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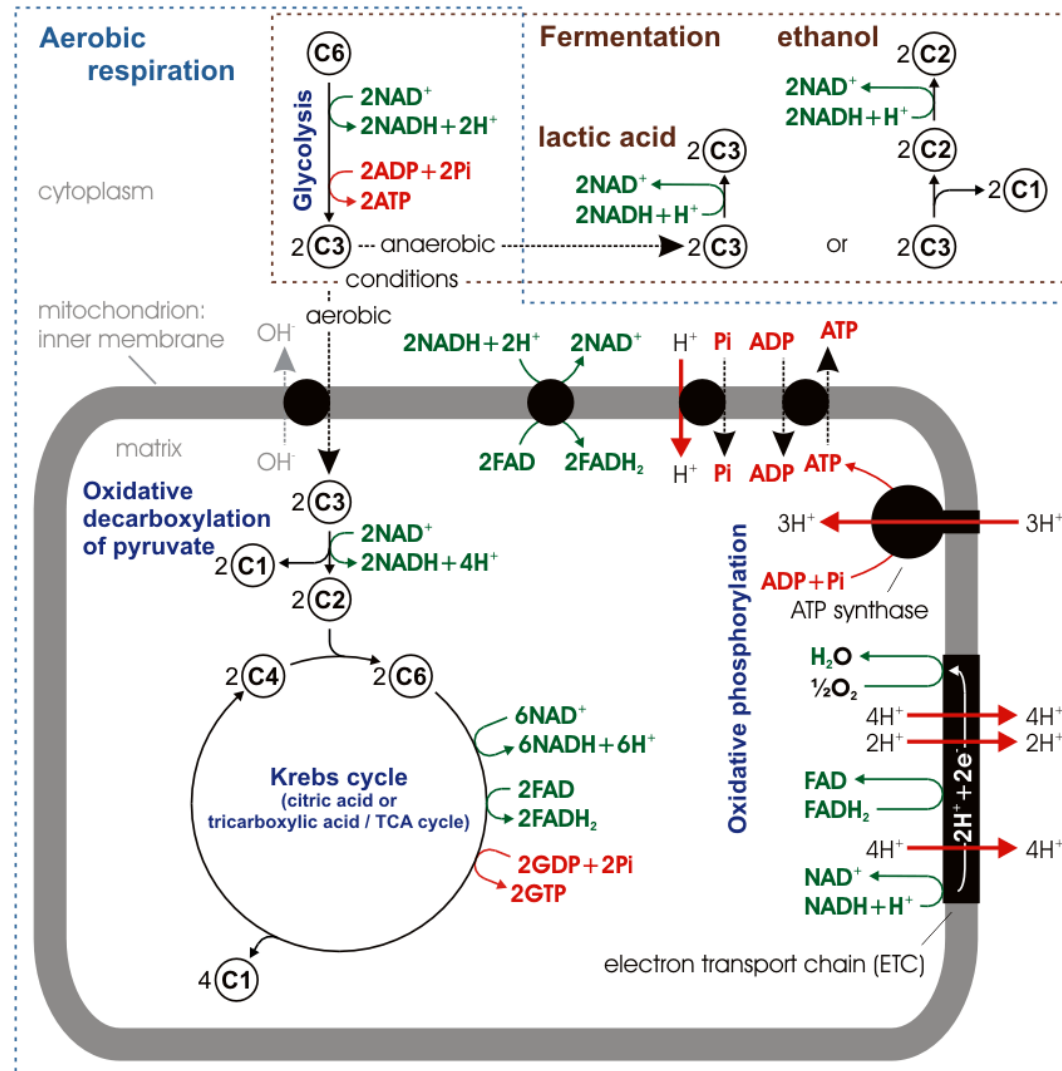
# (Kreb's, Citric Acid, TCA) Cycle

# Objectives

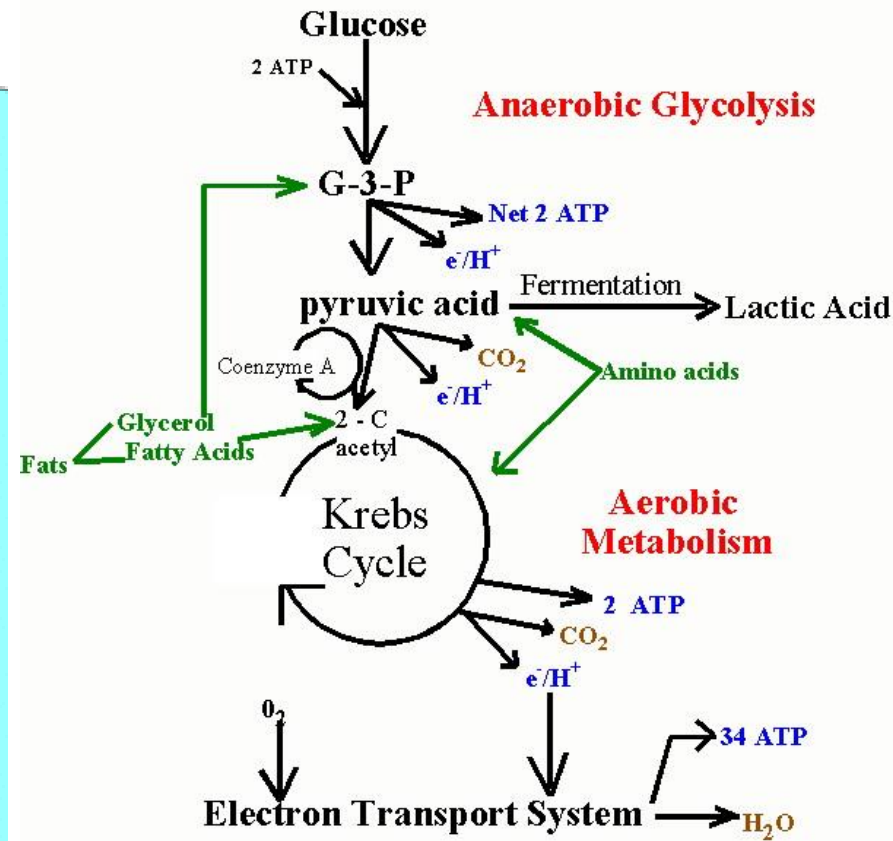
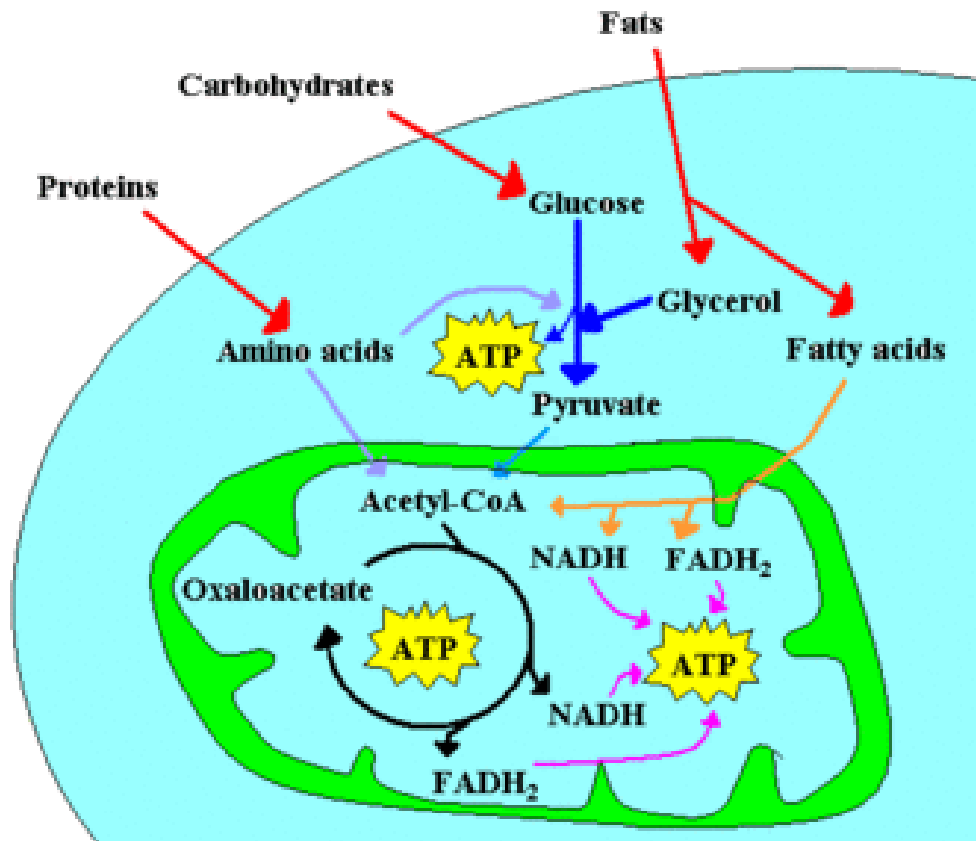
- Why do we need it?
- Location & where is it in the picture?
- Electron (energy) carrying molecules
- Components
- Enzymes & cofactors
- Regulation
- Intermediates & anaplerotic reactions

# Why do we need it?

- A route to make ATP eventually (most ATP is generated through oxidative phosphorylation)
- There is no life without TCA cycle!



