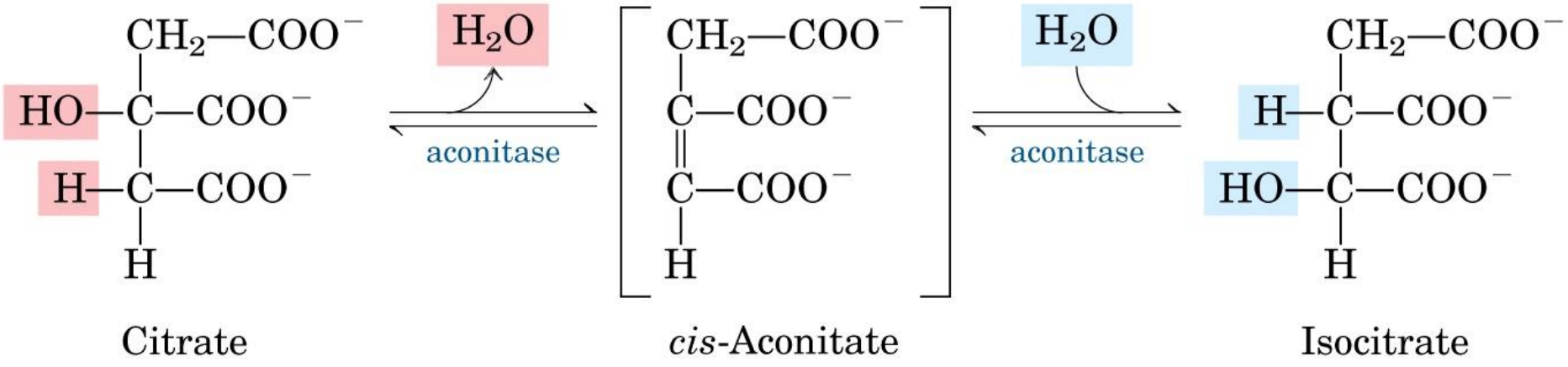
Inhibited by:

- ATP
- NADH
- Succinyl-CoA
- Citrate - competitive inhibitor of oxaloacetate



Citrate ↔ isocitrate

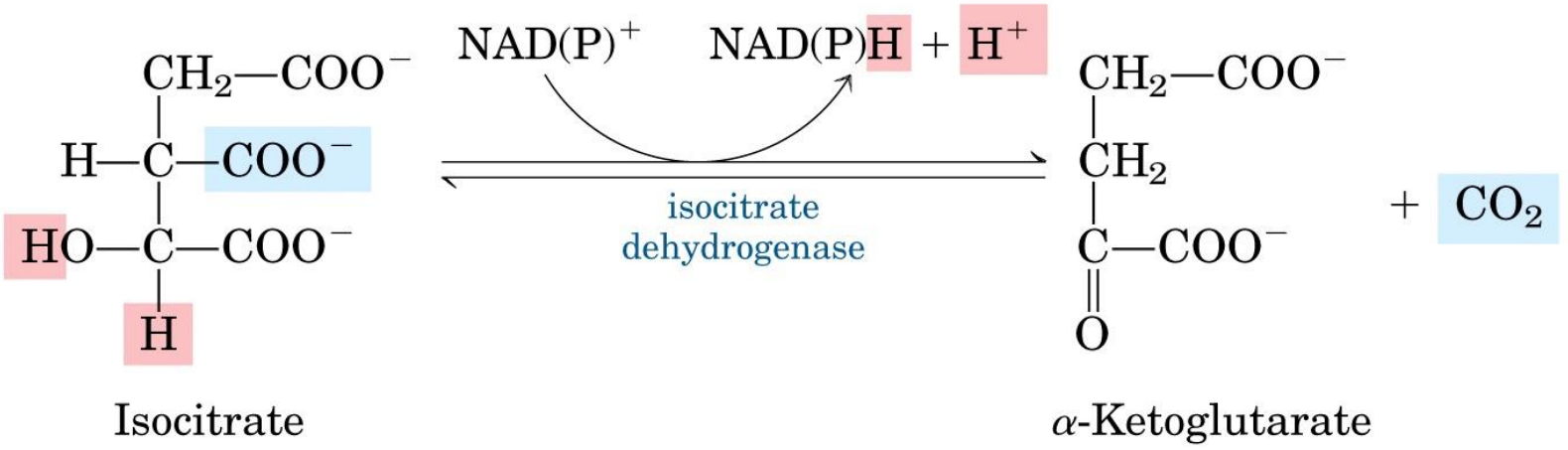
Citrate isomerised to isocitrate in two reactions (dehydration and hydration)
Equilibrium reactions catalysed by aconitase
Results in interchange of H and OH. Changes structure and energy distribution within molecule. Makes easier for next enzyme to remove hydrogen



