

# BIOCHEMISTRY LECTURES(II)

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# LECTURE CONTENT

- **INTRODUCTION**
- **CITRIC ACID CYCLE (T.C.A)**
- **PRODUCTION OF ACETYL CoA**
- **REACTIONS OF THE CITIRIC ACID CYCLE**
- **THE AMPHIBOLIC NATURE OF THE T.C.A CYCLE**
- **THE GLYOXYLATE CYCLE**
- **THE T.C.A AND GLYOXYLATE CYCLE ARE COORDINATELY REGULATED**
- **THE ELECTRON TRANSPORT CHAIN**
- **THE MITOCHONDRIA AND ITS ANATOMY**
- **SEQUENCE OF ELECTRON TRANSPORT**
- **COMPONENTS OF THE ELECTRON TRANSPORT**
- **QUESTIONS AND COMMENTS**

# INTRODUCTION

- 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
- In the second stage, the acetyl groups are fed into the citric acid cycle, which enzymatically oxidizes them to  $\text{CO}_2$ ; the energy released is conserved in the reduced electron carriers  $\text{NADH}$  and  $\text{FADH}_2$
- In the third stage of respiration, these reduced coenzymes are themselves oxidized, giving up protons ( $\text{H}^+$ ) and electrons. The electrons are transferred to  $\text{O}_2$  the final electron acceptor via a chain of electron carrying molecules known as the respiratory chain

# Steps of Respiration

- Steps of respiration:

## 1. glycolysis

- cytosol

## Coenzyme Junction

## 2. Citric acid cycle

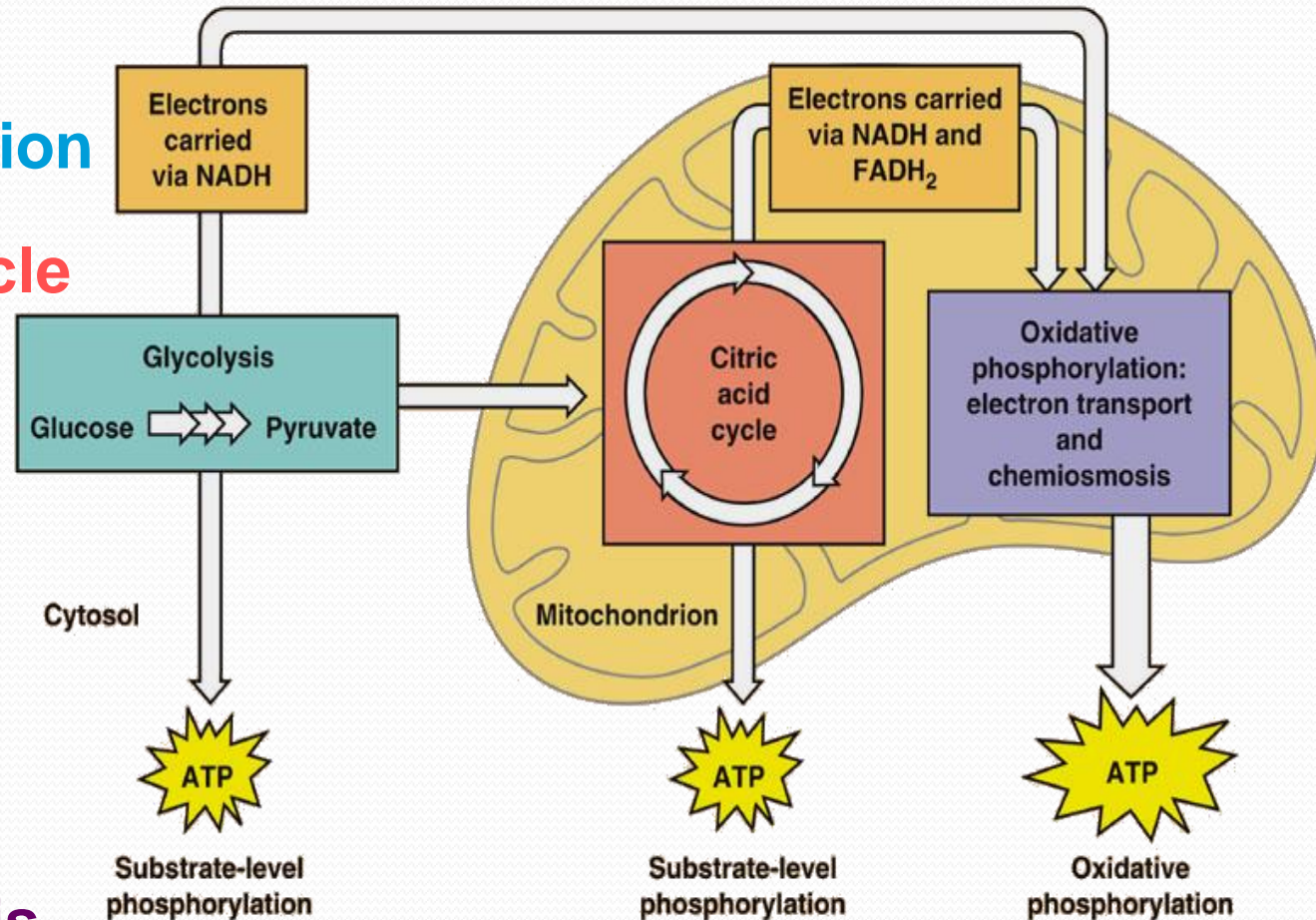
- mitochondrial matrix

## 3. ETC

- inner mitochondrial membrane

## 4. Chemiosmosis

- inner membrane to intermembrane space

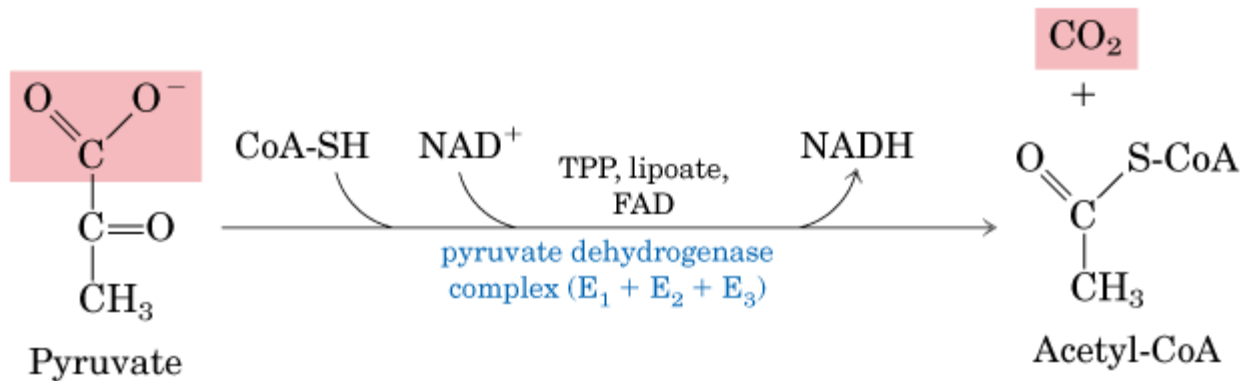


# THE CITRIC ACID CYCLE

- Citric acid cycle, also called the TRICARBOXYLIC ACID (T.C.A) cycle, or the KREBS cycle (after its discoverer, Hans Krebs)
- The Citric acid cycle is a HUB in metabolism, with degradative pathways leading in and anabolic pathways leading out
- It accounts for the major portion of carbohydrate, fatty acid and amino acid oxidation and generates numerous biosynthetic precursors
- the citric acid cycle is therefore AMPHIBOLIC, that is, it operates both catabolically and anabolically
- **The citric acid cycle enzymes are found in the matrix of the mitochondria**

# PRODUCTION OF ACETYL CoA

- Reaction of pyruvate dehydrogenase complex (PDC)
- 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
- Here we focus on how pyruvate, derived from glucose and other sugars by glycolysis, is oxidized to acetyl-coA by the **PYRUVATE DEHYDROGENASE COMPLEX**



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

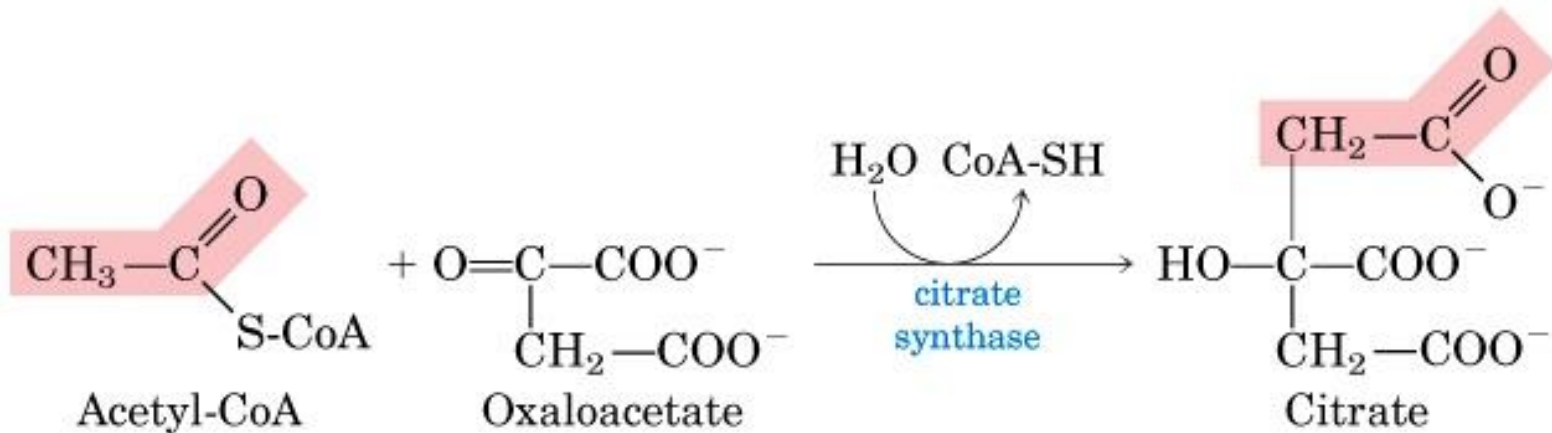
# PYRUVATE DEHYDROGENASE

- **Pyruvate dehydrogenase Complex (PDC)**
- **It is a multi-enzyme complex containing three enzymes associated together non-covalently:**
- **E-1 : Pyruvate dehydrogenase, uses Thiamine pyrophosphate as cofactor bound to E1**
- **E-2 : Dihydrolipoyl transacetylase, Lipoic acid bound, CoA as substrate**
- **E-3 : Dihydrolipoyl Dehydrogenase FAD bound, NAD<sup>+</sup> as substrate**
- **Advantages of multi-enzyme complex:**
  1. **Higher rate of reaction: Because product of one enzyme acts as a substrate of other, and is available for the active site of next enzyme without much diffusion.**
  2. **Minimum side reaction.**
  3. **Coordinated control.**