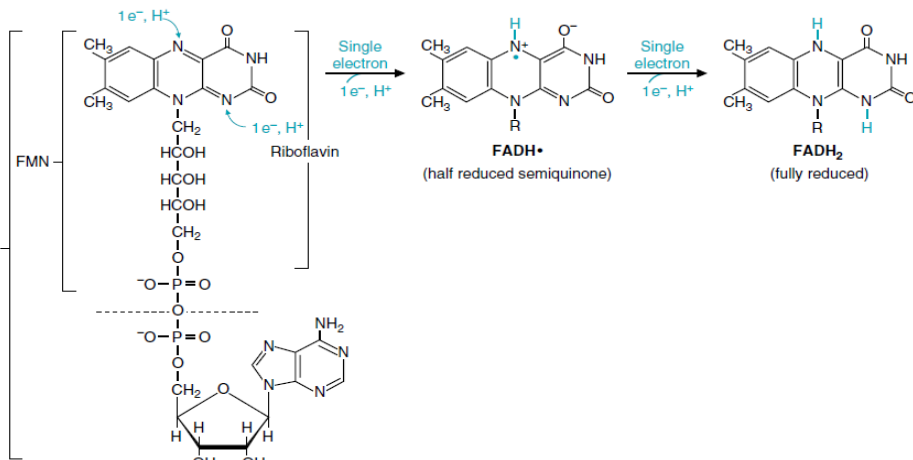
# How does it fit?



# Electron (energy) Carrying Molecules (NAD<sup>+</sup>, FAD)

## ➤ FAD

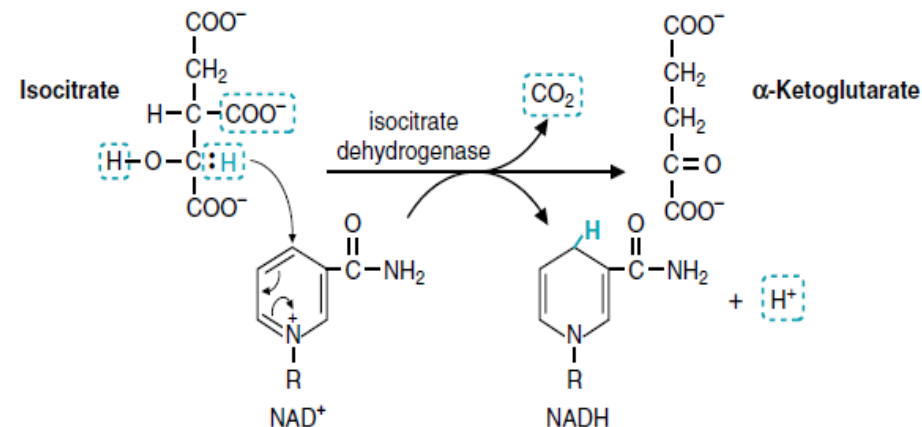
- Single electrons (H•), different sources
- Succinate to fumarate, lipoate to lipoate disulfide in  $\alpha$ -KG
- FAD must remain tightly, sometimes covalently, attached to its enzyme
- $E^0$  for enzyme-bound FAD varies



Flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN)

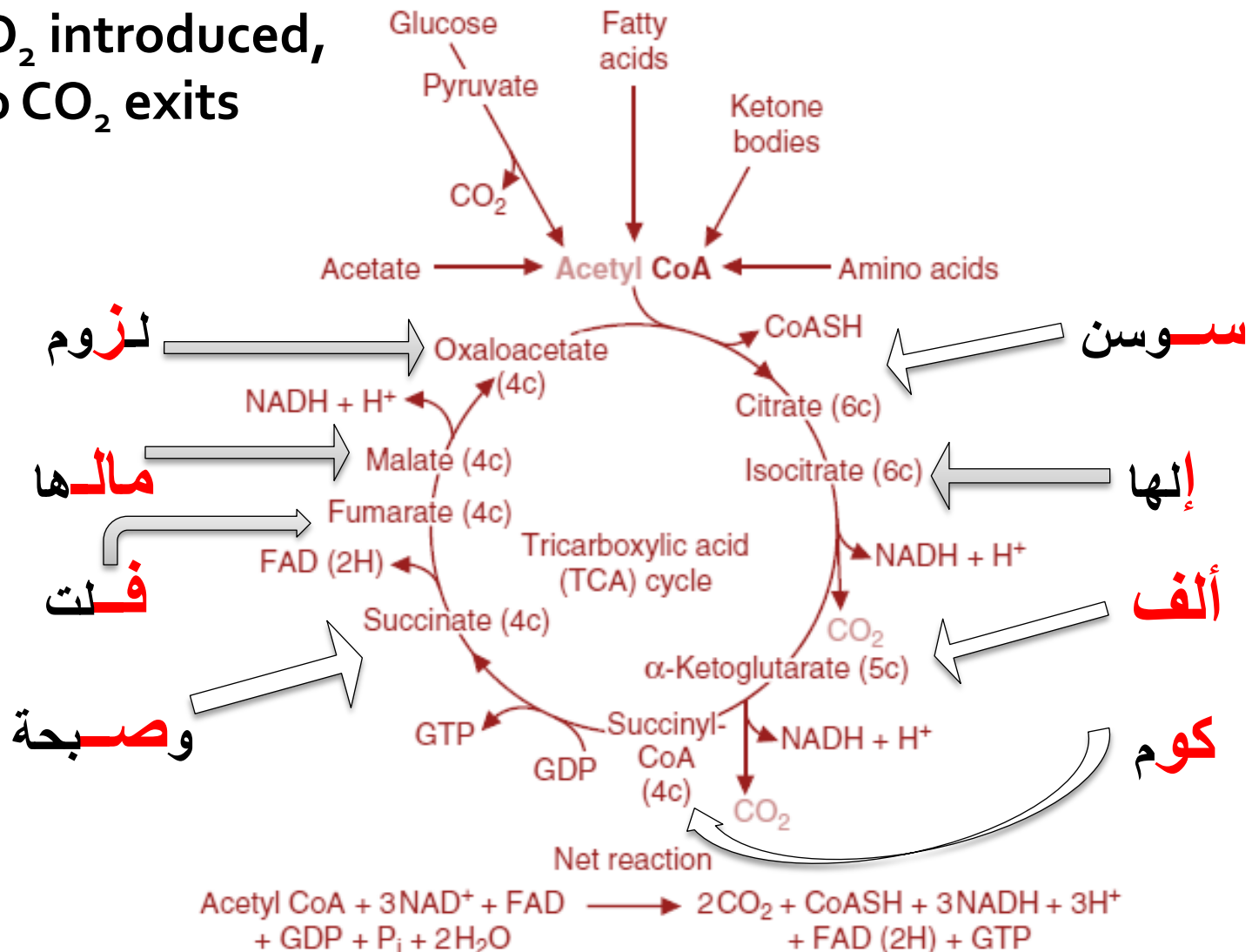
## ➤ NAD

- Pair of electrons (H<sup>-</sup>), same source
- Alcohols to ketones by malate dehydrogenase & isocitrate dehydrogenase
- NADH plays a regulatory role in balancing energy metabolism