isocitrate → α-ketoglutarate

Isocitrate dehydrogenated and decarboxylated to give α-ketoglutarate

Stimulated by isocitrate, NAD+, ADP,
Inhibited by NADH and ATP



α-ketoglutarate → succinyl CoA

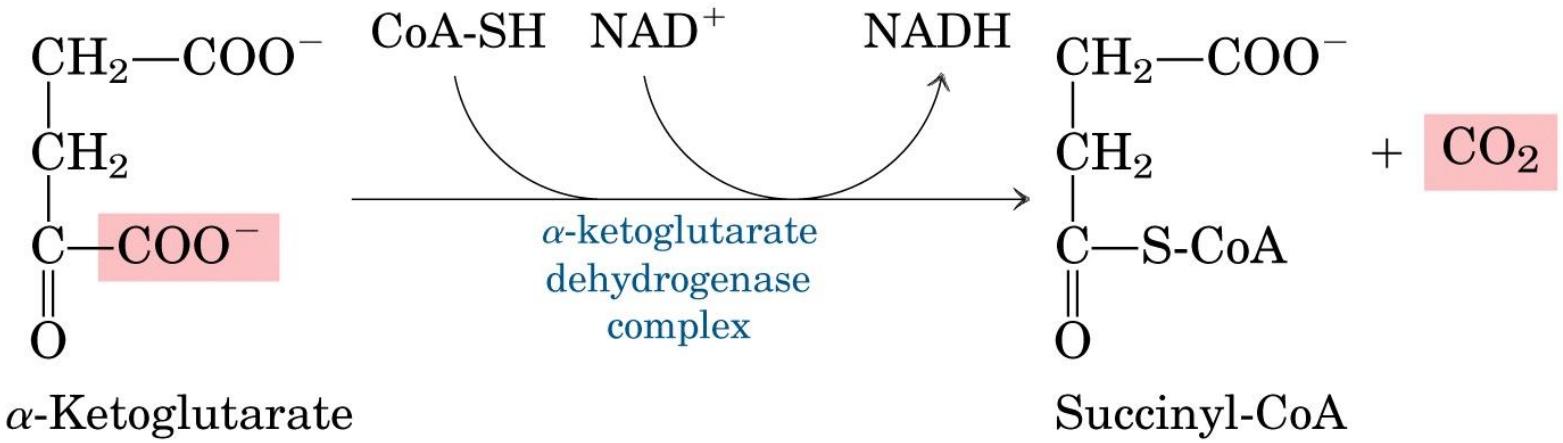
Series of reactions result in decarboxylation, dehydrogenation and incorporation of CoASH