# REACTIONS OF THE T.C.A CYCLE

## 1. Citrate synthase: Formation of Citroyl CoA intermediate.

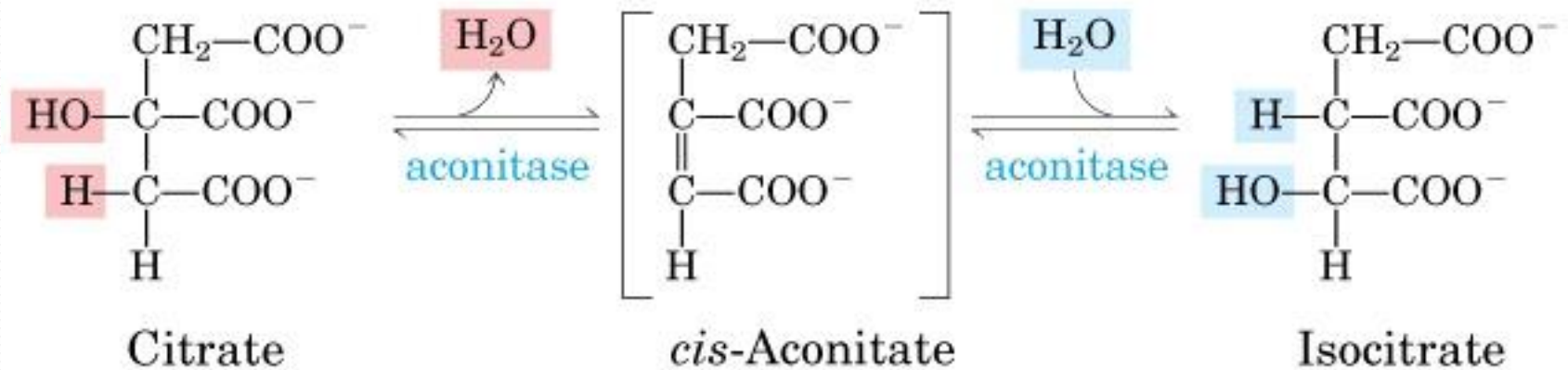
Binding of Oxaloacetate to the enzyme results in conformational change which facilitates the binding of the next substrate, the acetyl Coenzyme A. There is a further conformational change which leads to formation of products. This mechanism of reaction is referred to as induced fit model.



$$\Delta G'^{\circ} = -32.2 \text{ kJ/mol}$$

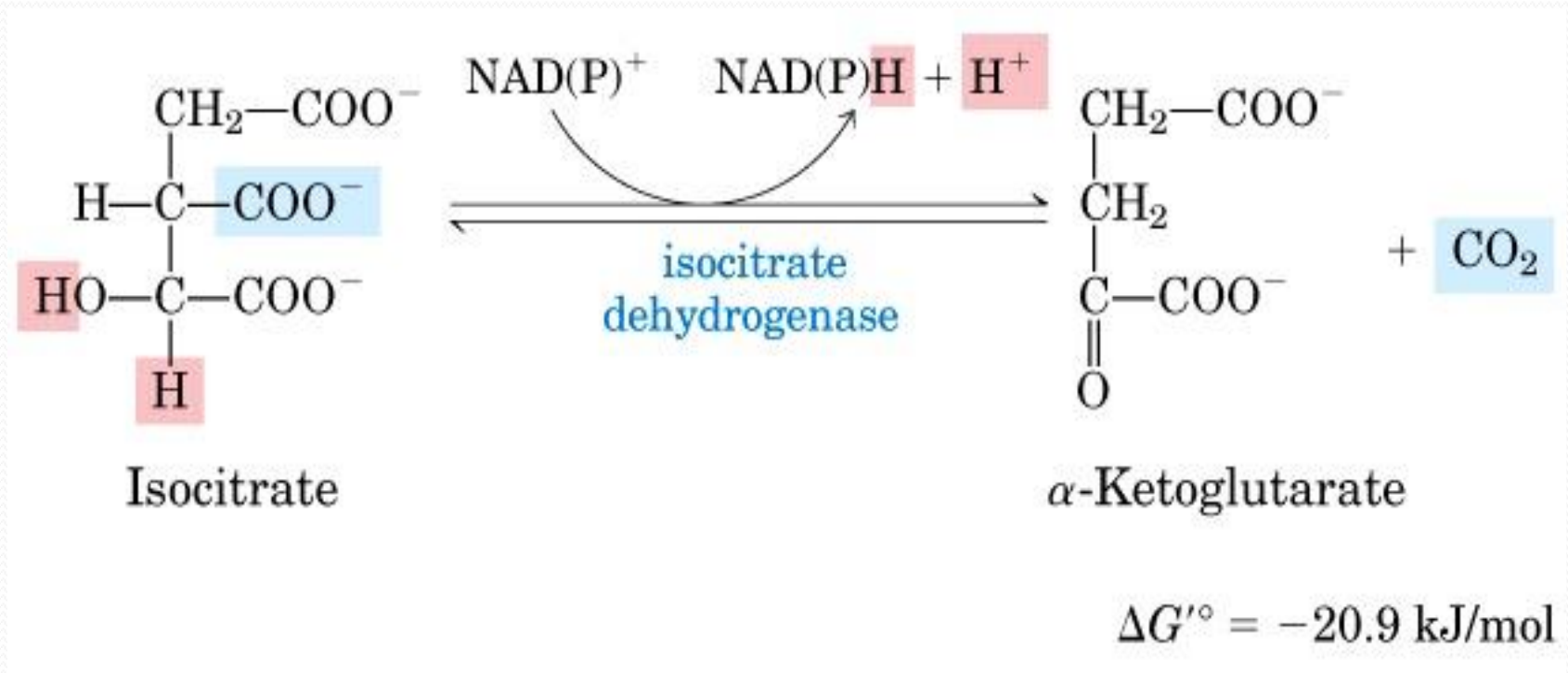


**2. Aconitase:** This enzyme catalyses the isomerization reaction by removing and then adding back the water ( H and OH ) to cis-aconitate in at different positions. Isocitrate is consumed rapidly by the next step thus driving the reaction in the forward direction.

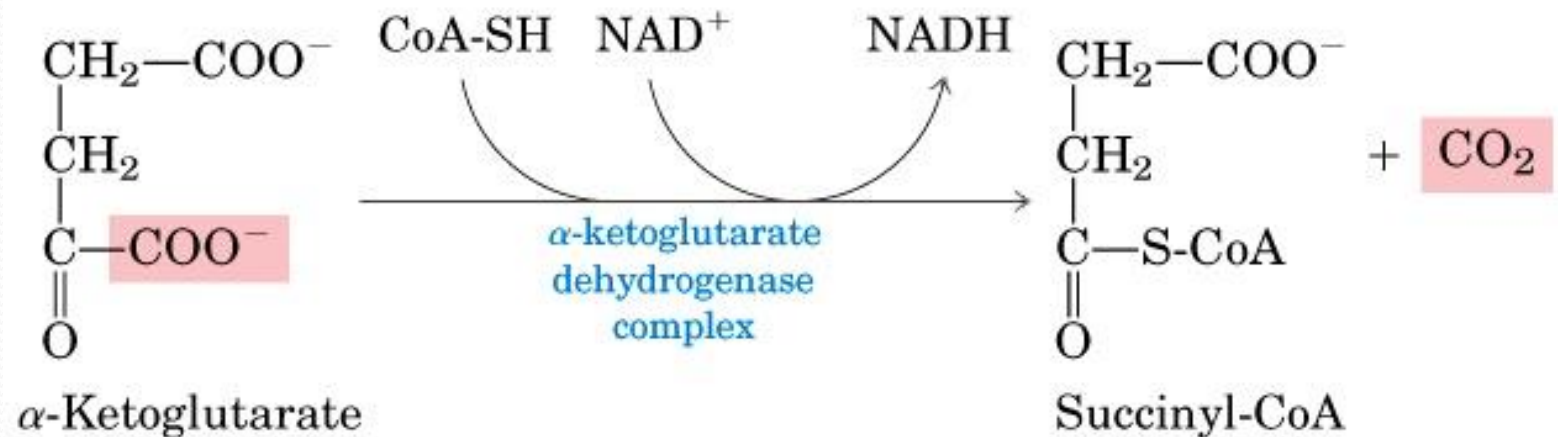


$$\Delta G'^{\circ} = 13.3 \text{ kJ/mol}$$

**3. Isocitrate dehydrogenase:** There are two isoforms of this enzyme, one uses  $\text{NAD}^+$  and other uses  $\text{NADP}^+$  as electron acceptor.

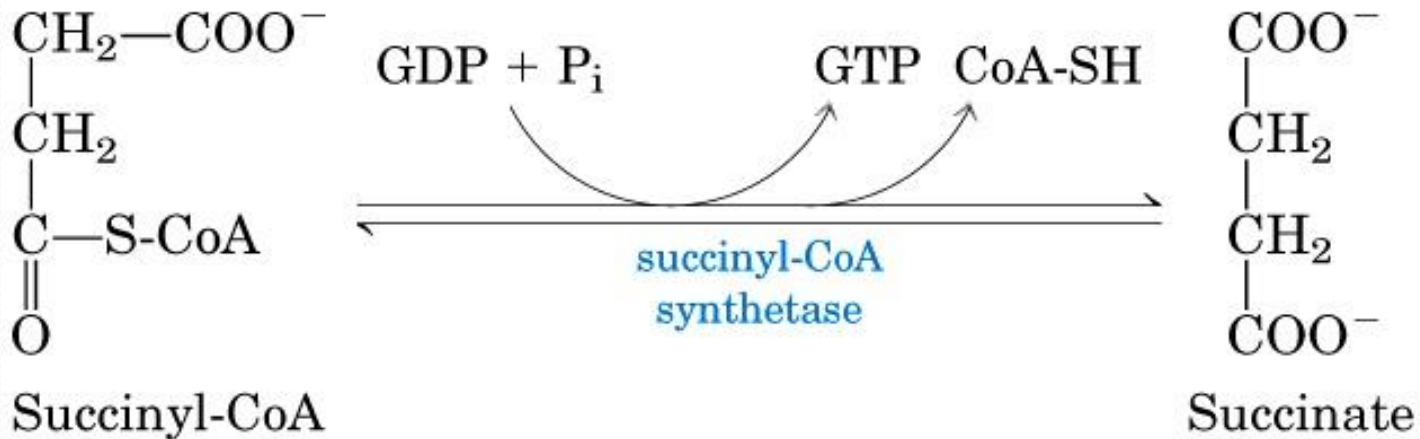


**4.  $\alpha$ -Ketoglutarate dehydrogenase:** This is a complex of different enzymatic activities similar to the pyruvate dehydrogenase complex. It has the same mechanism of reaction with E1, E2 and E3 enzyme units.  $\text{NAD}^+$  is an electron acceptor.



