BIOCHEMISTRY LECTURES(II)

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LECTURE CONTENT

- **INTRODUCTION**
- **CITRIC ACID CYCLE (T.C.A)**
- **PRODUCTION OF ACETYL CoA**
- **REACTIONS OF THE CITIRC 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 Co2; the energy released is conserved in the reduced electron carriers NADH and FADH2**
- **In the third stage of respiration, these reduced coenzymes are themselves oxidized, giving up protons (H+) and electrons. The electrons are transferred to o2 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

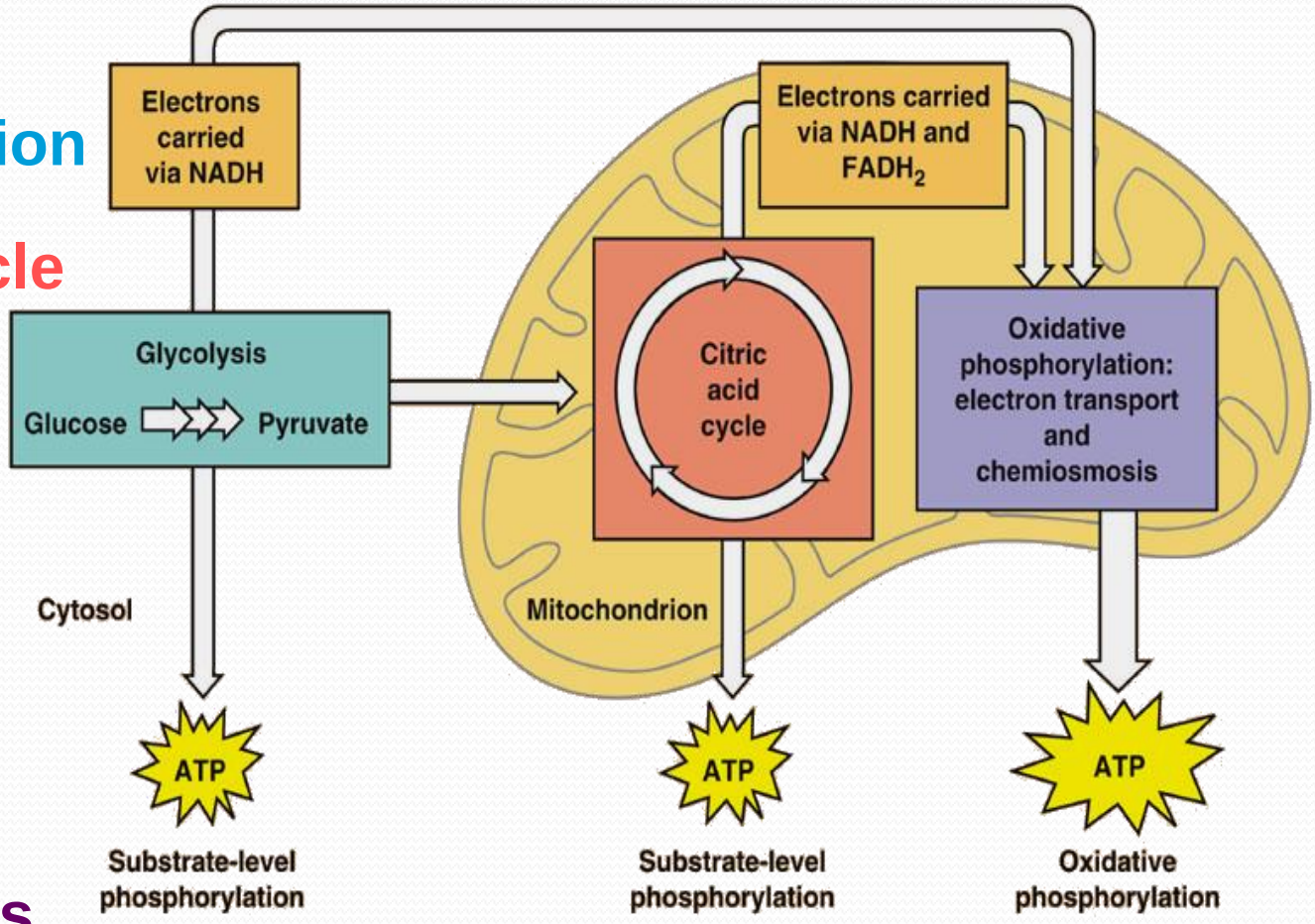
- mitochondrial matrix

3. ETC

- inner mitochondrial membrane

4. Chemiosmosis