Non-equilibrium reactions catalysed by α-ketoglutarate dehydrogenase complex

Results in formation of: CO2 +NADH and High energy bond

Stimulated by Ca2+

Inhibited by NADH, ATP, Succinyl CoA

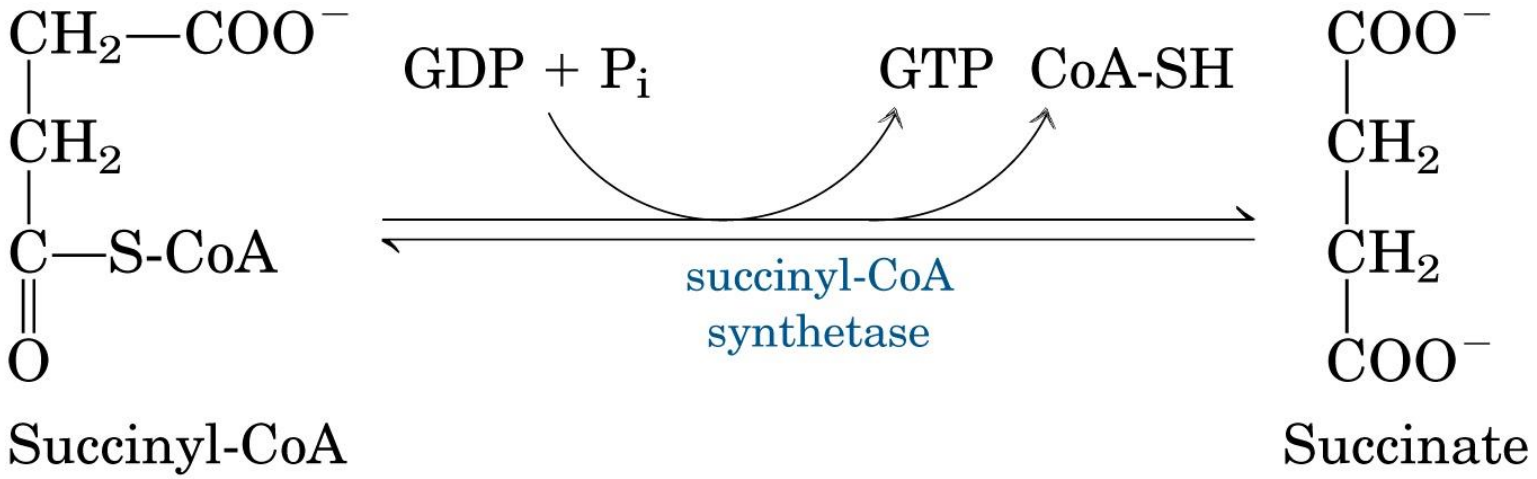


Succinyl CoA ↔ succinate

Equilibrium reaction, the succinyl thioester of CoA =energy rich bond.

Results in formation of: CoA-SH , GTP

GTP + ADP → GDP + ATP(nucleoside diphosphokinase)



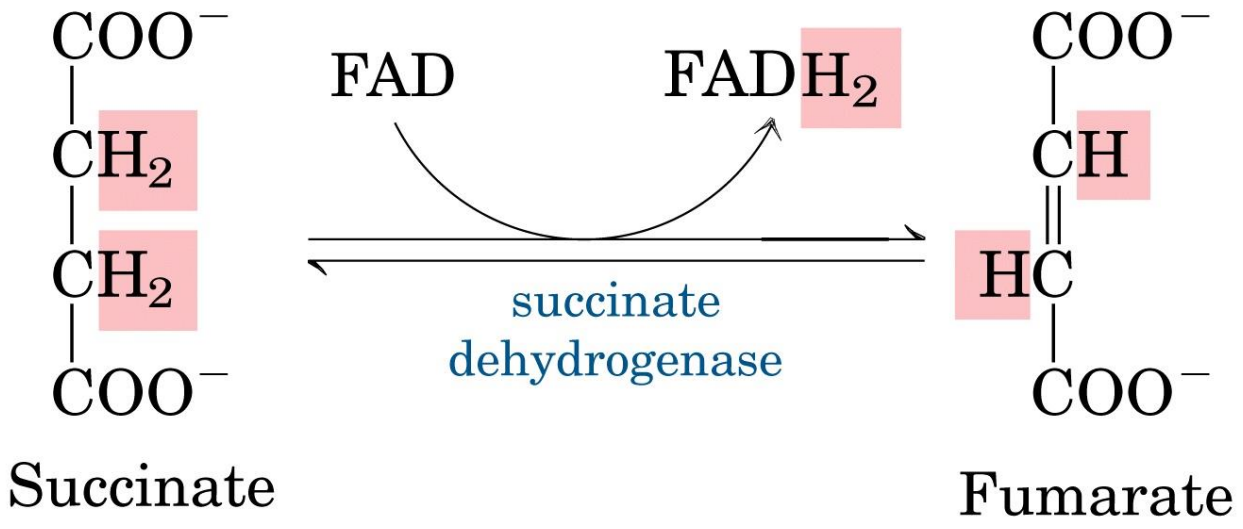
Succinate ↔ fumarate

Oxidation of succinate to fumarate reduces FAD to FADH2

Succinate dehydrogenated to form fumarate

Equilibrium reaction catalysed by succinate dehydrogenase. Only Krebs enzyme contained within inner mitochondrial membrane

Results in formation of FADH2.