$$\Delta G'^{\circ} = -33.5 \text{ kJ/mol}$$

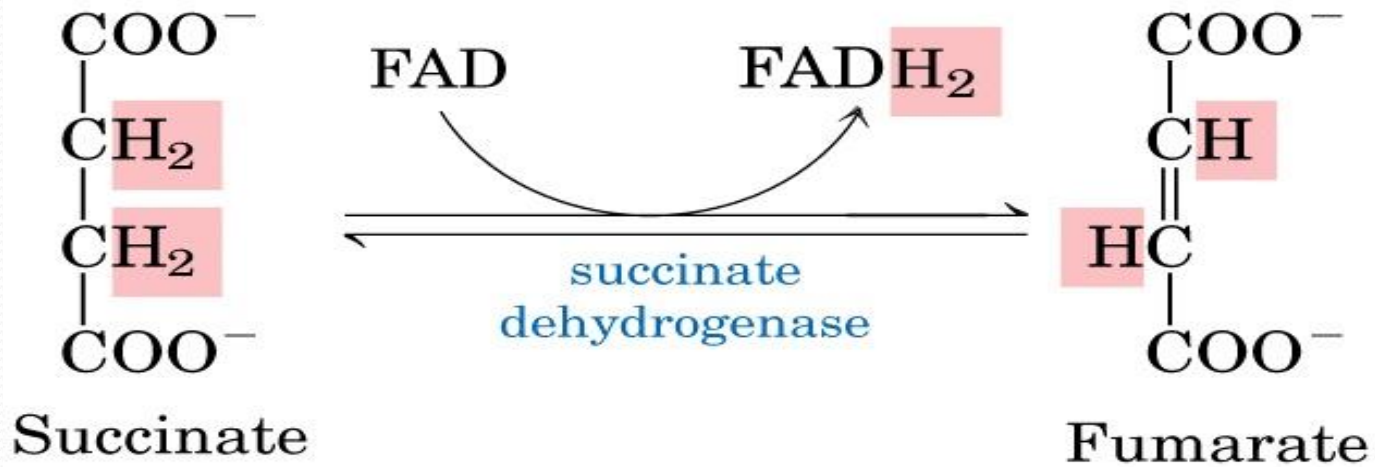
**5. Succinyl CoA synthetase:** Succinyl CoA, like Acetyl CoA has a thioester bond with very negative free energy of hydrolysis. In this reaction, the hydrolysis of the thioester bond leads to the formation of phosphoester bond with inorganic phosphate. This phosphate is transferred to Histidine residue of the enzyme and this high energy, unstable phosphate is finally transferred to GDP resulting in the generation of GTP.



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

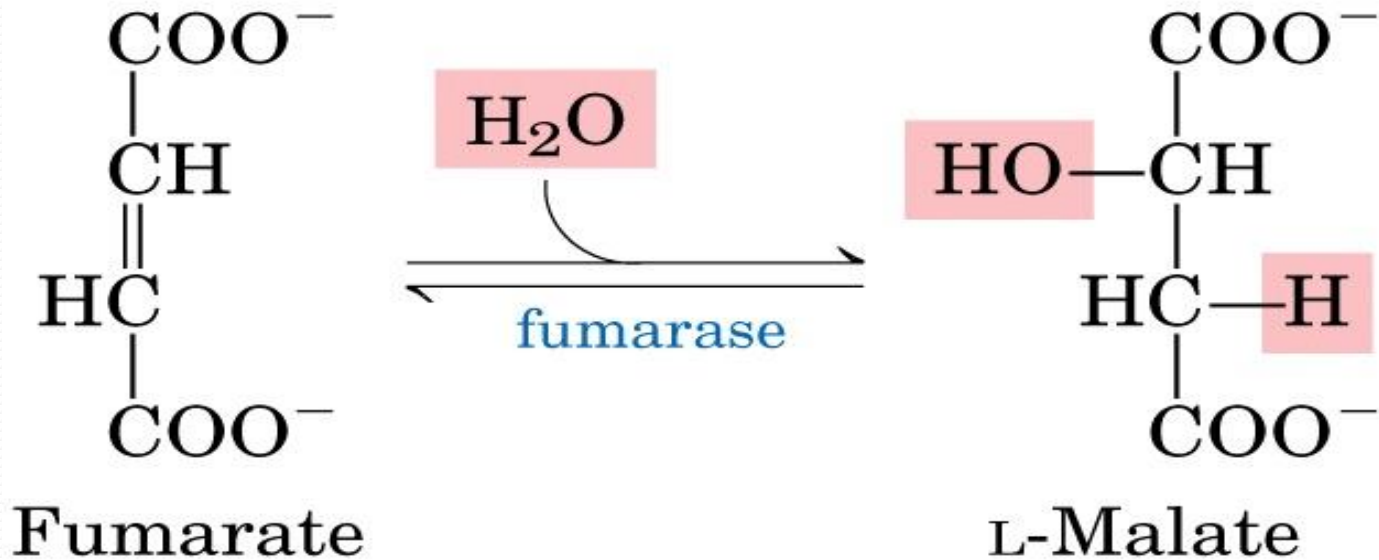
**6. Succinate Dehydrogenase:** Oxidation of succinate to fumarate. This is the only citric acid cycle enzyme that is tightly bound to the inner mitochondrial membrane. It is an FAD dependent enzyme.

Malonate has similar structure to Succinate, and it competitively inhibits Succinate Dehydrogenase.



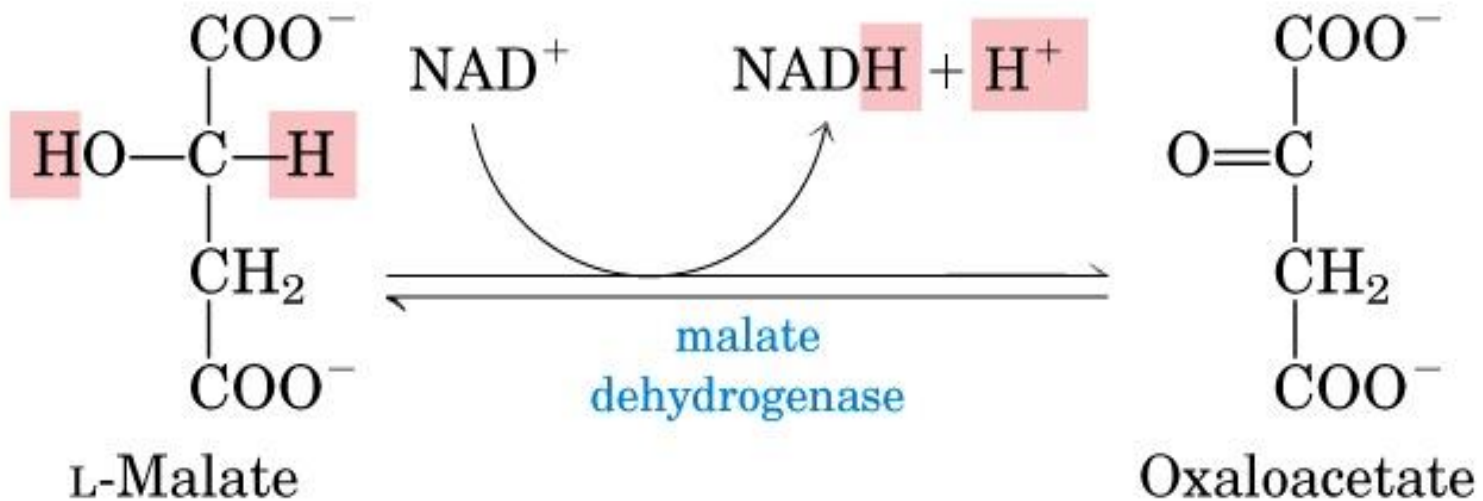
$$\Delta G'^{\circ} = 0 \text{ kJ/mol}$$

**7. Fumarase:** Hydration of Fumarate to malate: It is a highly stereospecific enzyme. *cis*-Maleate (the *cis* form of fumarate is not recognized by this enzyme.

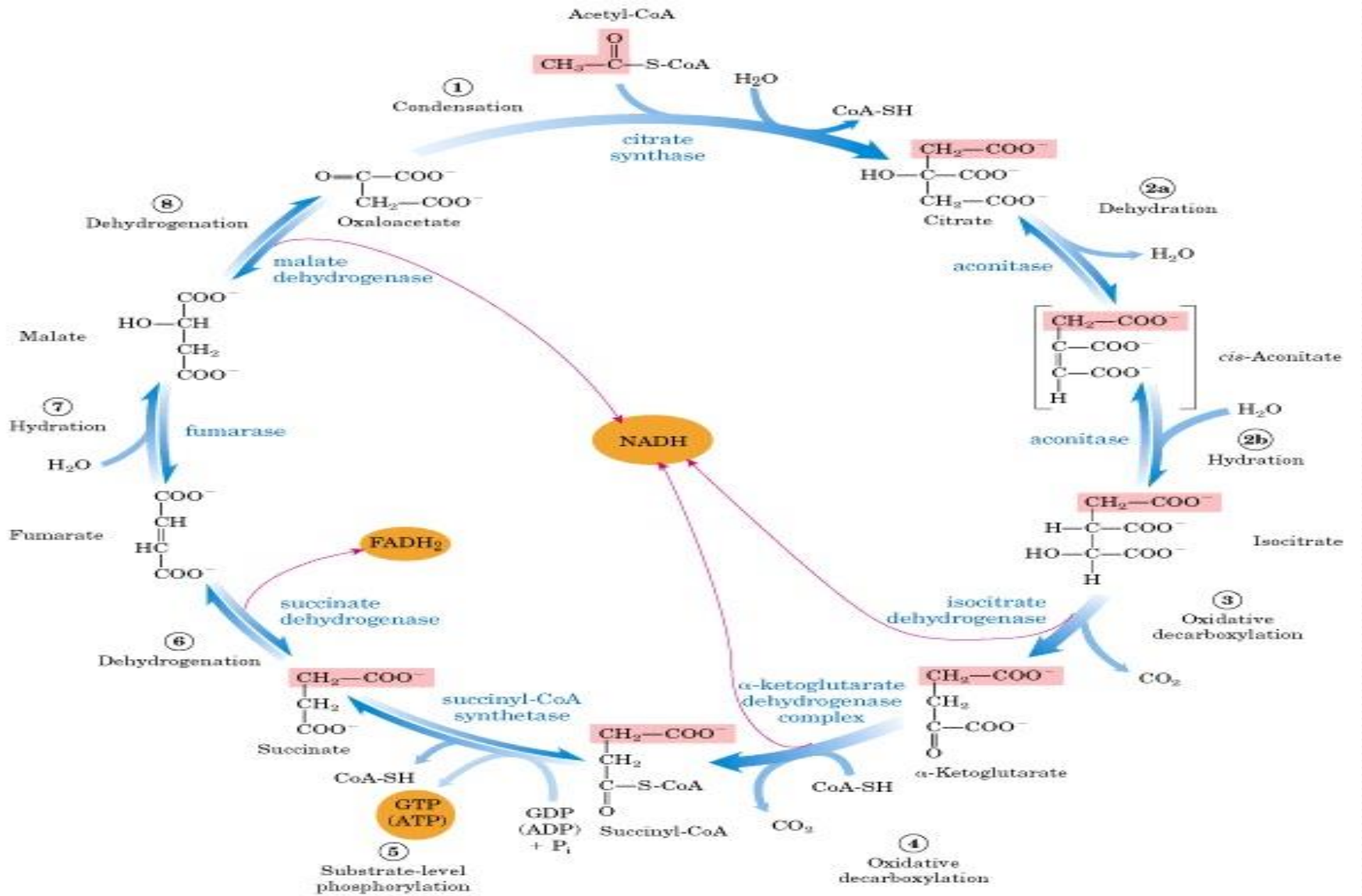


$$\Delta G'^{\circ} = -3.8 \text{ kJ/mol}$$

**8. L-Malate dehydrogenase: Oxidation of malate to oxaloacetate:** It is an  $\text{NAD}^+$  dependent enzyme. Reaction is pulled in forward direction by the next reaction (citrate synthase reaction) as the oxaloacetate is depleted at a very fast rate.