- inner membrane to intermembrane space

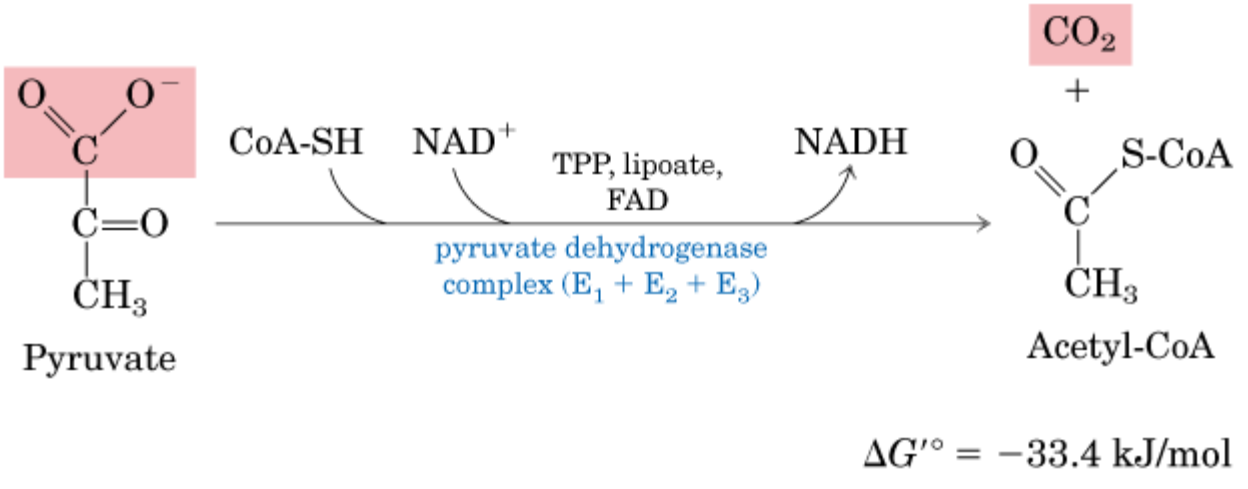


THE CITRIC ACID CYLCE

- **Citric acid cycle, also called the TRICABOXYLIC 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**



PYRUVATE DEHYDROGENASE

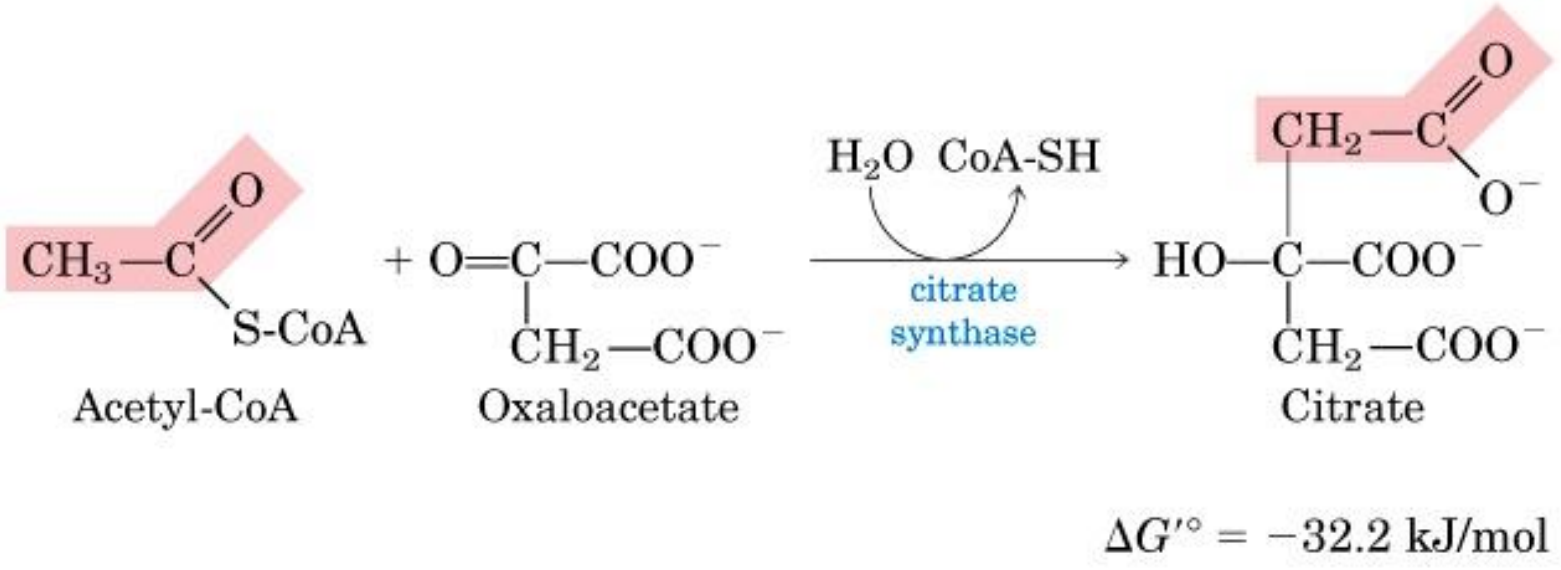
- **Pyruvate dehydrogenase Complex (PDC)**
- **It is a multi-enzyme complex containing three enzymes associated together non-covalently:**
- **E-1 : Pyruvate dehydrogenase, uses Thiamine pyrophosphate as cofactor bound to E1**
- **E-2 : Dihydrolipoyl transacetylase, Lipoic acid bound, CoA as substrate**
- **E-3 : Dihydrolipoyl Dehydrogenase FAD bound, NAD^+ 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.**