# Components & stepwise reactions

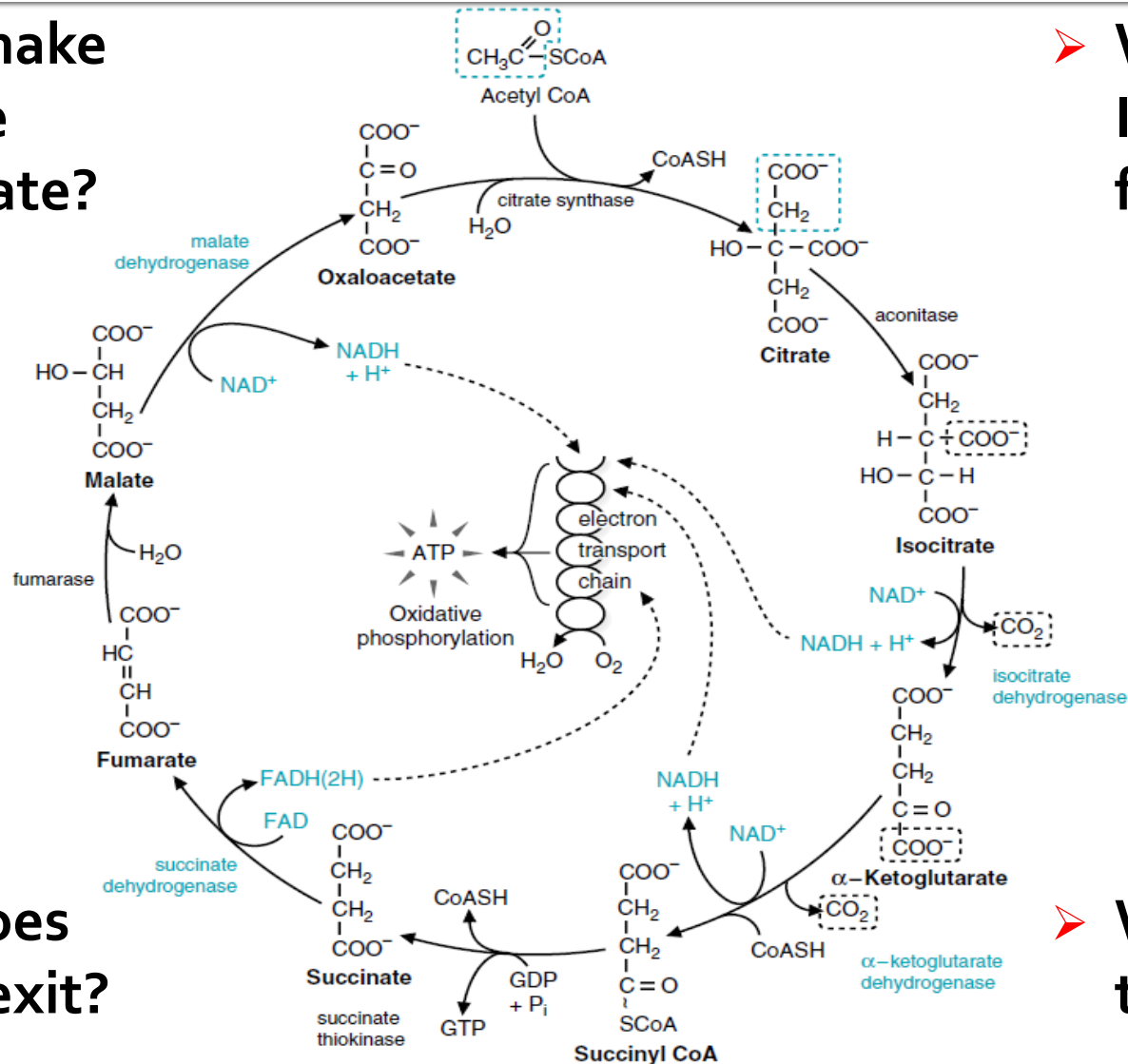
- No O<sub>2</sub> introduced, two CO<sub>2</sub> exits



# Does Acetyl-CoA exit as 2 CO<sub>2</sub>?

➤ Why to make Isocitrate from citrate?

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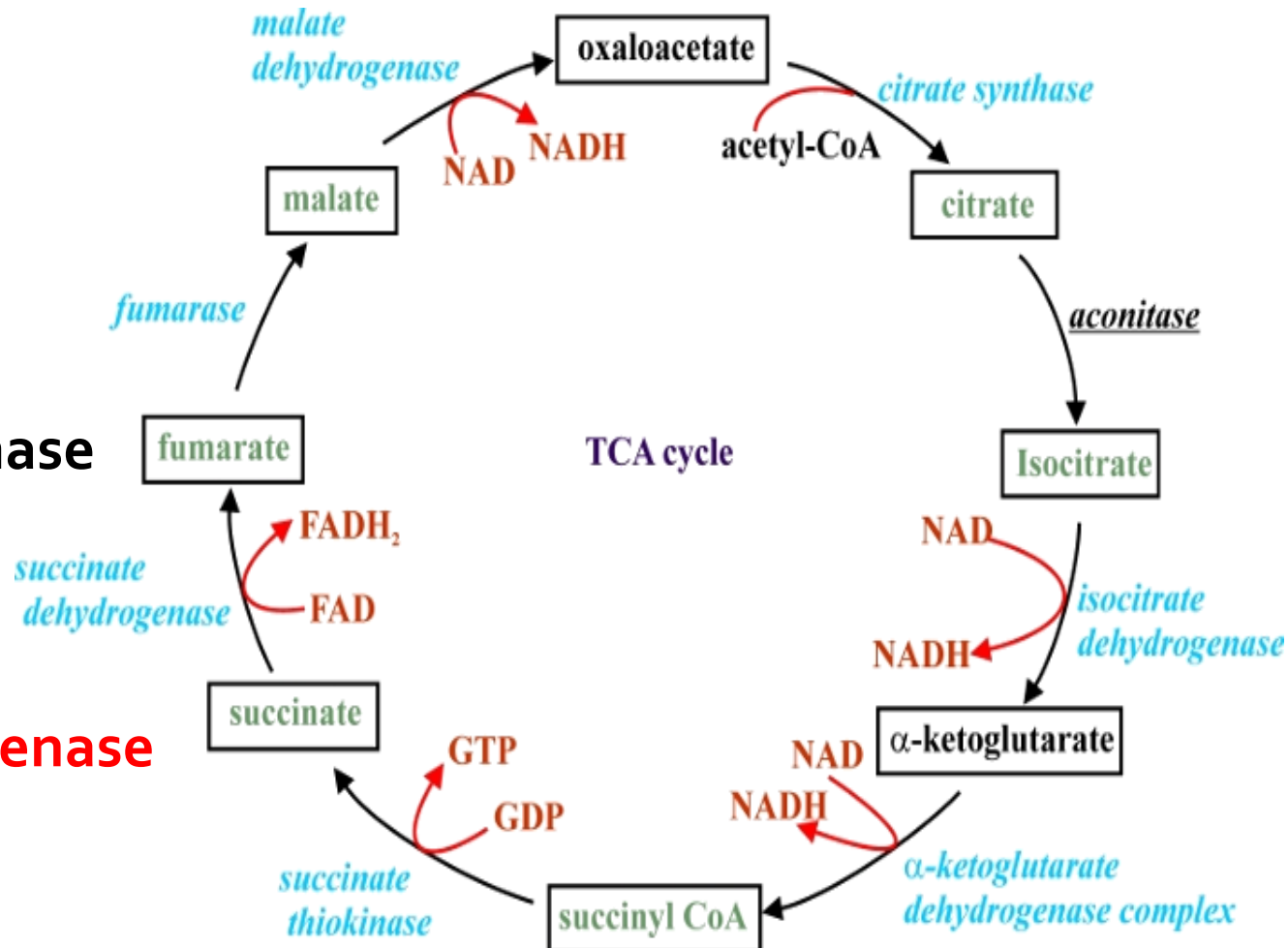


➤ Where does the CO<sub>2</sub> exit?

➤ Where does the CO<sub>2</sub> exit?

# Enzymes of the TCA Cycle

- Citrate synthase
- Aconitase
- Isocitrate dehydrogenase
- $\alpha$ -ketoglutarate dehydrogenase
- Succinate thiokinase
- Succinate dehydrogenase
- Fumarase
- Malate dehydrogenase