$$\Delta G'^{\circ} = 29.7 \text{ kJ/mol}$$





# REGULATION OF THE T.C.A CYCLE

The regulation of key enzymes in metabolic pathways by **ALLOSTERIC EFFECTORS** and **COVALENT MODIFICATION** ensures the production intermediates at the rate required to keep the cell in a stable steady state while avoiding wasteful overproduction . The key enzymes are:

## (1) PYRUVATE DEHYDROGENASE:

The activators are AMP, CoA, NAD<sup>+</sup>, The inhibitors are ATP, Acetyl-CoA, NADH and Fatty acids

## (2) CITRATE SYNTHASE:

The activator is ADP, The inhibitors are NADH, succinyl-CoA, Citrate and ATP

## (3) ISOCITRATE DEHYDROGENASE:

The activators are Ca<sup>2+</sup> and ADP. The inhibitor is ATP

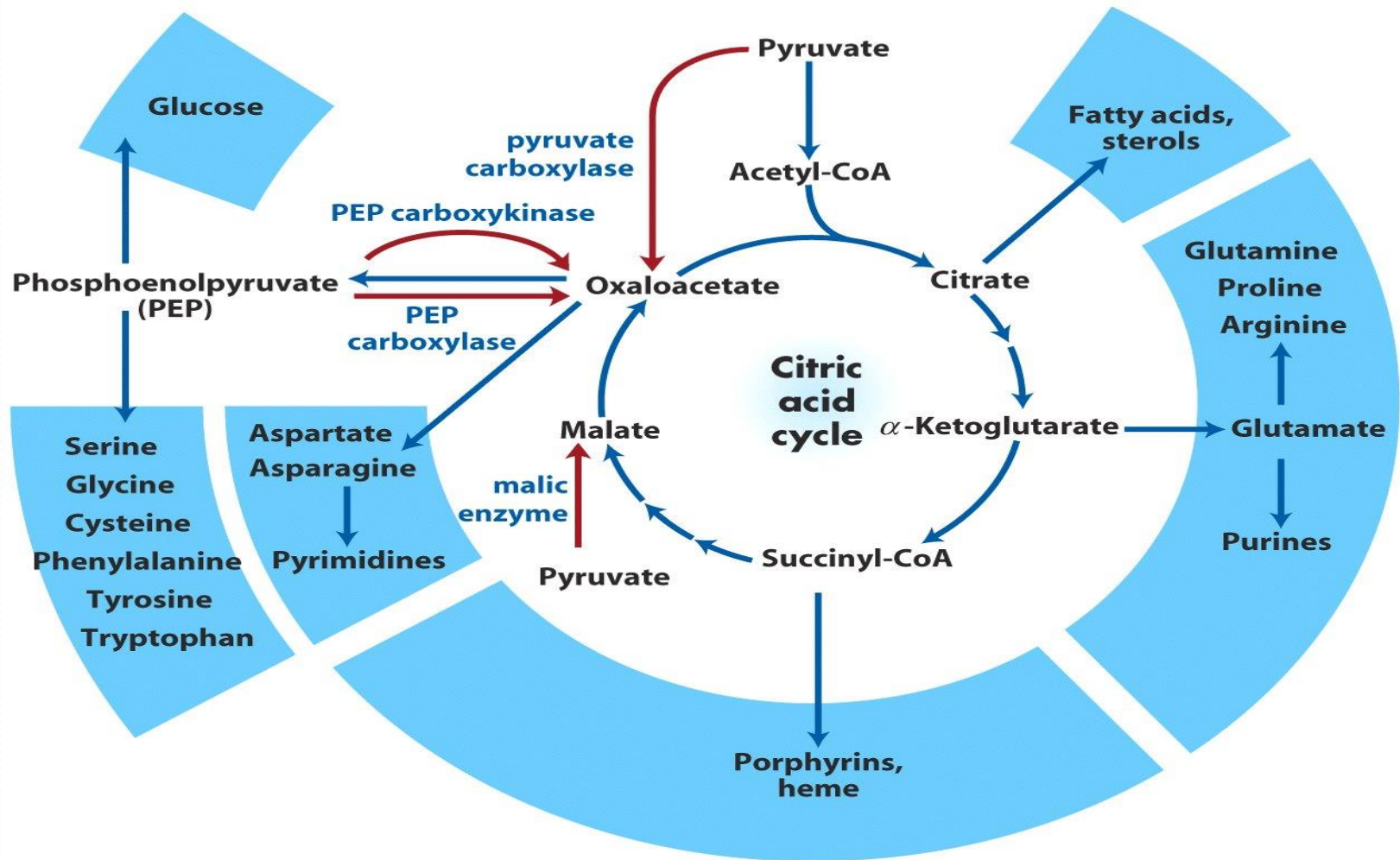
## (4) $\alpha$ -KETOGLUTARATE DEHYDROGENASE COMPLEX:

The activator is Ca<sup>2+</sup>. The inhibitors are succinyl-CoA and NADH

The flow of metabolites through the T.C.A cycle is under stringent regulations.

Three factors govern the flux through the cycle. (1) Substrate availability (2) inhibition by accumulating products and (3) allosteric inhibition of key enzymes

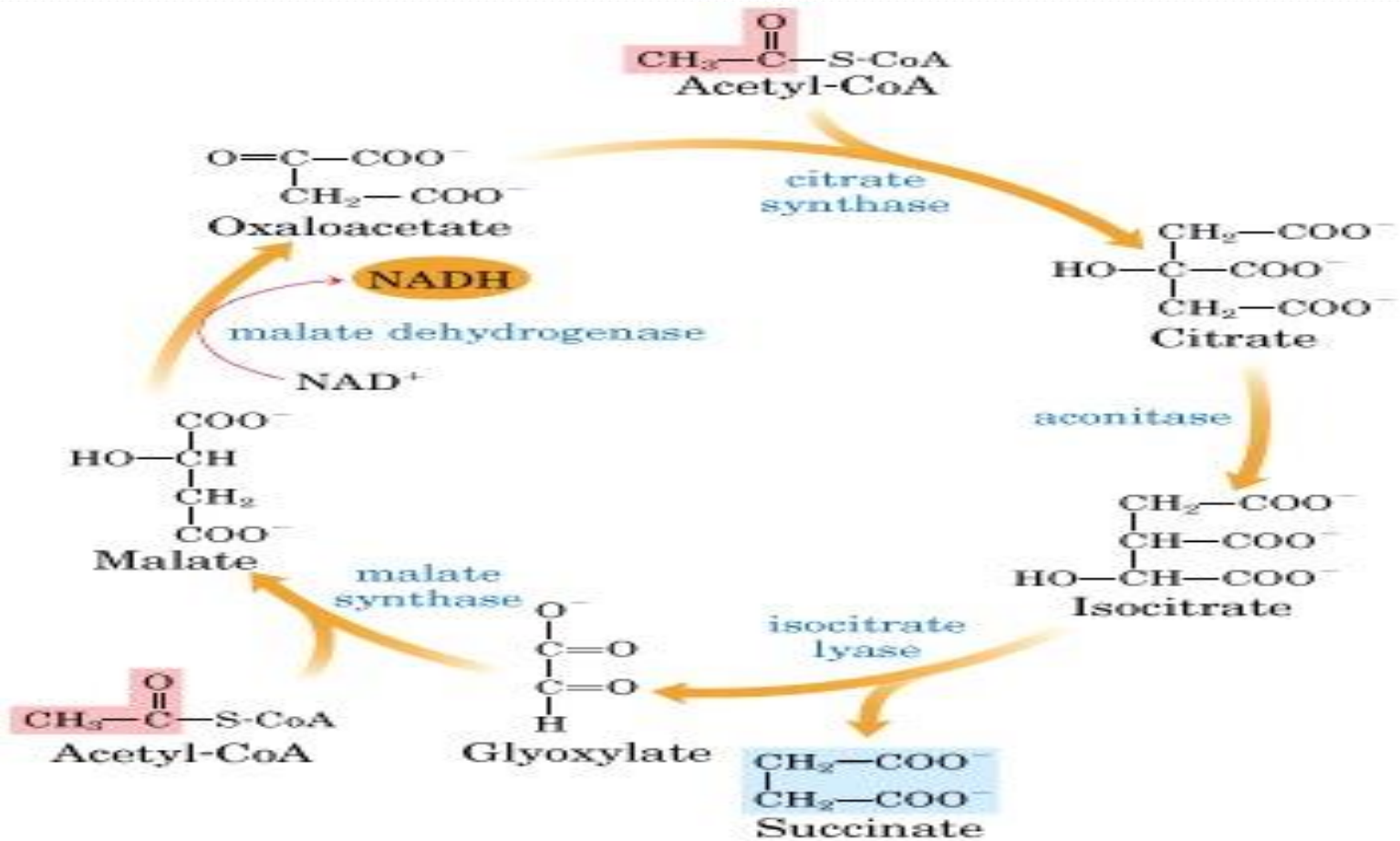
# THE AMPHIBOLIC NATURE OF THE T.C.A CYCLE