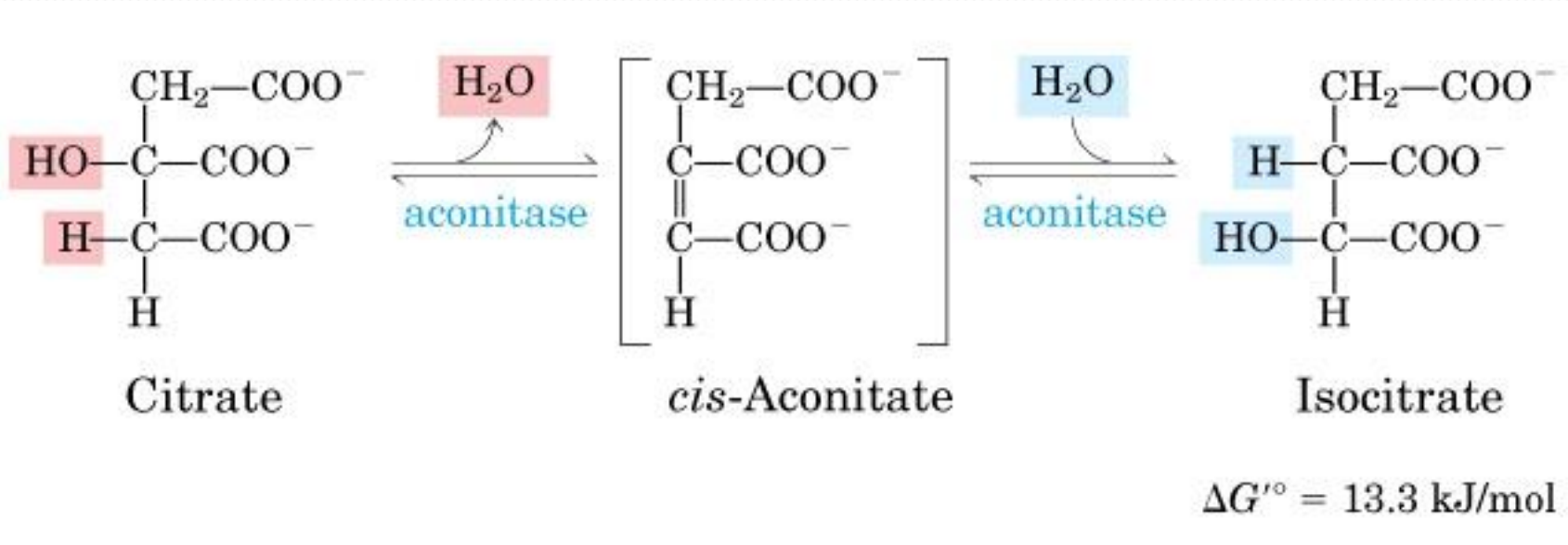
REACTIONS OF THE T.C.A CYCLE

1. Citrate synthase: Formation of Citroyl CoA intermediate.

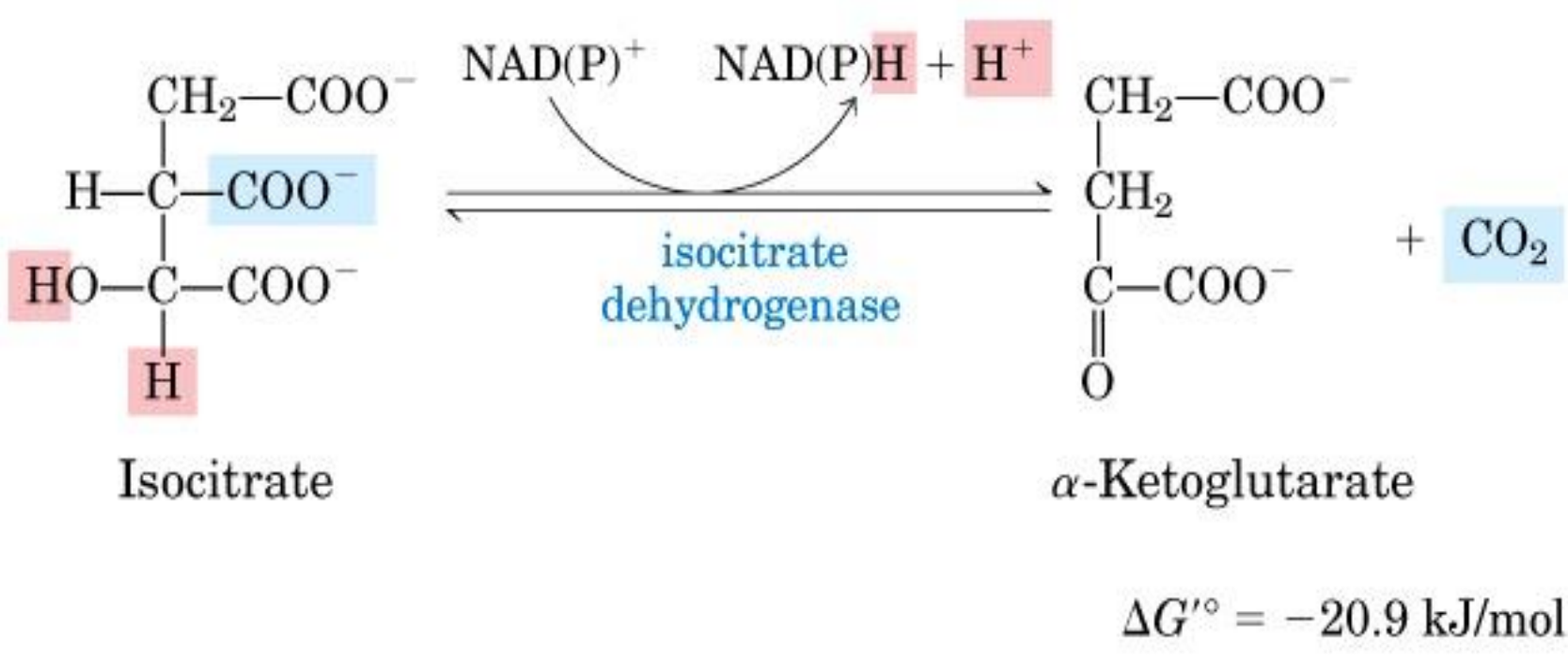
Binding of Oxaloacetate to the enzyme results in conformational change which facilitates the binding of the next substrate, the acetyl Coenzyme A. There is a further conformational change which leads to formation of products. This mechanism of reaction is referred to as induced fit model.