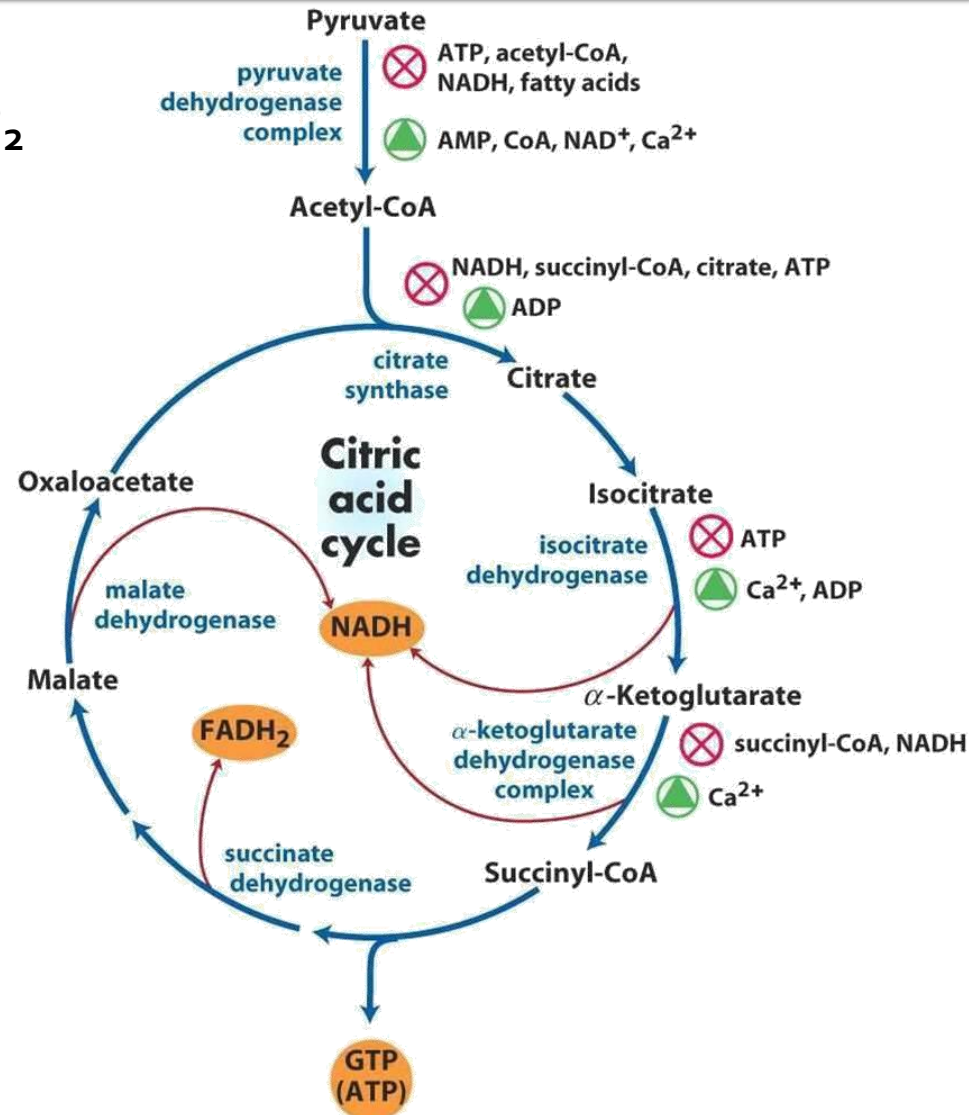
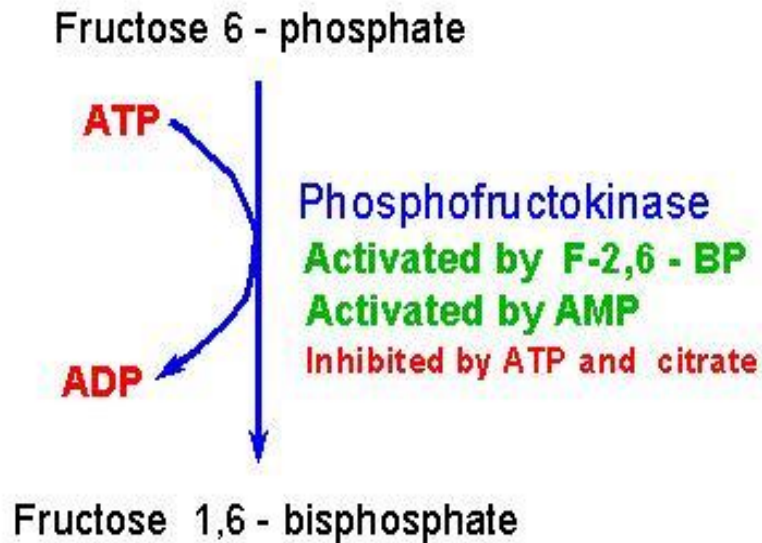




# Formation and Oxidation of Isocitrate

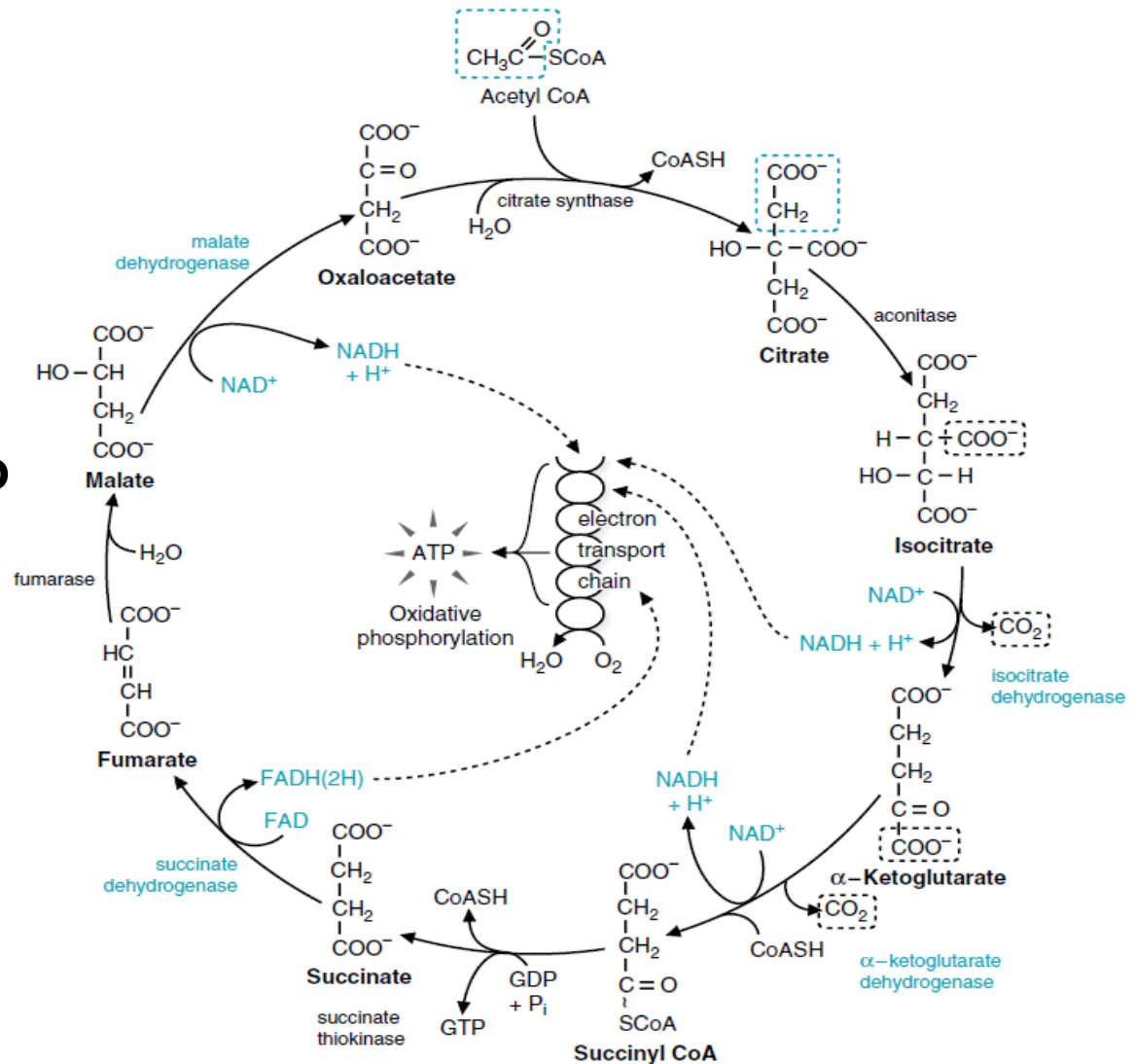
- Oxidative decarboxylation,  $\text{CO}_2$
- $3^\circ$  to  $2^\circ$  alcohol