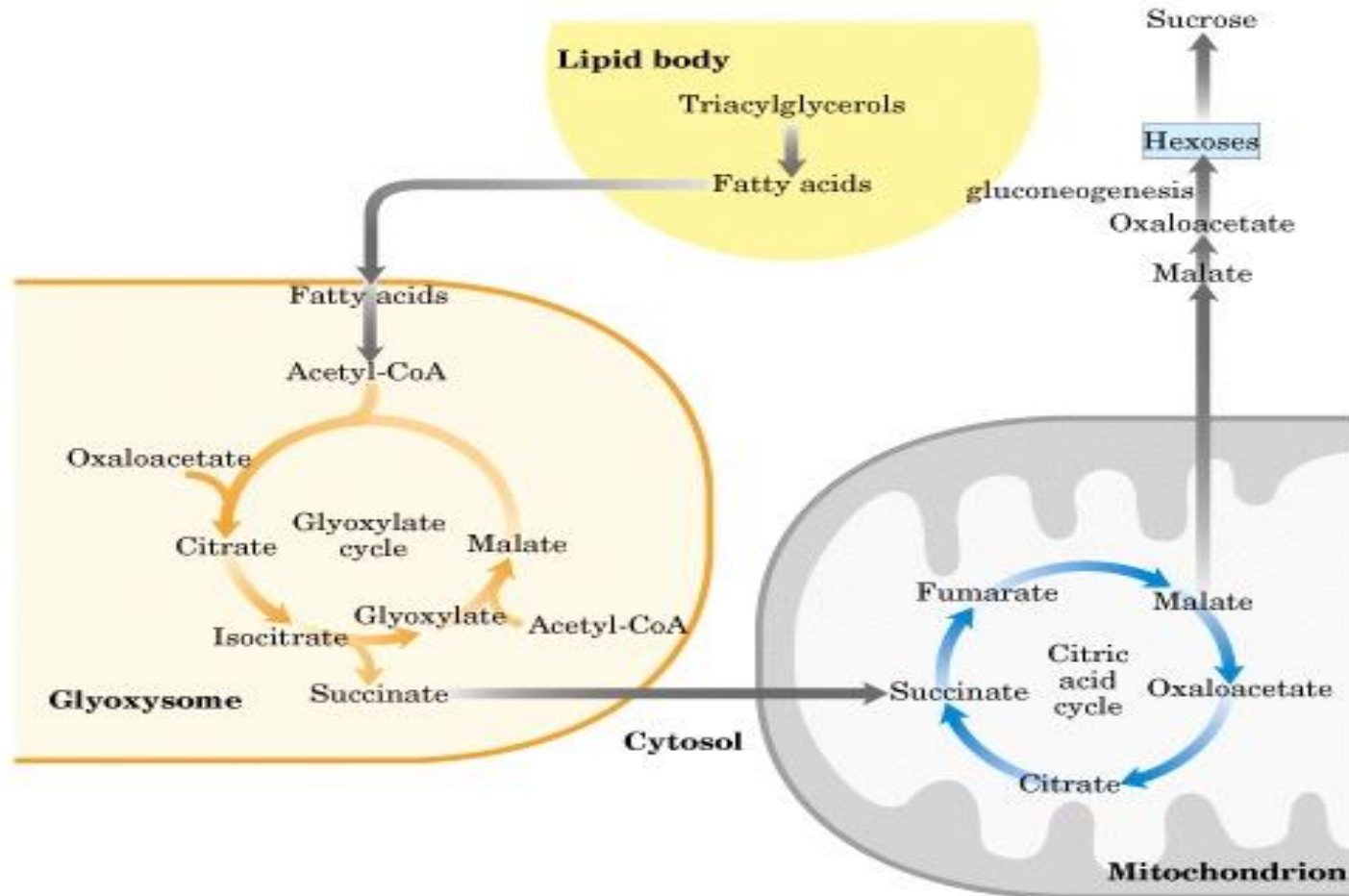
# GLYOXYLATE CYCLE

- **Vertebrates cannot convert fatty acids, or the acetate derived from them to carbohydrates**
- **Conversion of PEP and pyruvate to acetyl-CoA are so exergonic to be essentially irreversible . If a cell cannot convert acetate into PEP, acetate cannot serve as the starting material for the gluconeogenic pathway which leads from PEP to glucose**
- **In plants, certain invertebrates and some microorganism acetate can serve both as an energy rich fuel and as a source of PEP for carbohydrates**
- **In these organisms, enzymes of the GLYOXYLATE CYCLE Catalyze the net conversion of acetate to succinate**
- **In plants, the enzymes of the glyoxylate cycle are sequestered in membrane-bounded organelles called GLYOXYSOME**
- **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**

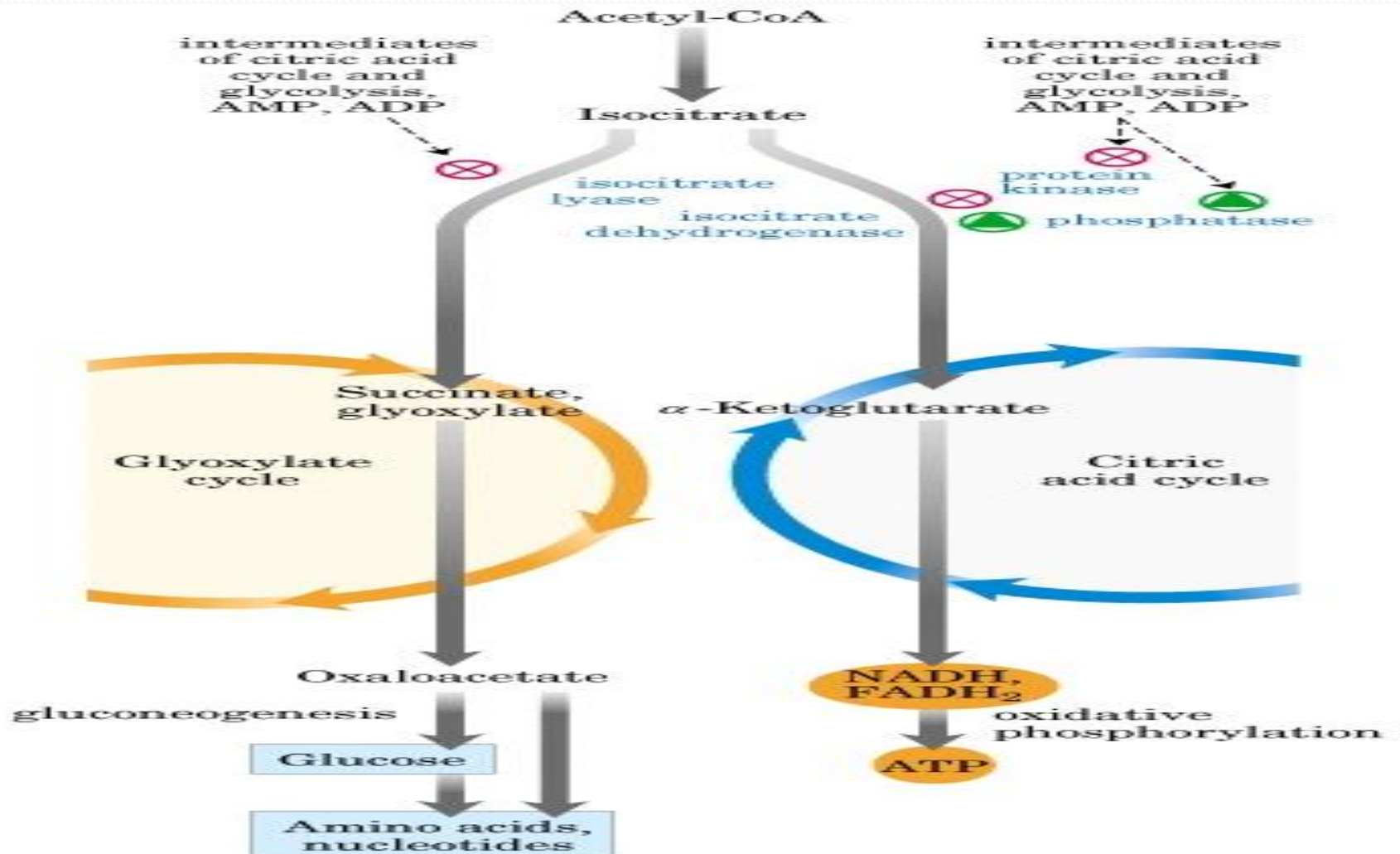
# GLYOXYLATE CYCLE



# GLYOXYLATE CYCLE CONT'D

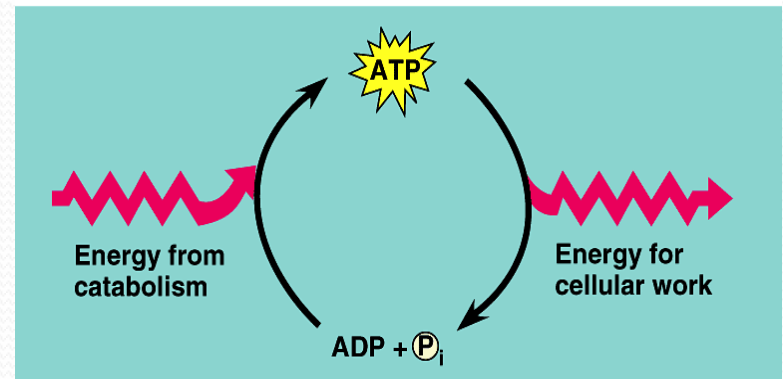
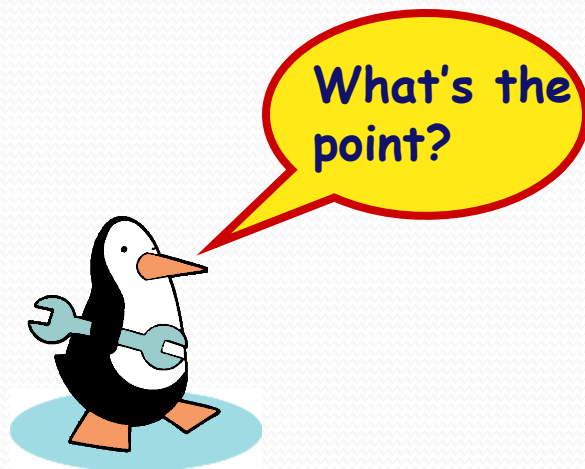


# THE T.C.A CYCLE AND GLYOXYLATE CYCLES ARE COORDINATELY REGULATED



# ATP accounting so far...

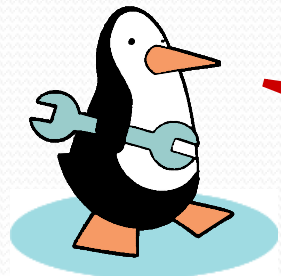
- Glycolysis → **2 ATP**
- Krebs's cycle → **2 ATP**
- Life takes a lot of energy to run, need to extract more energy than **4 ATP!**
- **There's got to be a better way!**



A working muscle recycles over 10 million ATPs per second

# THERE IS A BETTER WAY!

- **Electron Transport Chain**
  - series of molecules built into inner mitochondrial membrane
    - along cristae
    - transport proteins & enzymes
  - transport of electrons down ETC linked to pumping of  $H^+$  to create  $H^+$  gradient
  - yields ~34 ATP from 1 glucose!
  - only in presence of  $O_2$  (aerobic respiration)



That  
sounds more  
like it!