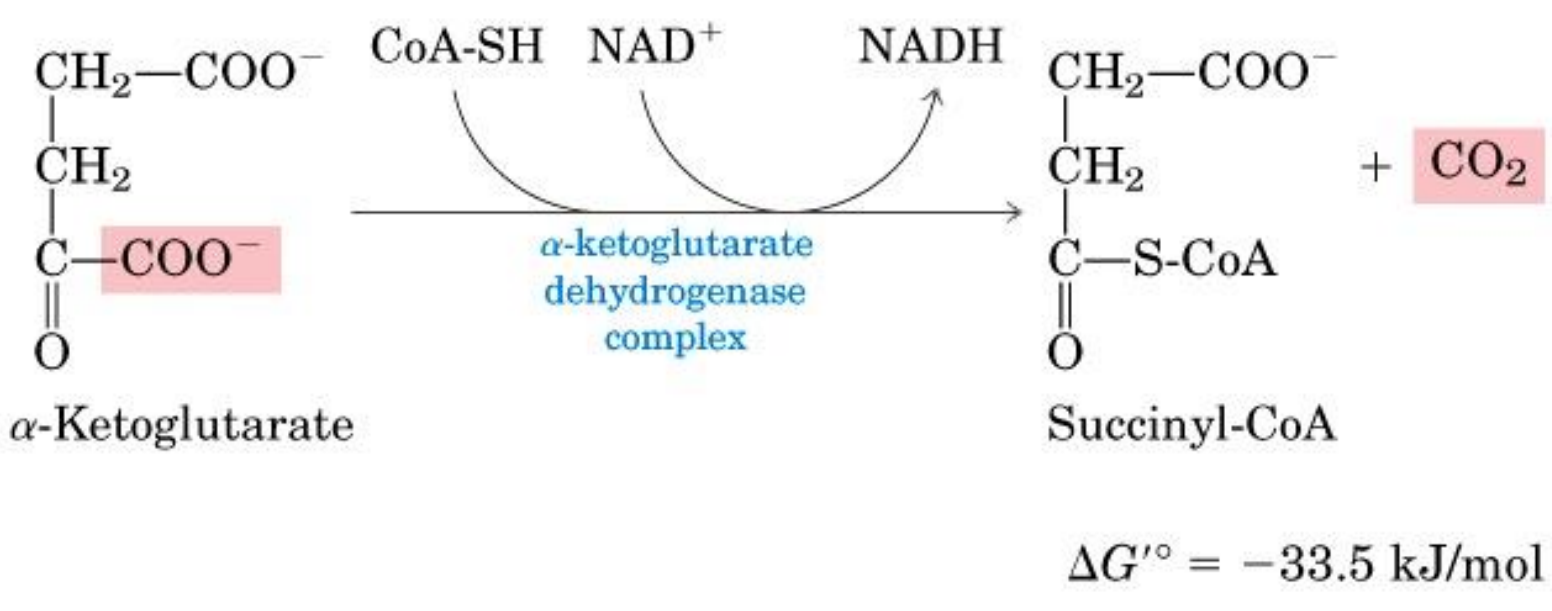
2. Aconitase: This enzyme catalyses the isomerization reaction by removing and then adding back the water (H and OH) to cis-aconitate in at different positions. Isocitrate is consumed rapidly by the