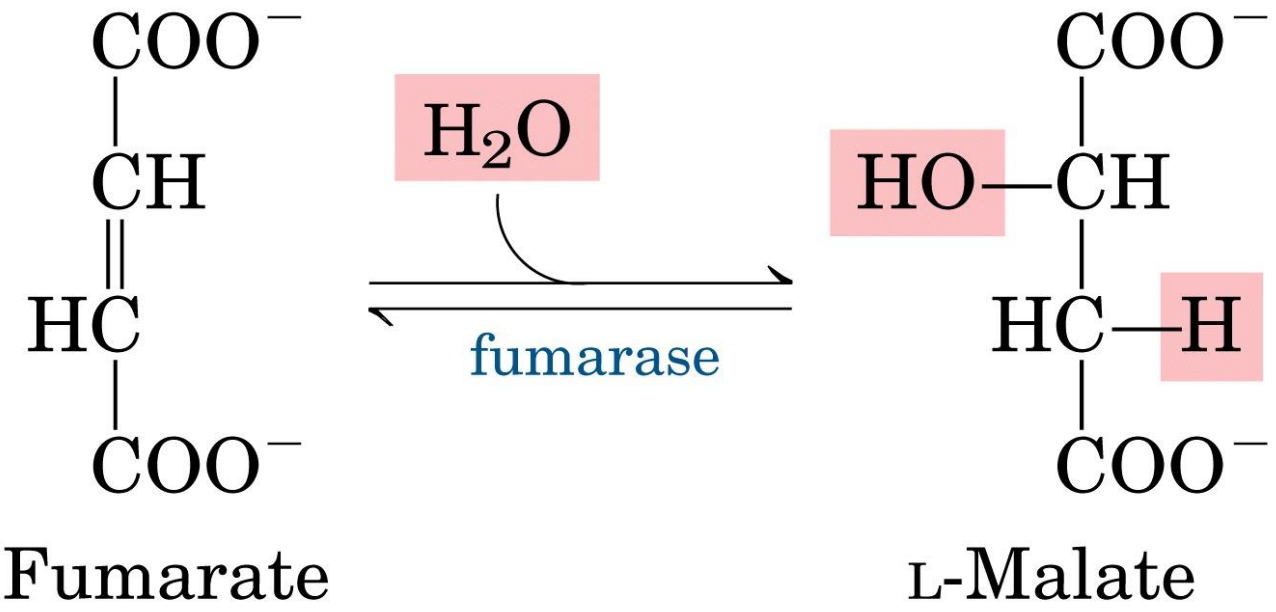
Fumarate ↔ malate

Addition of water to the double bond, to make the alcohol

Fumarate hydrated to form malate

Equilibrium reaction catalysed by fumarase

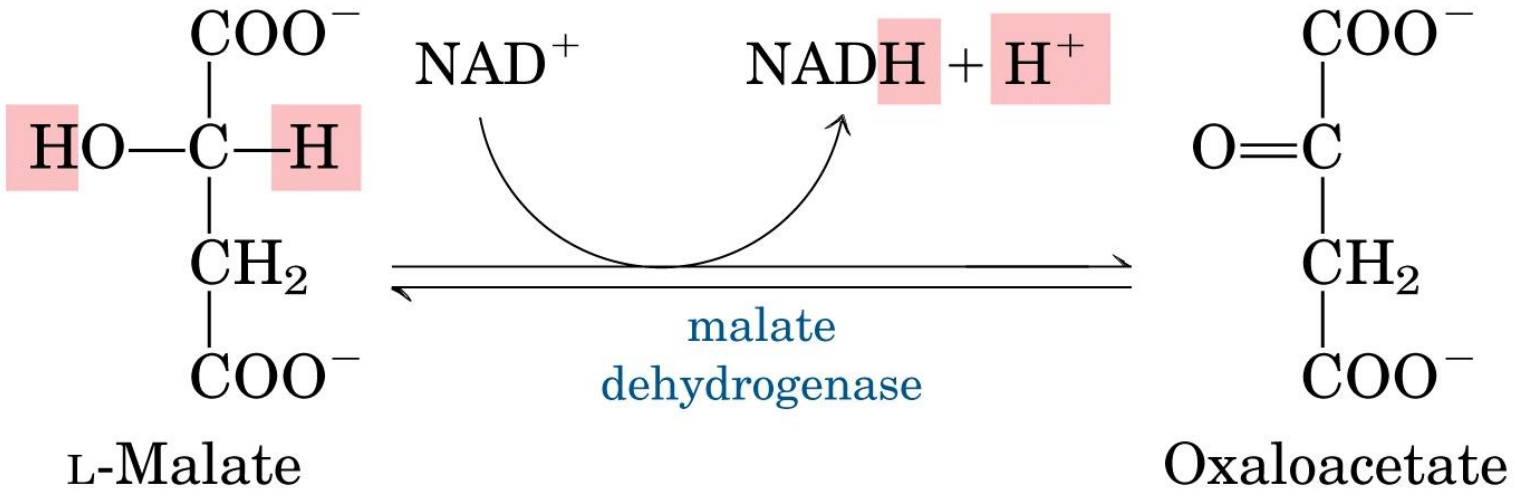
Results in redistribution of energy within molecule so next step can remove hydrogen