# THE ELECTRON TRANSPORT CHAIN

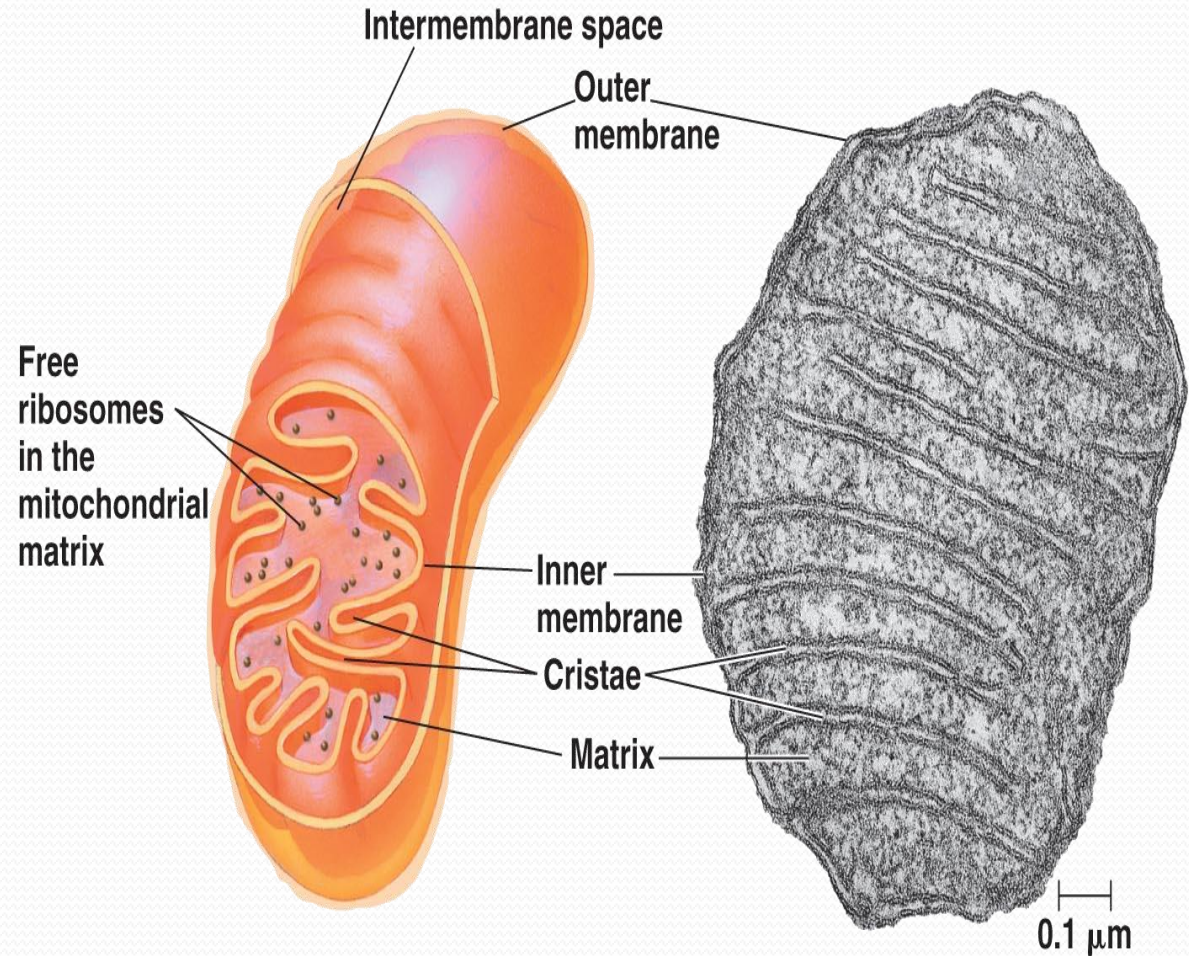
- The 12 electron pairs involved in glucose oxidation are not transferred directly to  $O_2$ .
- Rather, as we have seen, they are transferred to the coenzymes  $NAD^+$  and  $FAD$  to form  $10NADH$  and  $2FADH_2$
- In the reactions catalyzed by the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase, pyruvate dehydrogenase and the citric acid enzymes :isocitrate dehydrogenase,  $\alpha$ -ketoglutarate dehydrogenase, succinate dehydrogenase (the only  $FAD$  reduction) and malate dehydrogenase
- The electrons then pass into the **ELECTRON-TRANSPORT CHAIN**, where through re-oxidation of  $NADH$  and  $FADH_2$ , they participate in the sequential oxidation-reduction of over 10 redox centers before reducing  $O_2$  to  $H_2O$

# THE MITOCHONDRION

- The mitochondria vary considerably in size and shape depending on their source and metabolic state
- The mitochondria is the site of eukaryotic oxidative metabolism
- It contains the enzymes that mediate this process, including pyruvate dehydrogenase, the citric acid cycle enzymes, the enzymes catalyzing fatty acid oxidation and the enzymes and redox proteins involved in electron transport and oxidative phosphorylation
- It is therefore with good reasons that the mitochondrion is described as the cell's “power plant”

# THE MITOCHONDRIAL ANATOMY

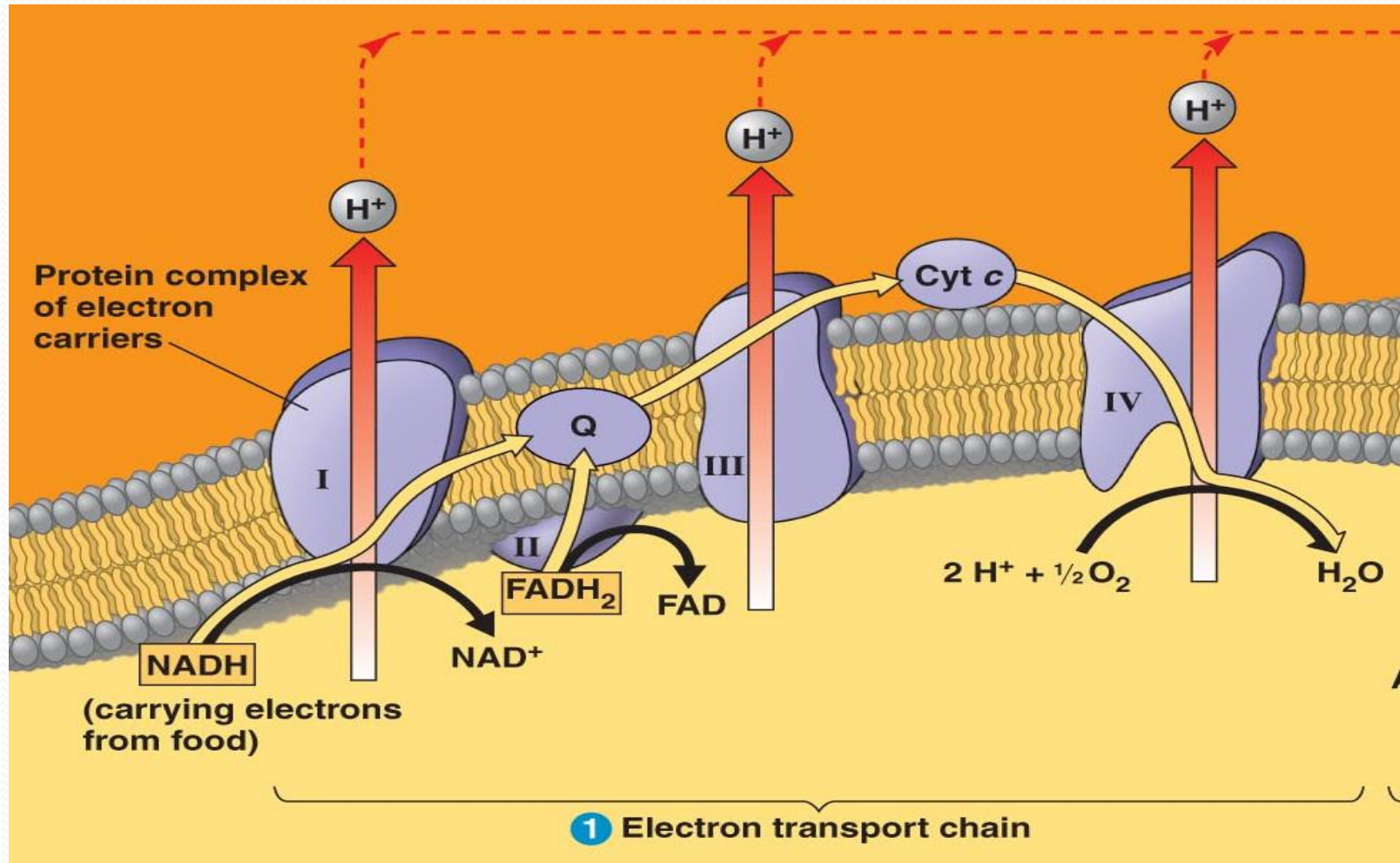
- Double membrane
  - outer membrane
  - inner membrane
    - highly folded cristae
    - enzymes & transport proteins
  - intermembrane space
    - fluid-filled space between membranes