next step thus driving the reaction in the forward direction.



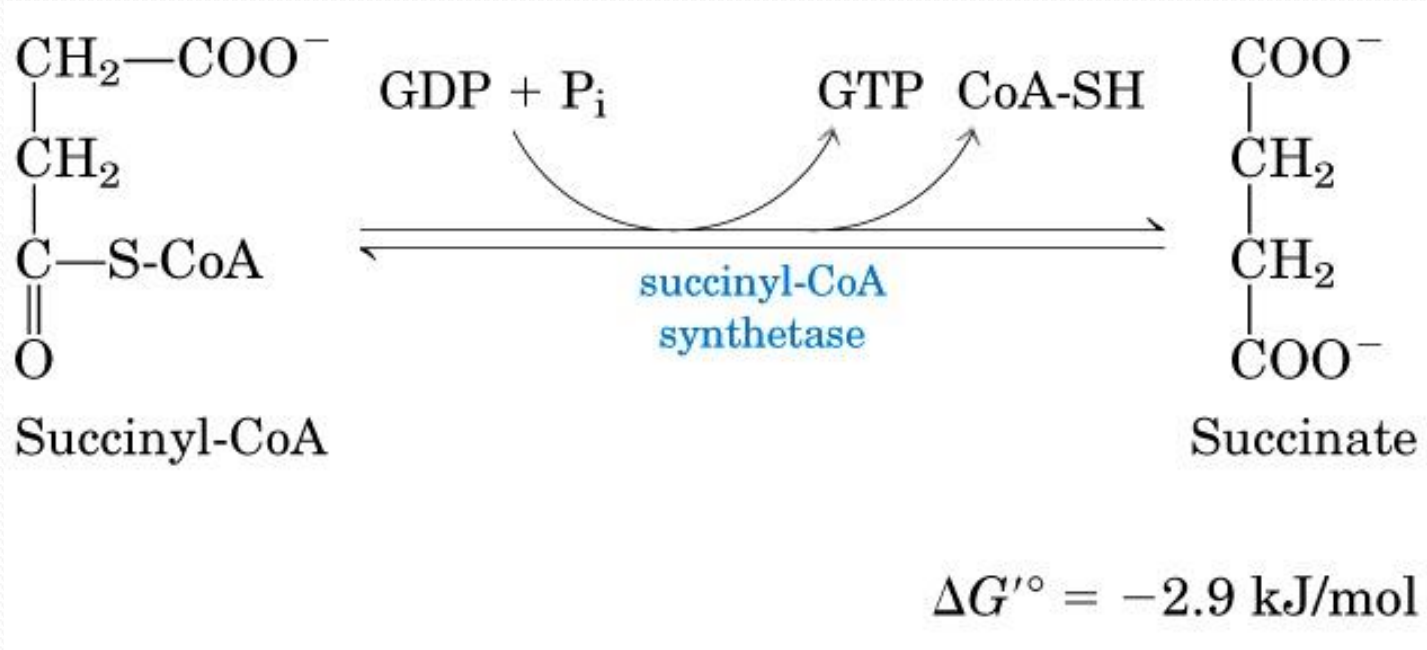
3. Isocitrate dehydrogenase: **There are two isoforms of this enzyme, one uses NAD^+ and other uses $NADP^+$ as electron acceptor.**