Control at the committed step of glycolysis



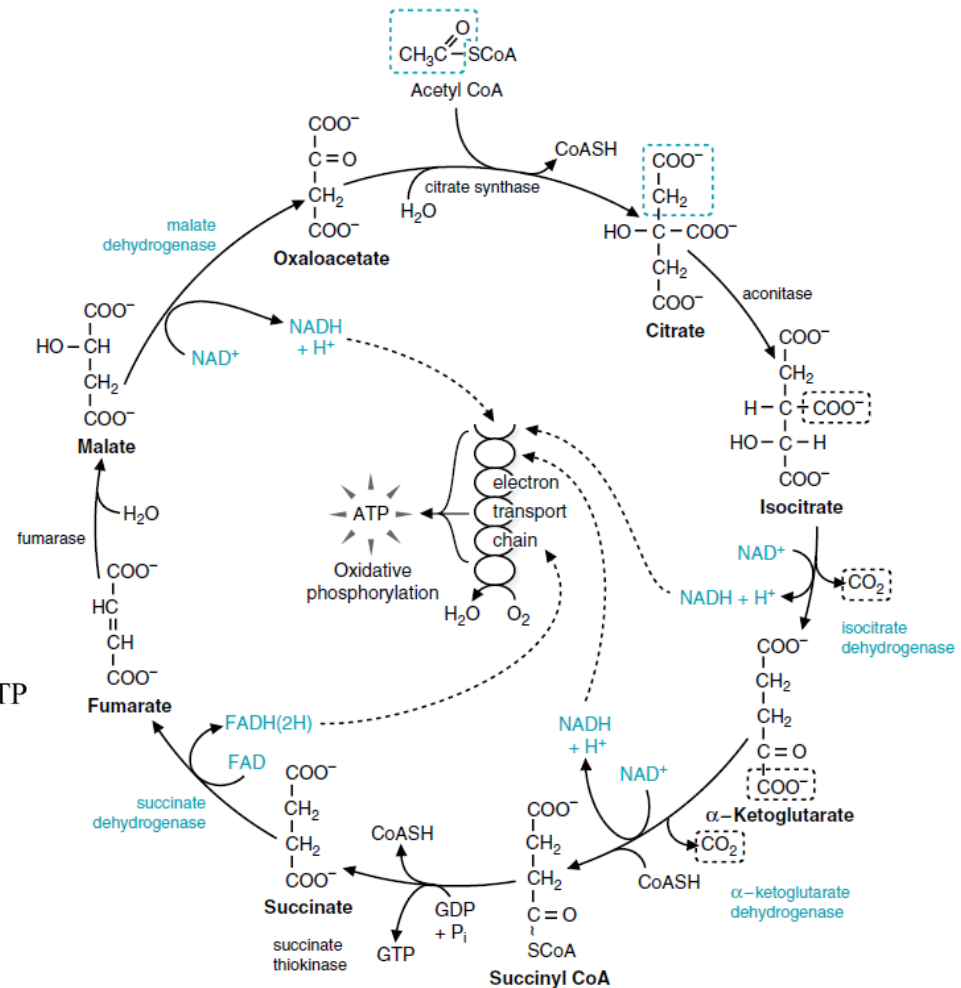
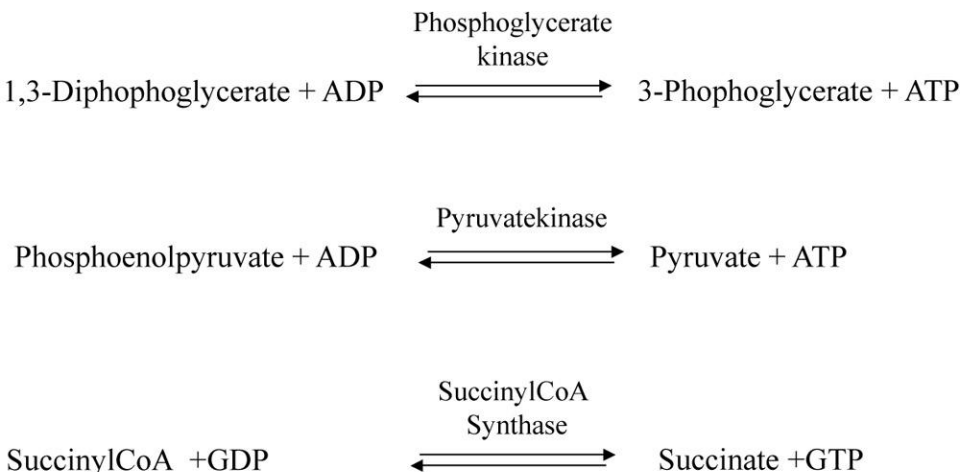
# $\alpha$ -Ketoglutarate to Succinyl CoA

- Oxidative decarboxylation
- Thiamine pyrophosphate, lipoic acid, and FAD
- Keto group oxidized to acid, CoA-SH, succinyl CoA
- Energy conserved as NADH, thioester bond



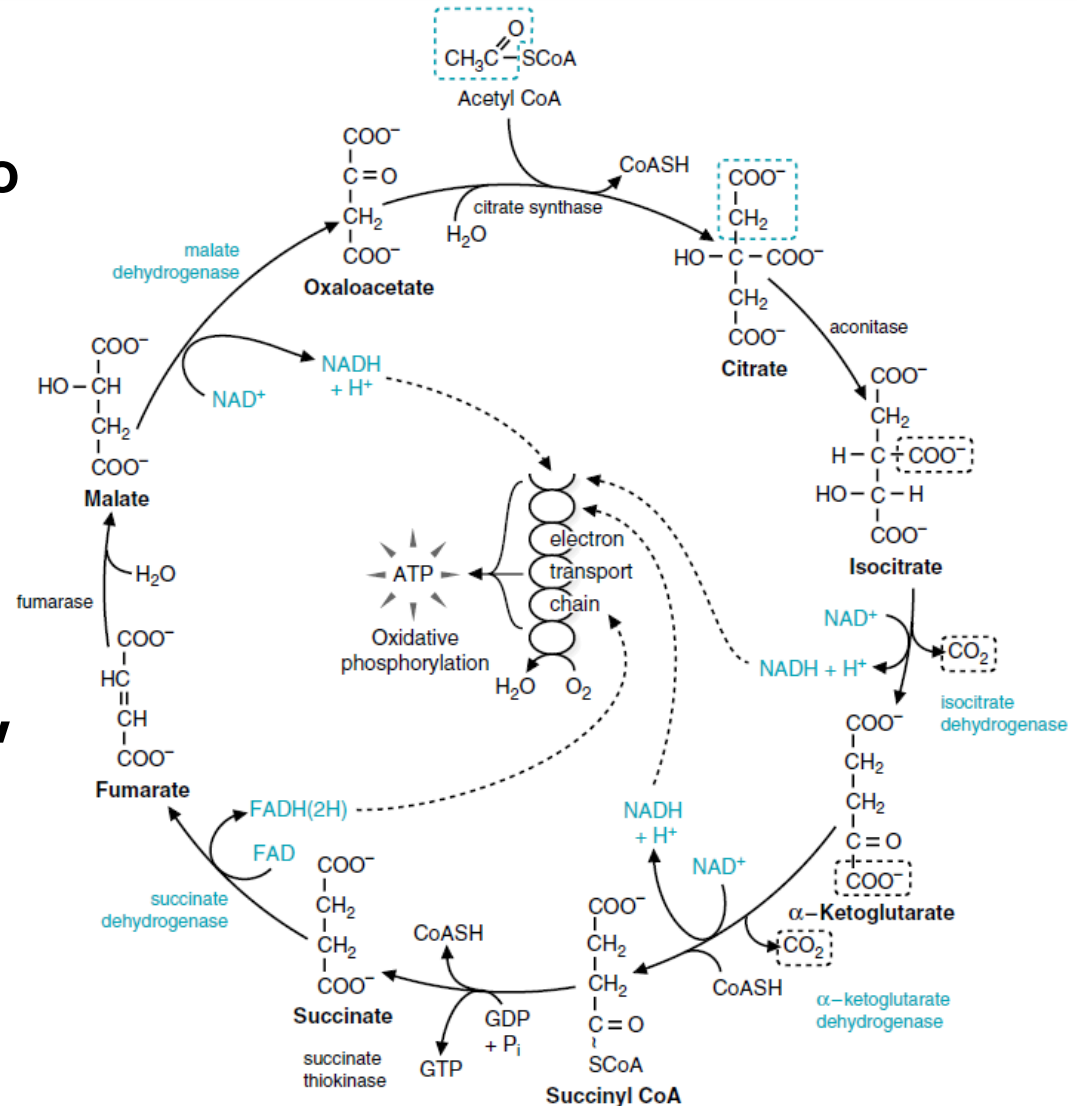
# Generation of GTP

➤ **Succinyl CoA thioester bond, succinate thiokinase, substrate level phosphorylation**



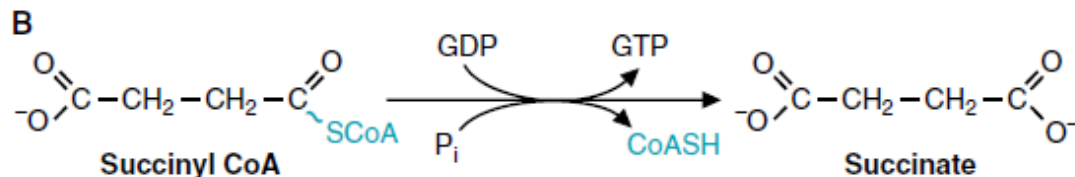
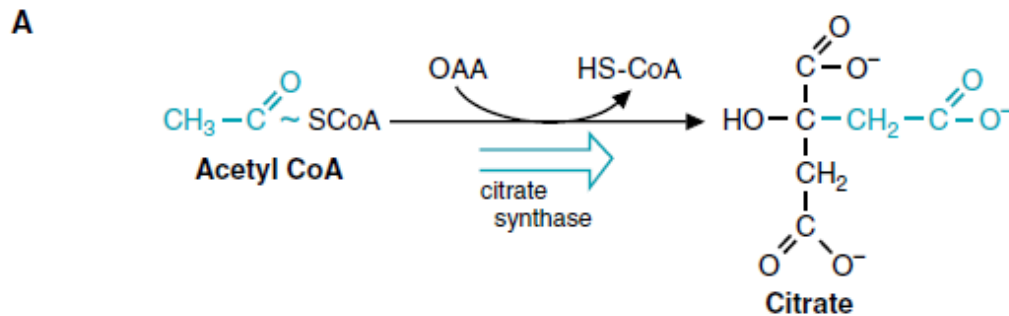
# Oxidation of Succinate to Oxaloacetate