# SEQUENCE OF ELECTRON TRANSPORT

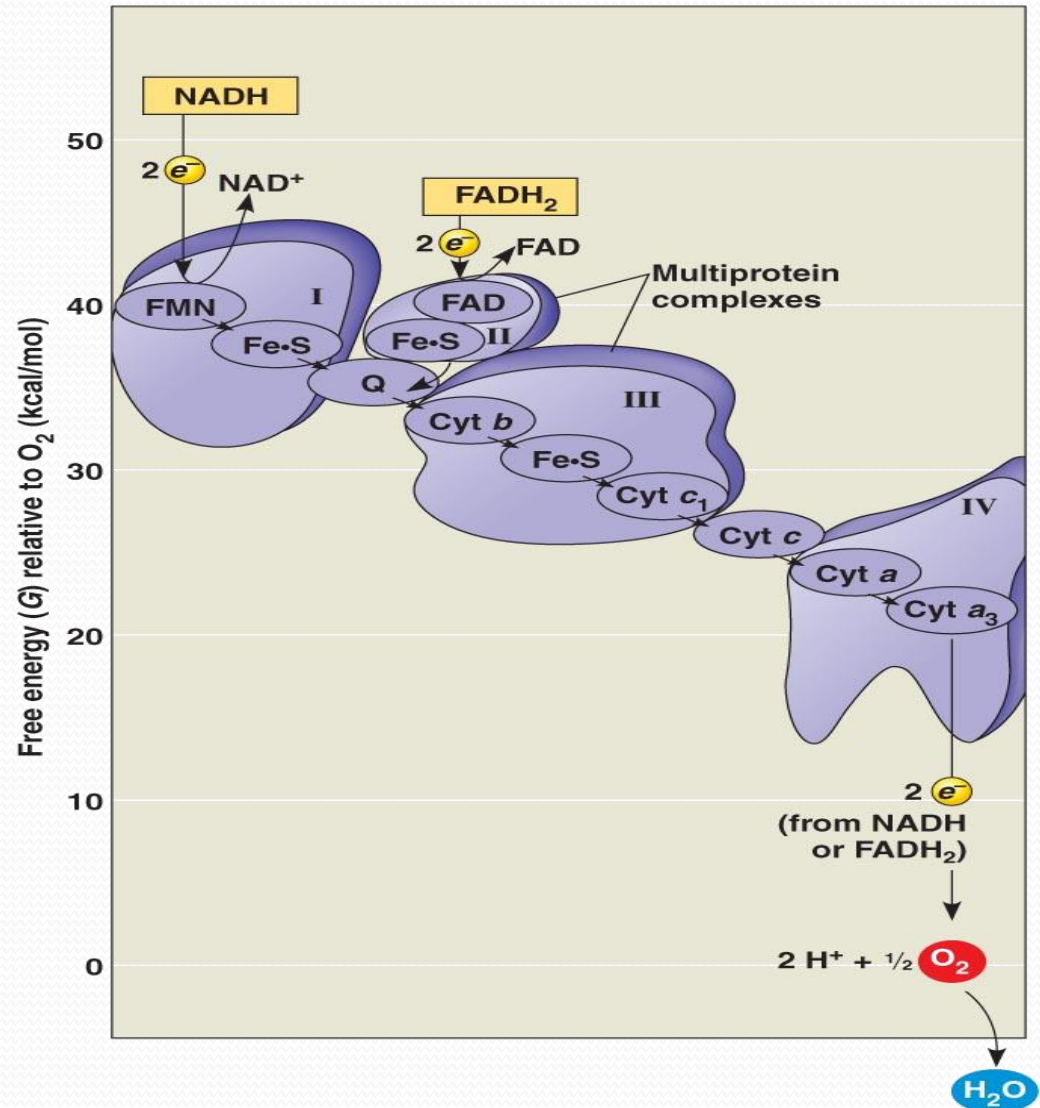
- The free energy necessary to generate ATP is extracted from the oxidation of NADH and FADH<sub>2</sub> by the electron-transport chain, a series of four protein complexes through which electrons pass from lower to higher standard reduction potentials.
- Electrons are carried from complexes I and II to complex III by coenzyme Q (CoQ or Ubiquinone) and from complex III to complex IV by the peripheral membrane protein Cytochrome c

# ELECTRON TRANSPORT CHAIN



# COMPONENTS OF THE ELECTRON TRANSPORT CHAIN

- Complex I (NADH: Coenzyme Q Oxidoreductase)
- Complex II (Succinate: coenzyme Q oxidoreductase)
- Complex III (Coenzyme Q: cytochrome c Oxidoreductase or cytochrome bc1 complex)
- Complex IV (cytochrome c Oxidase)

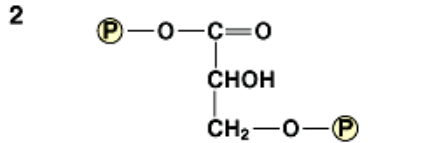
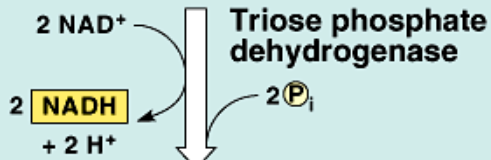


# REMEMBER THE ELECTRON CARRIERS?

## Glycolysis

glucose

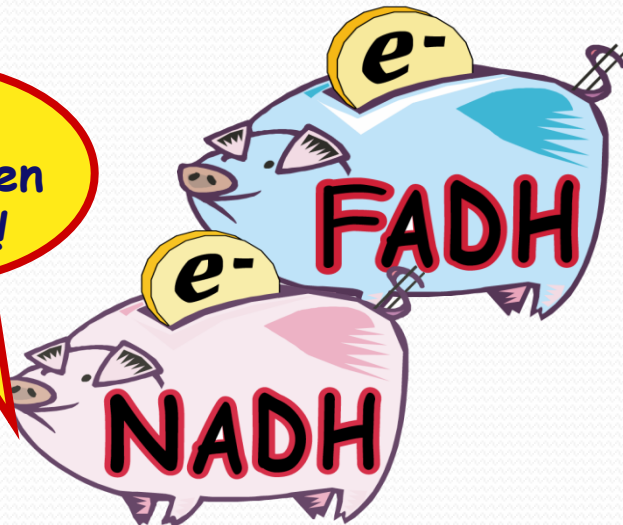
G3P



1, 3-Bisphosphoglycerate

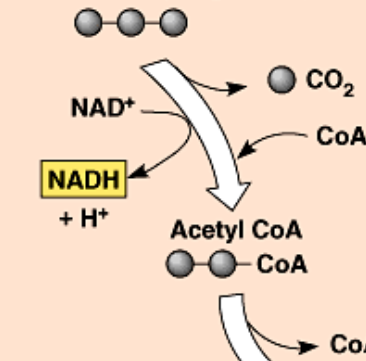
2 NADH

Time to break open the bank!

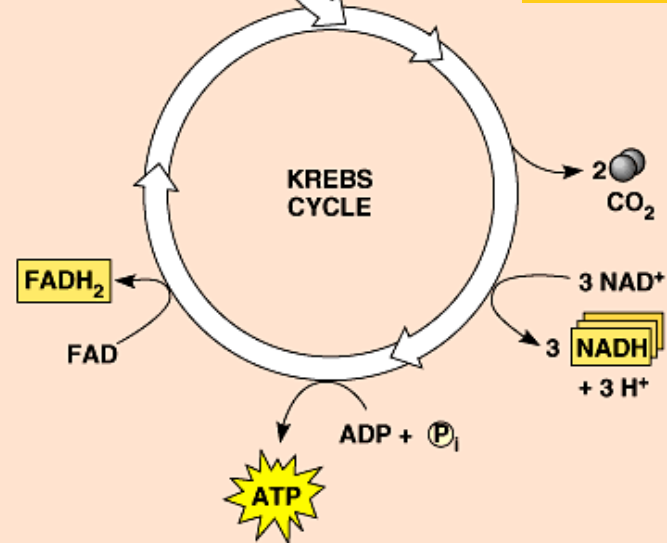


## Krebs cycle

Pyruvate (from glycolysis, 2 molecules per glucose)



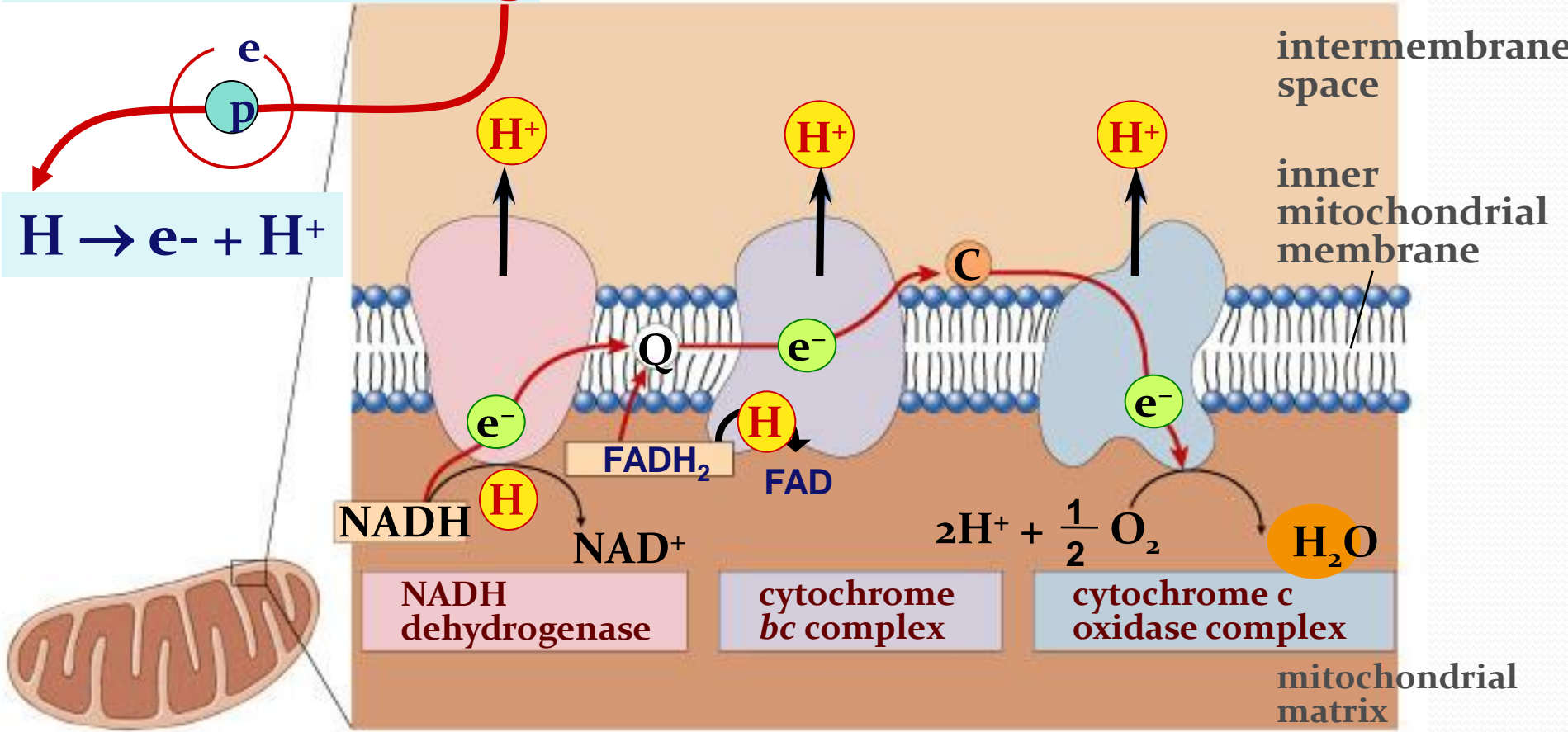
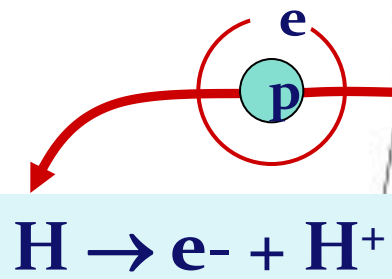
8 NADH  
2 FADH<sub>2</sub>