Malate ↔ oxaloacetate

Oxidation of Malate to Oxaloacetate reduces NAD+ to NADH

Malate dehydrogenated to form oxaloacetate
Equilibrium reaction catalysed by malate dehydrogenase
Results in formation of NADH + H+



Summary of TCA Cycle Reactions

acetyl CoA oxidized to $2CO_2$

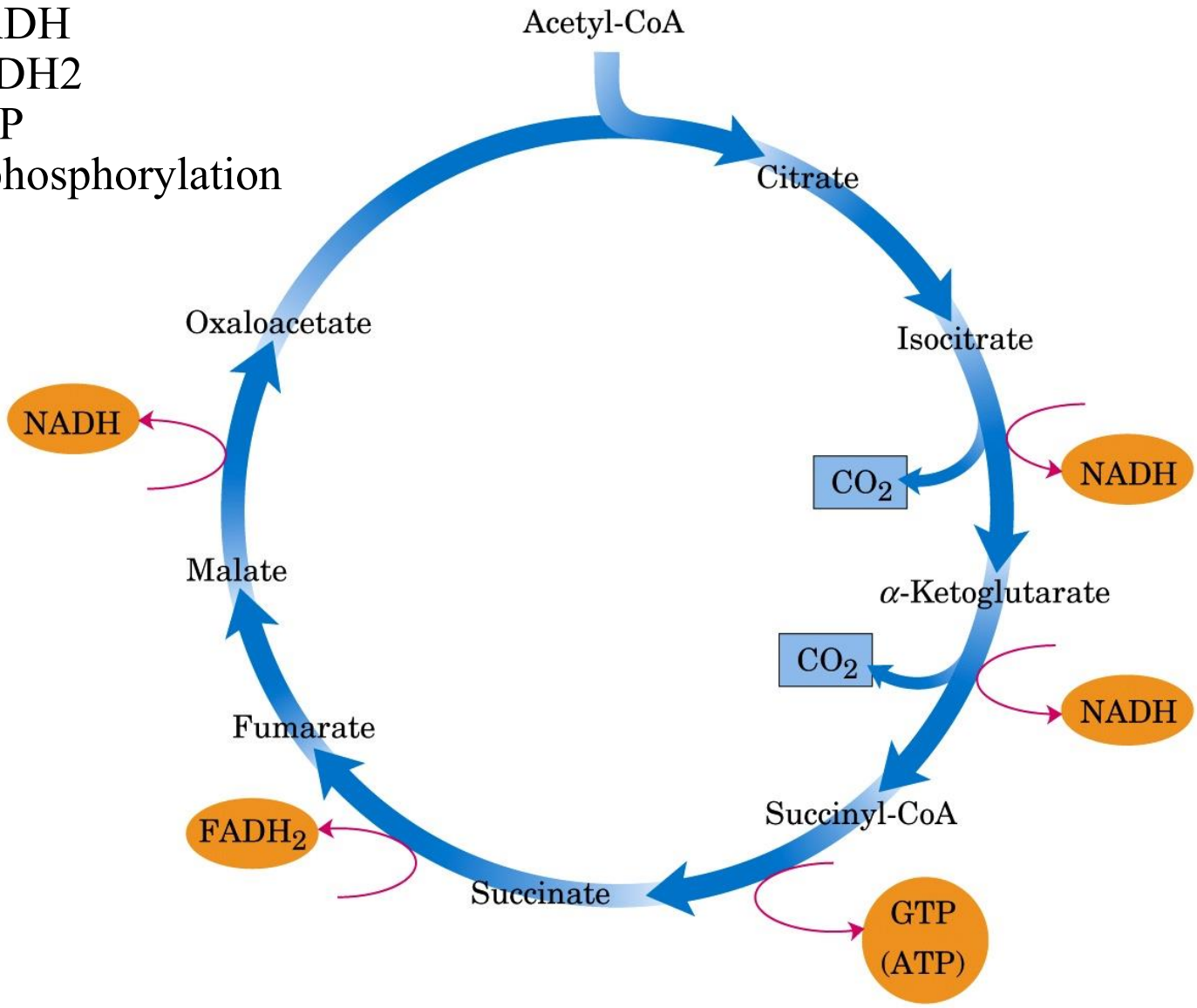
4 electron pairs

3NADH

1FADH2

1GTP

Coupled to oxidative phosphorylation



All of the carbons that are input as **Pyruvate** are released as **CO2**. This is as highly oxidized as carbon can get.