- Oxidation of succinate to fumarate, succinate dehydrogenase, FAD
- Fumarase,  $\text{OH} + \text{H}^+$  from water, fumarate to malate
- Alcohol group of malate oxidized to a keto group, NADH

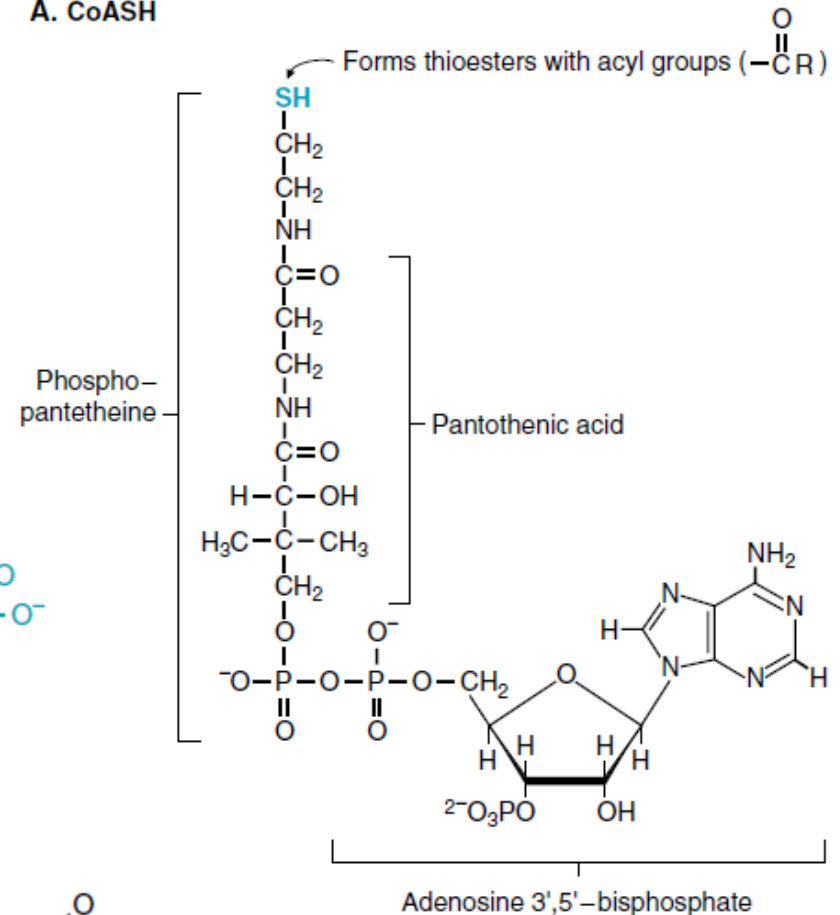


# CoA

- Forms a thioester bond, CoASH & an acyl group (e.g., acetyl CoA, succinyl CoA)
- Sulfur vs. oxygen (carbon can be activated, -13kcal, GTP, keeps the reaction going)

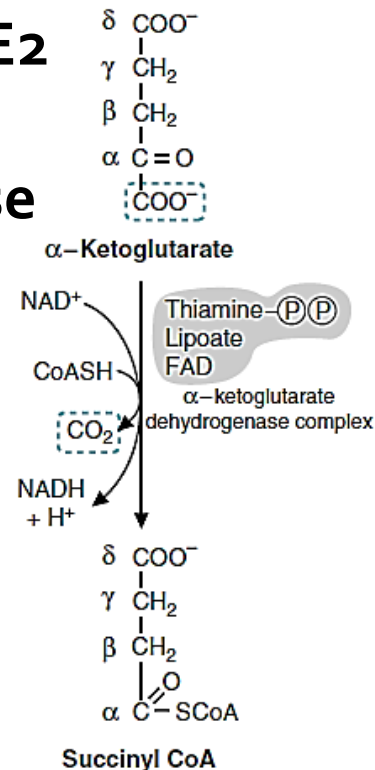
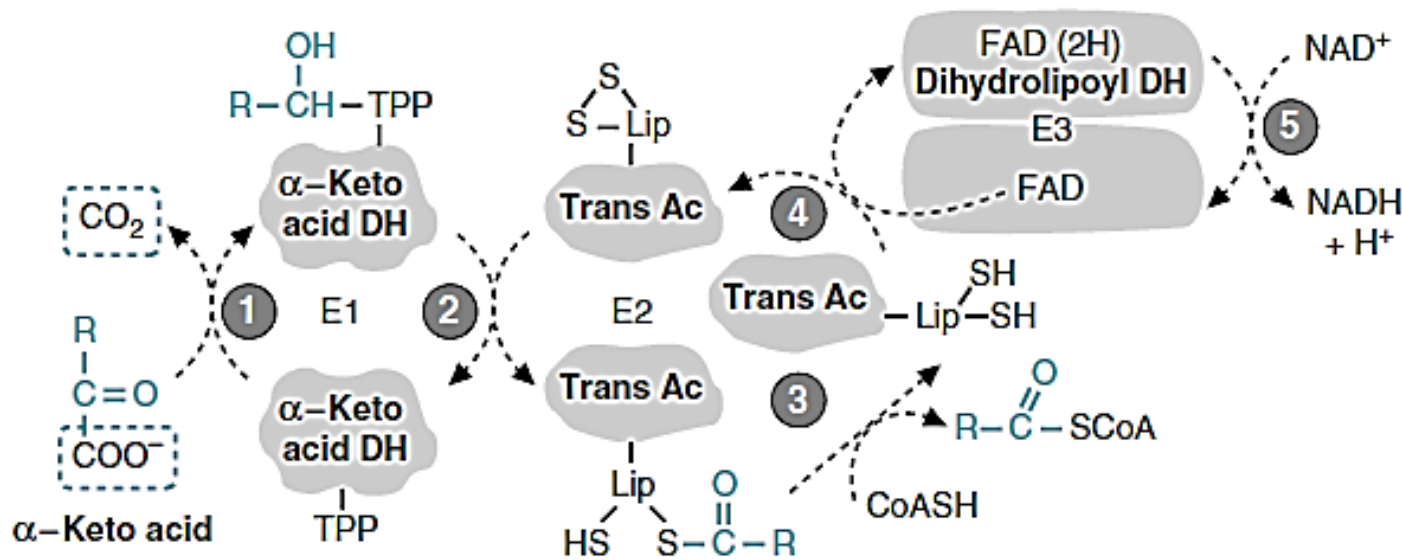


**A. CoASH**



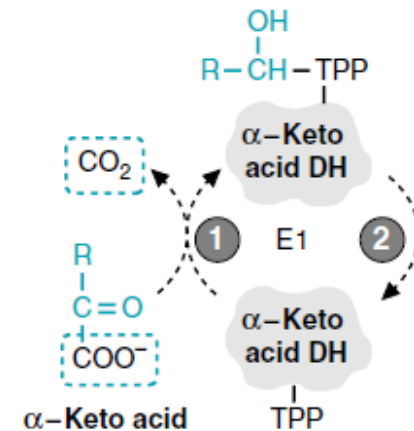
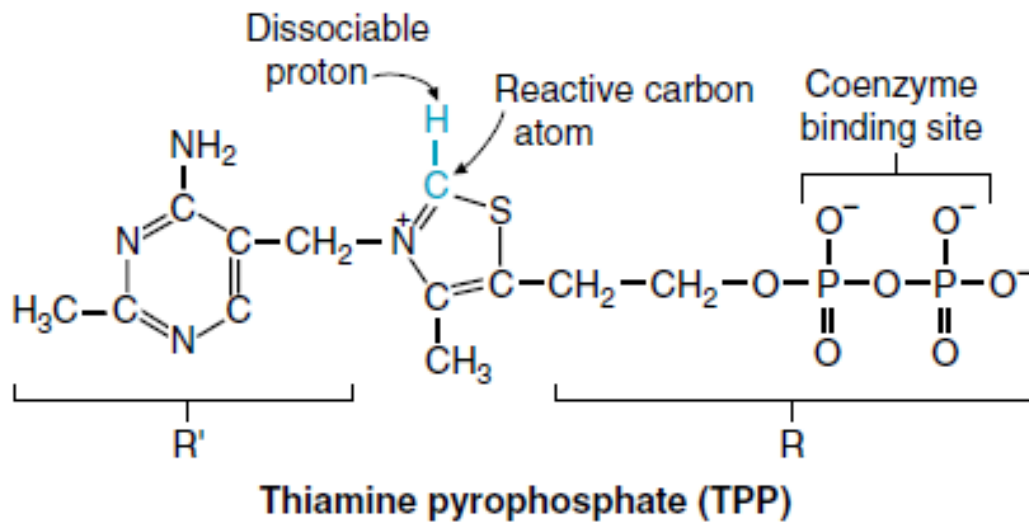
# $\alpha$ -Ketoacid Dehydrogenase Complexes (TLCFN)