# ELECTRON TRANSPORT CHAIN



**Building proton gradient!**

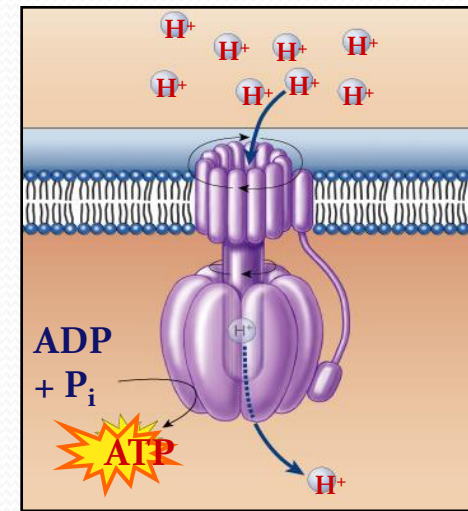
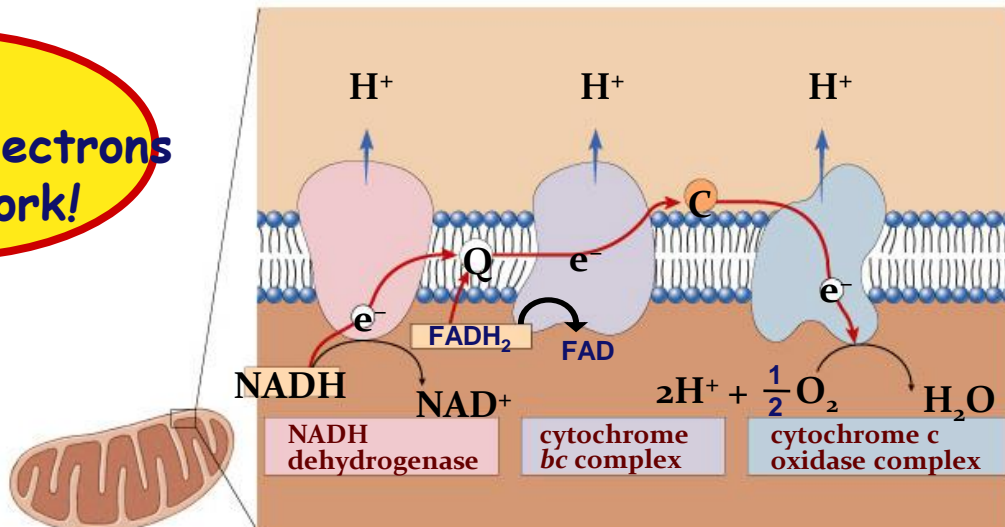
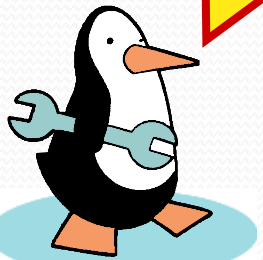




# STRIPPING H FROM ELECTRON CARRIERS

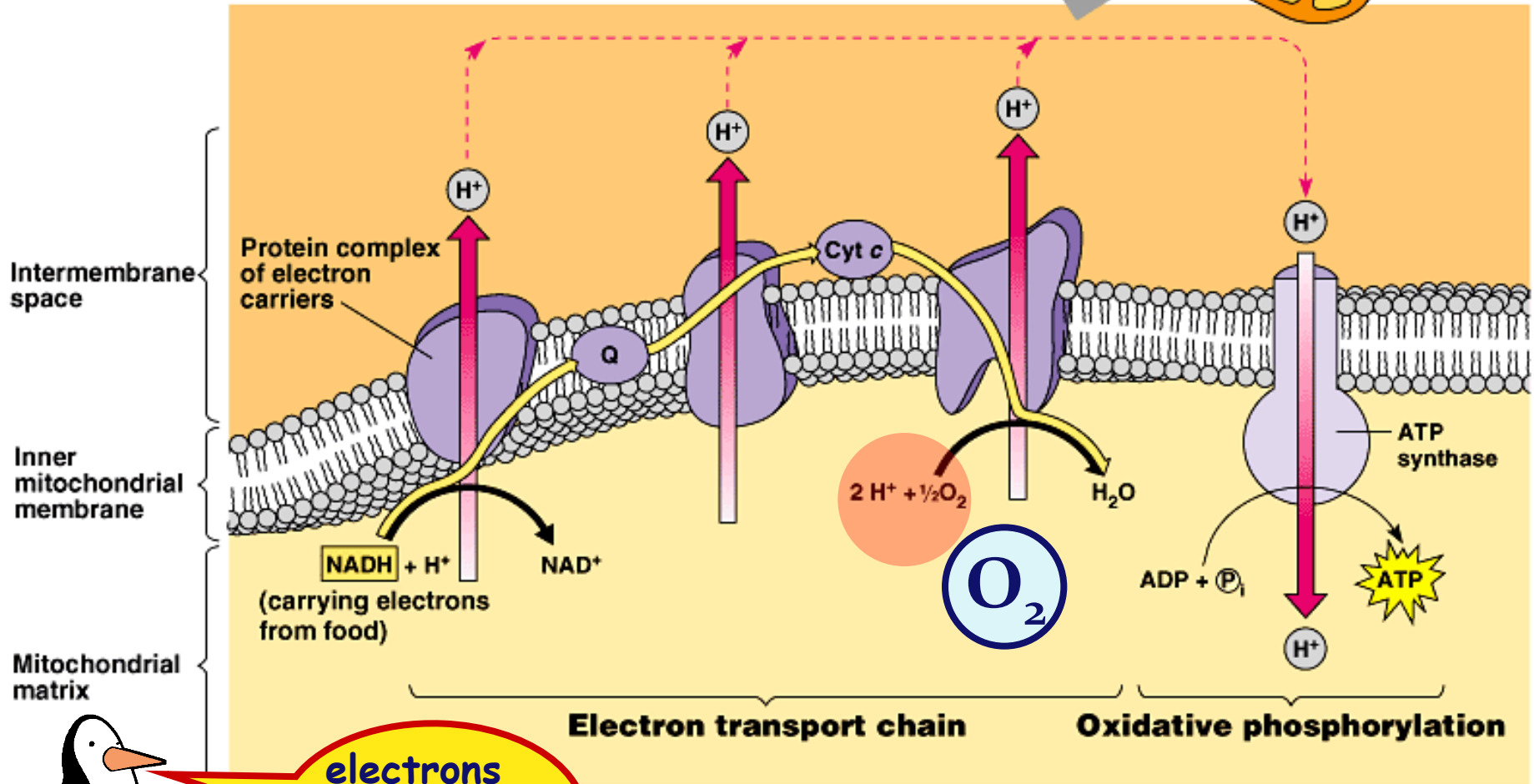
- NADH passes electrons to ETC
  - H cleaved off NADH & FADH<sub>2</sub>
  - electrons stripped from H atoms → H<sup>+</sup> (protons)
  - electrons passed from one electron carrier to next in mitochondrial membrane (ETC)
  - transport proteins in membrane pump H<sup>+</sup> (protons) across inner membrane to intermembrane space

TA-DA!!  
Moving electrons  
do the work!



# But what “pulls” the electrons down the ETC?

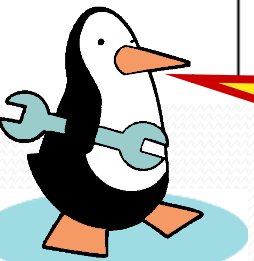
Inner mitochondrial membrane



Mitochondrial matrix

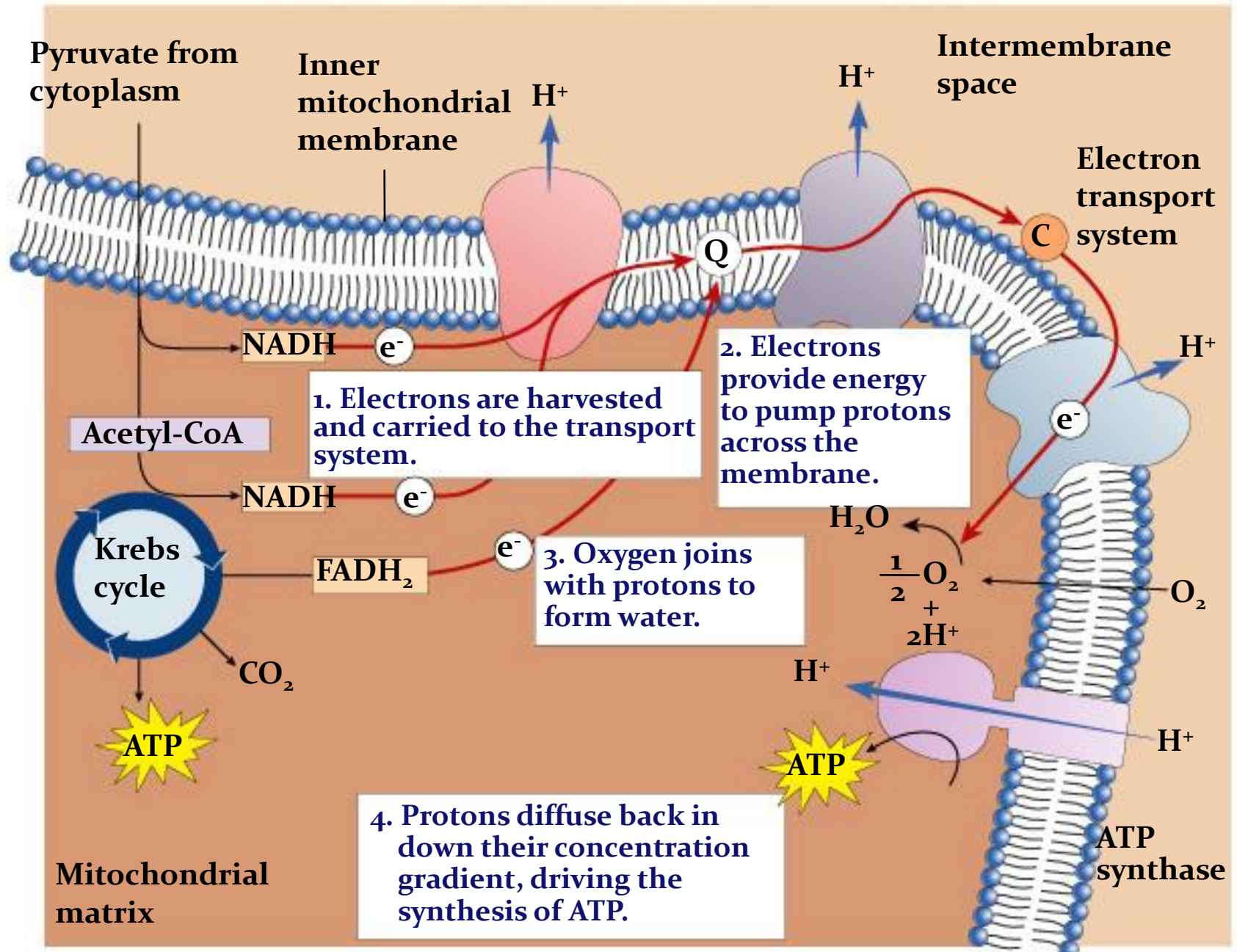
Intermembrane space

Inner mitochondrial membrane



electrons flow downhill to O<sub>2</sub>

# oxidative phosphorylation



## Summary of cellular respiration



- Where did the glucose come from?
- Where did the  $\text{O}_2$  come from?
- Where did the  $\text{CO}_2$  come from?
- Where did the  $\text{CO}_2$  go?
- Where did the  $\text{H}_2\text{O}$  come from?
- Where did the ATP come from?
- What else is produced that is not listed in this equation?
- Why do we breathe?