Oxidative decarboxylation: each time a CO2 is produced one NADH is produced.

Locations of CO2 release:

1. Pyruvate dehydrogenase: Pyruvate to Acetyl-CoA
2. Isocitrate dehydrogenase: Isocitrate to a-ketoglutarate
3. alpha-ketoglutarate dehydrogenase:a-ketoglutarate to succinyl-CoA

Regulation of PDH complex

1- inhibition by end products (NADH, Acetyl CoA). Increased levels of acetyl CoA and NADH inhibit E_2, E_3 in mammals and *E. coli*.

2- Feed back regulation by nucleotides (GTP)

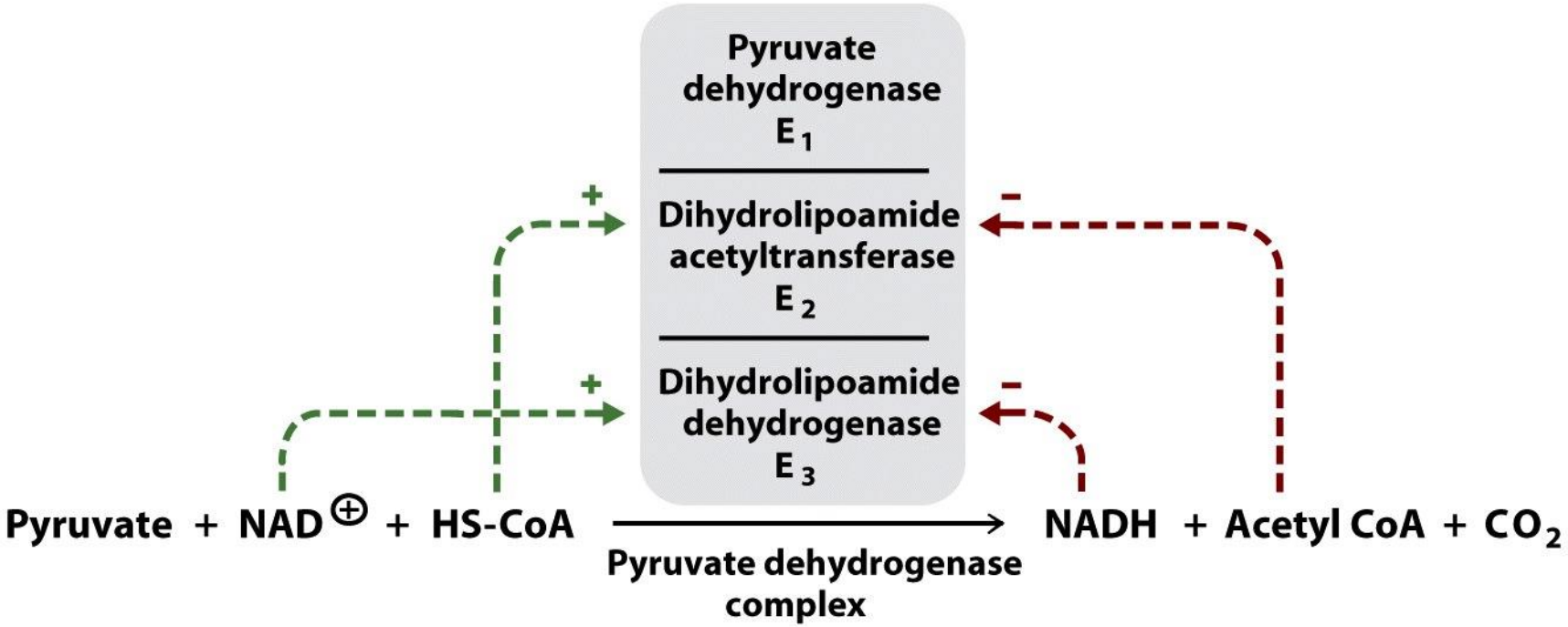


Figure 13-11 Principles of Biochemistry, 4/e

3- Regulation by reversible phosphorylation
phosphorylation/dephosphorylation of E_1