- ( $\alpha$ -ketoglutarate, pyruvate, and branched chain  $\alpha$ -keto acid) dehydrogenase complexes
- Huge enzyme complexes, multiple subunits of 3 different enzymes (no loss of energy, substrates for E2 and E3 remain bound  $\rightarrow$  higher rate)
- E1, E2, & E3 are a decarboxylase (TPP), a transacylase (lipoate), & a dehydrogenase (FAD)



# Thiamine Pyrophosphate

- Thiamine deficiency,  $\alpha$ -ketoglutarate, pyruvate, & branched chain  $\alpha$ -keto acids accumulate in the blood

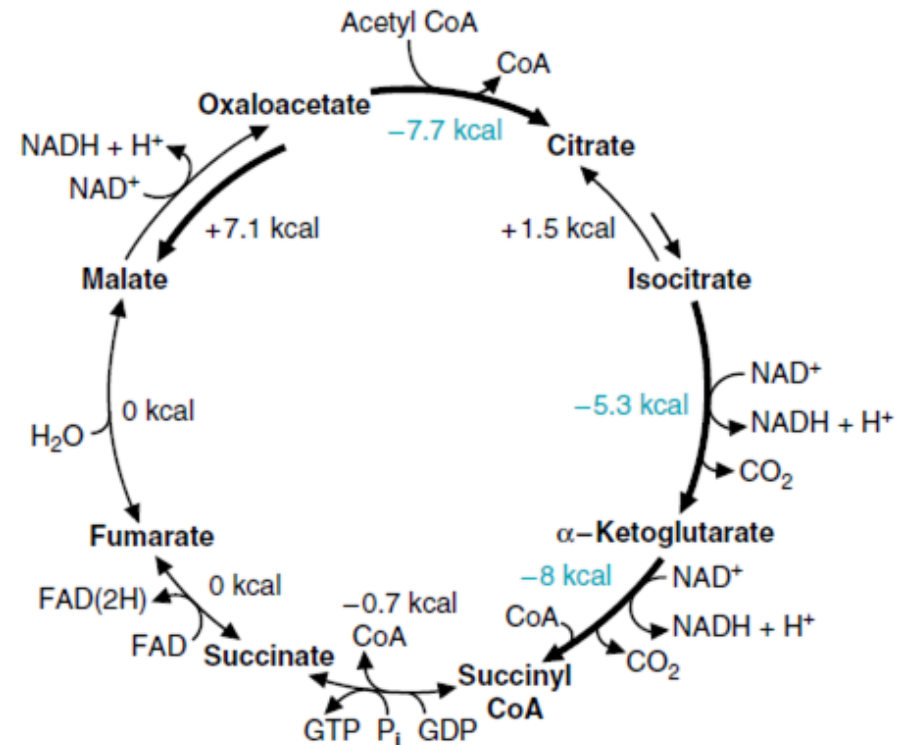




# Bioenergetics of the TCA Cycle

- Like all pathways, overall net -  $\Delta G$  (-228 kcal/mole), not 100%
- NADH, FAD(H<sub>2</sub>), and GTP (10ATP), 207 Kcal, 90%
- Three reactions have large (-ve) values
- Physiologically irreversible, low products
- Aconitase (+ $\Delta G$ ), maintains 20 times of citrate/Isocitrate, Acetyl CoA in cytosol for fatty acid and cholesterol synthesis
- Malate DH (+ $\Delta G$ ), favors malate over oxaloacetate, fasting, liver, substrate for gluconeogenesis

kcal/mole	
3 NADH:	$3 \times 53 = 159$
1 FAD(2H)	= 41
1 GTP	= 7
Sum	= 207



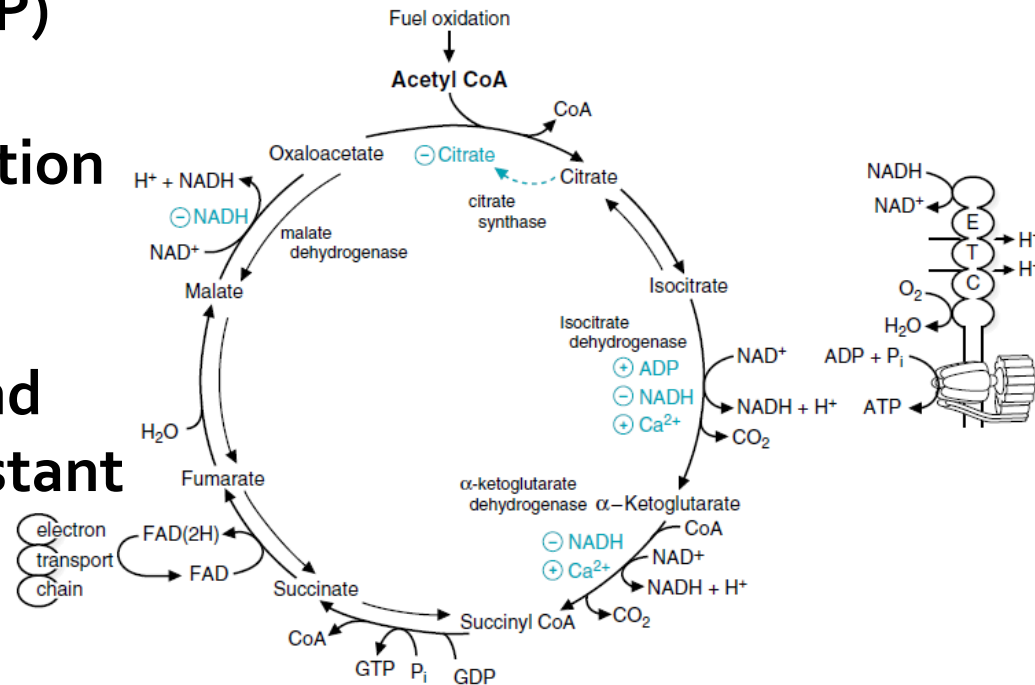


# Regulation of the TCA Cycle

- Correspond to ETC (ATP/ADP)
- Two major messengers (feedback): (a) phosphorylation state of adenines, (b) the reduction state of NAD
- Adenine nucleotides pool and NAD pool are relatively constant

## Citrate Synthase

- The first step, no allosteric regulation
- Rate regulated by oxaloacetate & citrate (inhibitor)
- **Malate-oxaloacetate** equilibrium has  $[ ] < K_m$ ,  $\text{NADH}/\text{NAD}^+$  (acetyl-CoA will be used for ketone bodies)

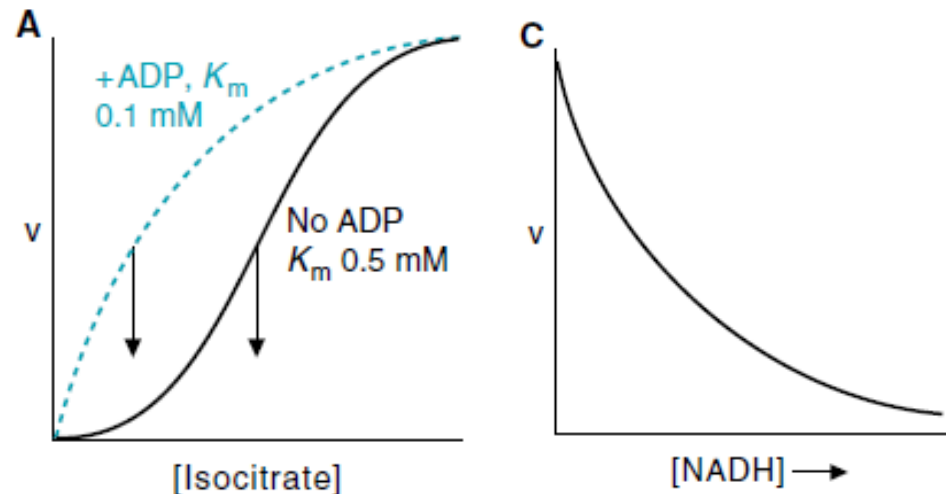
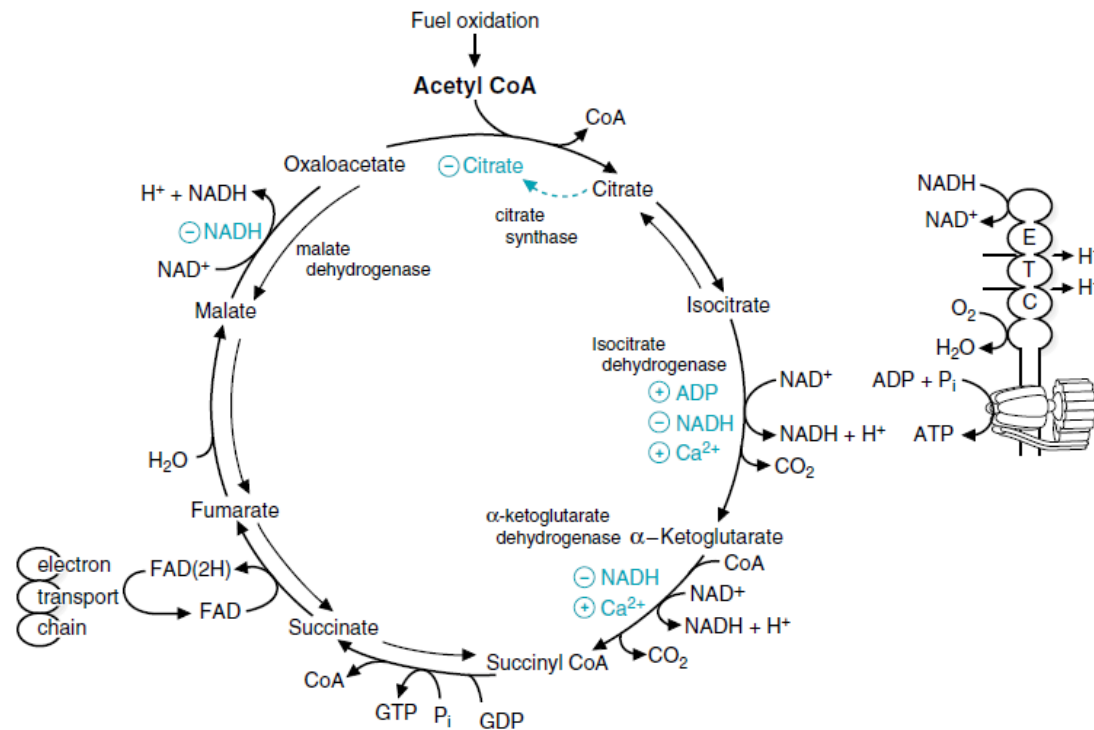


# Isocitrate DH

- Best regulation at rate-limiting step (Isocitrate DH)
- Allosterically: activated (ADP,  $\text{Ca}^{+2}$ )
- Inhibition (NADH)
- No ADP vs. ADP ( $K_m$  less), a small change in ADP, great effect

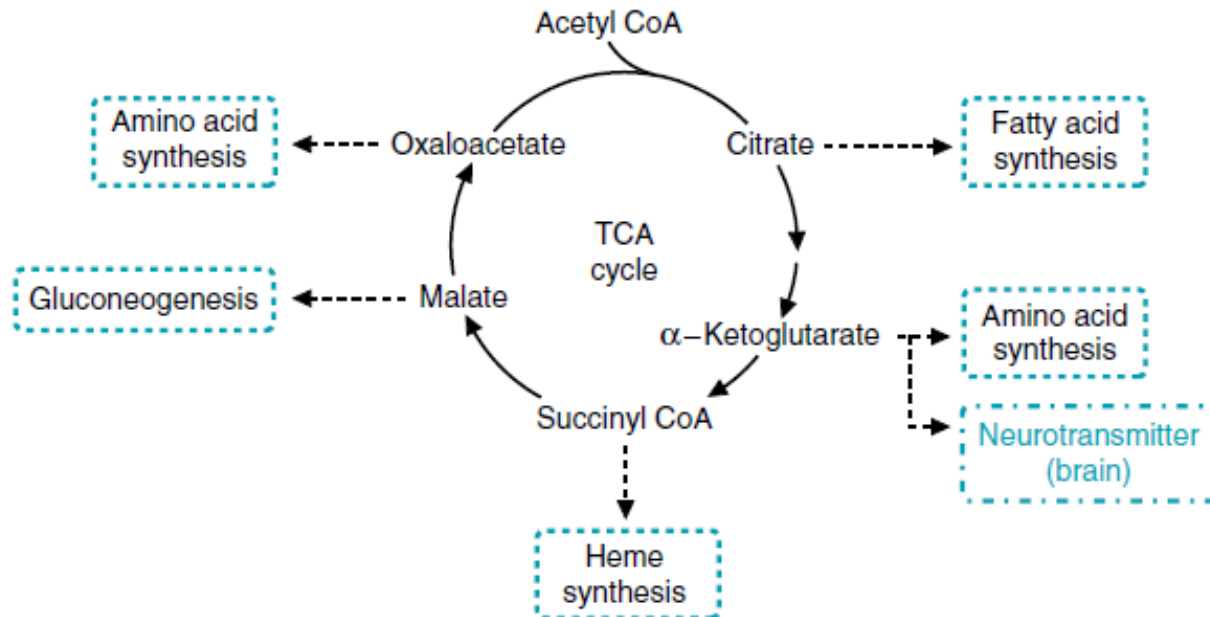
## $\alpha$ -Ketoglutarate DH

- Inhibited by NADH and succinyl CoA, GTP
- Activated by  $\text{Ca}^{+2}$ , muscle contraction



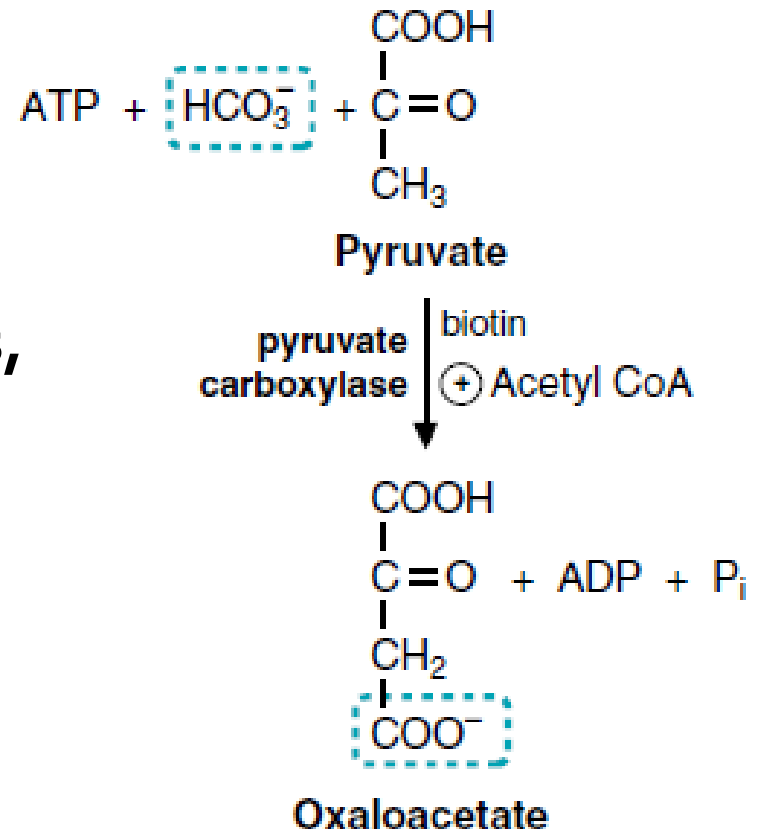
# TCA Cycle Intermediates

- Intermediates are Precursors for Biosynthetic Pathways (citrate, acetyl CoA, fatty acid synthesis, liver) (fasting, malate, gluconeogenesis, liver) (Succinyl CoA, heme biosynthesis, bone marrow) ( $\alpha$ -ketoglutarate, glutamate, GABA, a neurotransmitter, brain) ( $\alpha$ -ketoglutarate, glutamine, skeletal muscle to other tissues for protein synthesis)



# Anaplerotic Reactions

- Pathways or reactions that replenish the intermediates of the TCA cycle
- Pyruvate Carboxylase is a major anaplerotic enzyme (requires biotin)
- Found in many tissues, liver, kidneys, brain, adipocytes, and fibroblasts
- Very high conc. In liver and kidney (gluconeogenic pathway)
- Activated (acetyl CoA)



# Other Anaplerotic Routes (Amino Acid Degradation)