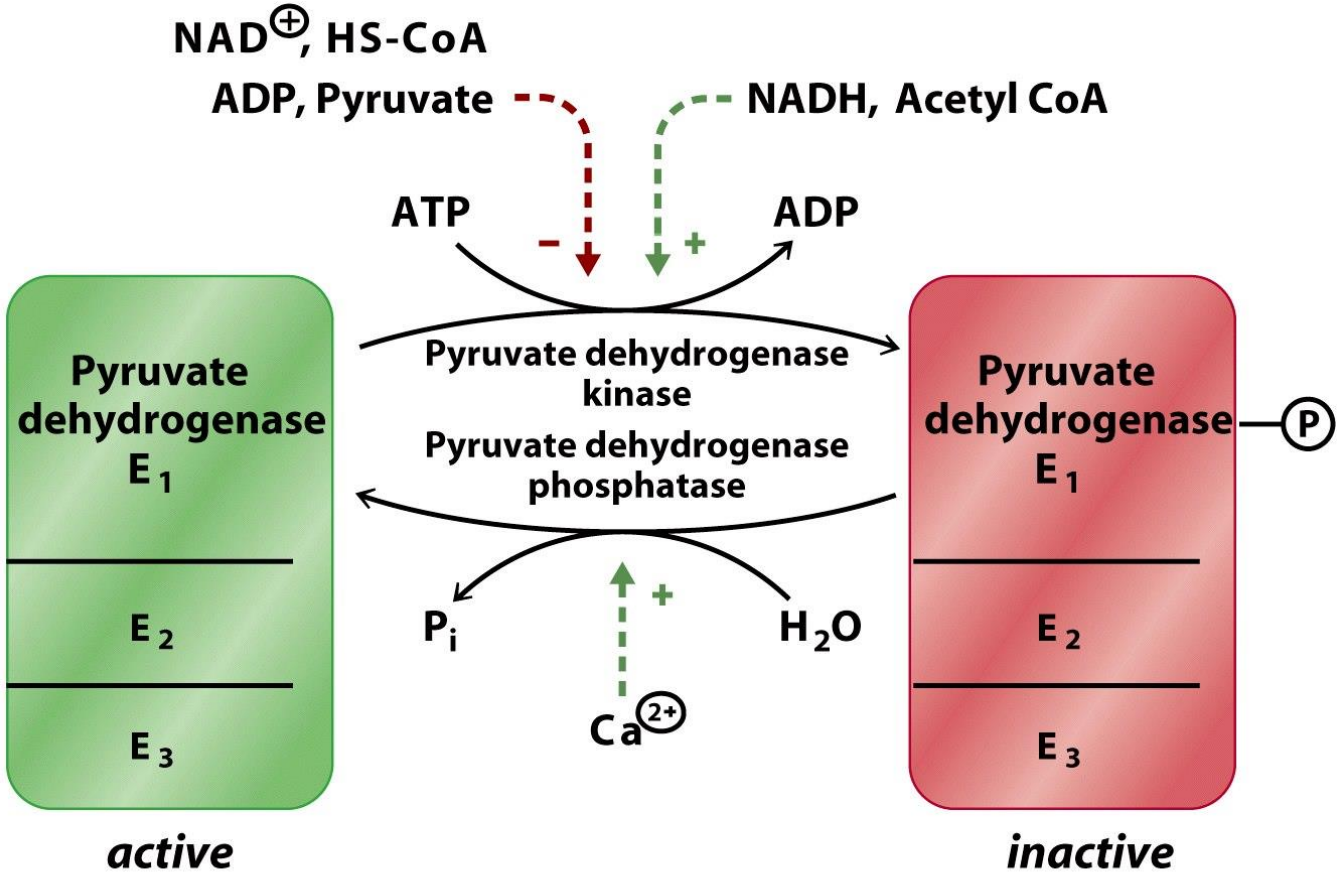


Figure 13-12 Principles of Biochemistry, 4/e

Regulation of Krebs Cycle

- Cycle always proceeds in same direction due to presence of 3 non-equilibrium reactions catalysed by
  - **Citrate synthase**

    - NADH, ATP, citrate, succinyl CoA

    + NAD+, ADP
  - **Isocitrate dehydrogenase**

    - NADH, ATP

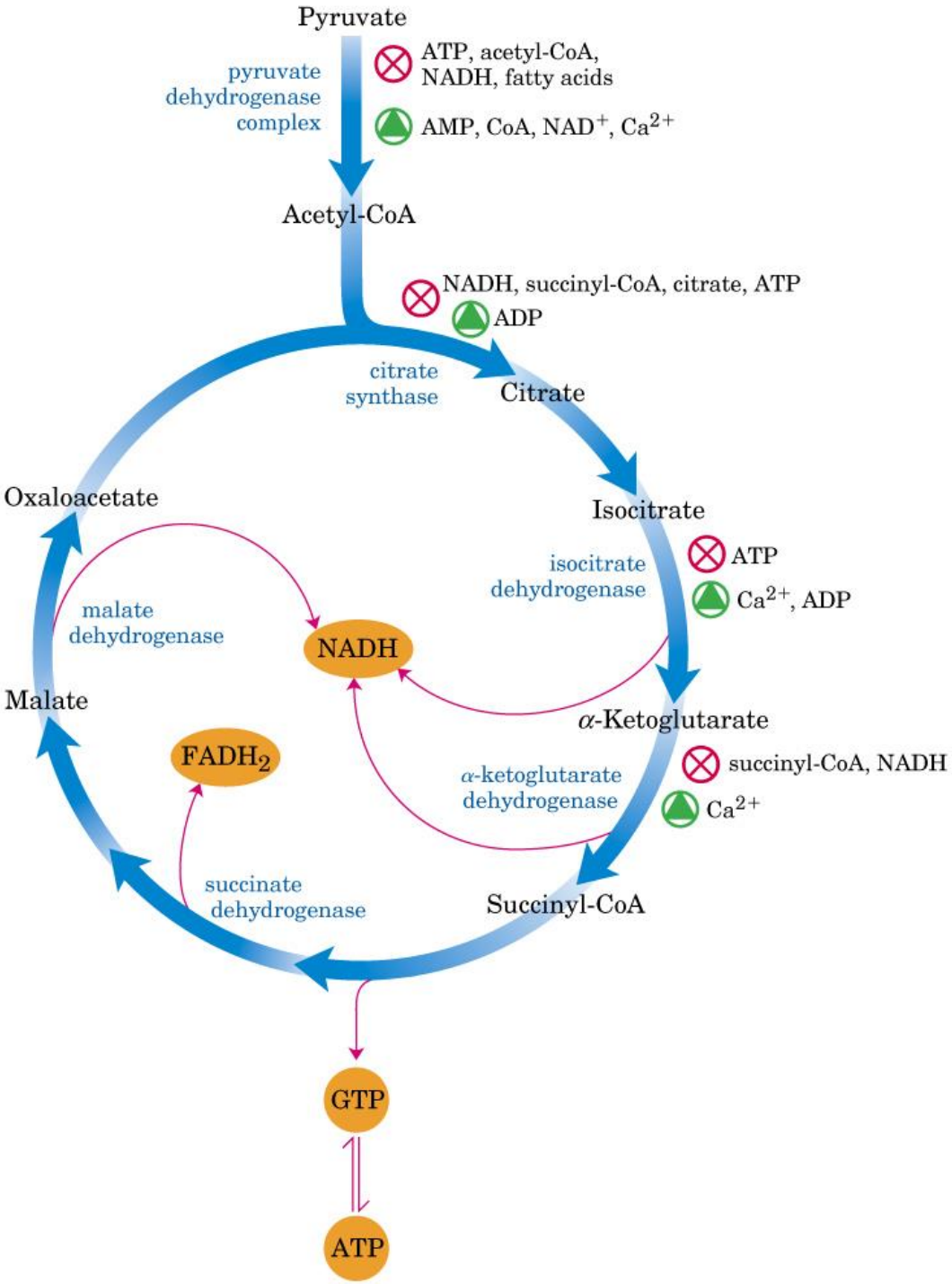
    + NAD+, ADP, Ca2+
  - **α-ketoglutarate dehydrogenase**

    - NADH, Succinyl CoA

    + NAD+, Ca2+

Regulation of Krebs Cycle

- Flux through KC increases during exercise i.e as contractile activity increases
- 3 non-equilibrium enzymes inhibited by NADH
  - KC tightly coupled to ETC
    - If NADH decreases due to increased oxidation in ETC flux through KC increases
- Isocitrate dehydrogenase and α-ketoglutarate dehydrogenase also stimulated by $Ca2^{+}$


Pyruvate
pyruvate dehydrogenase complex
ATP, acetyl-CoA, NADH, fatty acids
AMP, CoA, NAD+, Ca2+
Acetyl-CoA
NADH, succinyl-CoA, citrate, ATP
ADP
citrate synthase
Citrate
Isocitrate
isocitrate dehydrogenase
ATP
Ca2+, ADP
α-Ketoglutarate
α-ketoglutarate dehydrogenase
succinyl-CoA, NADH
Ca2+
Succinyl-CoA
succinate dehydrogenase
FADH2
Malate
malate dehydrogenase
NADH
Oxaloacetate
GTP
ATP