4. α-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. NAD+ is an electron acceptor.

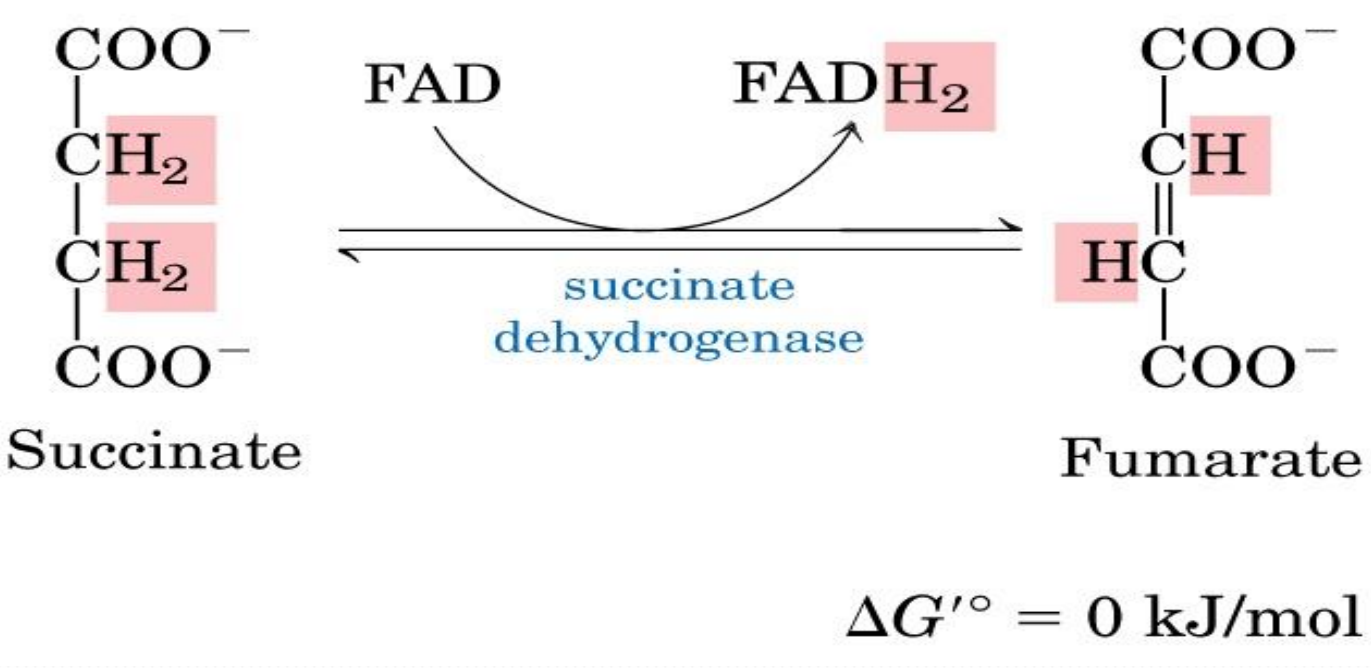


5. Succinyl CoA synthetase: Succinyl CoA, like Acetyl CoA has a thioester bond with very negative free energy of hydrolysis. In this reaction, the hydrolysis of the thioester bond leads to the formation of phosphoester bond with inorganic phosphate. This phosphate is transferred to Histidine residue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

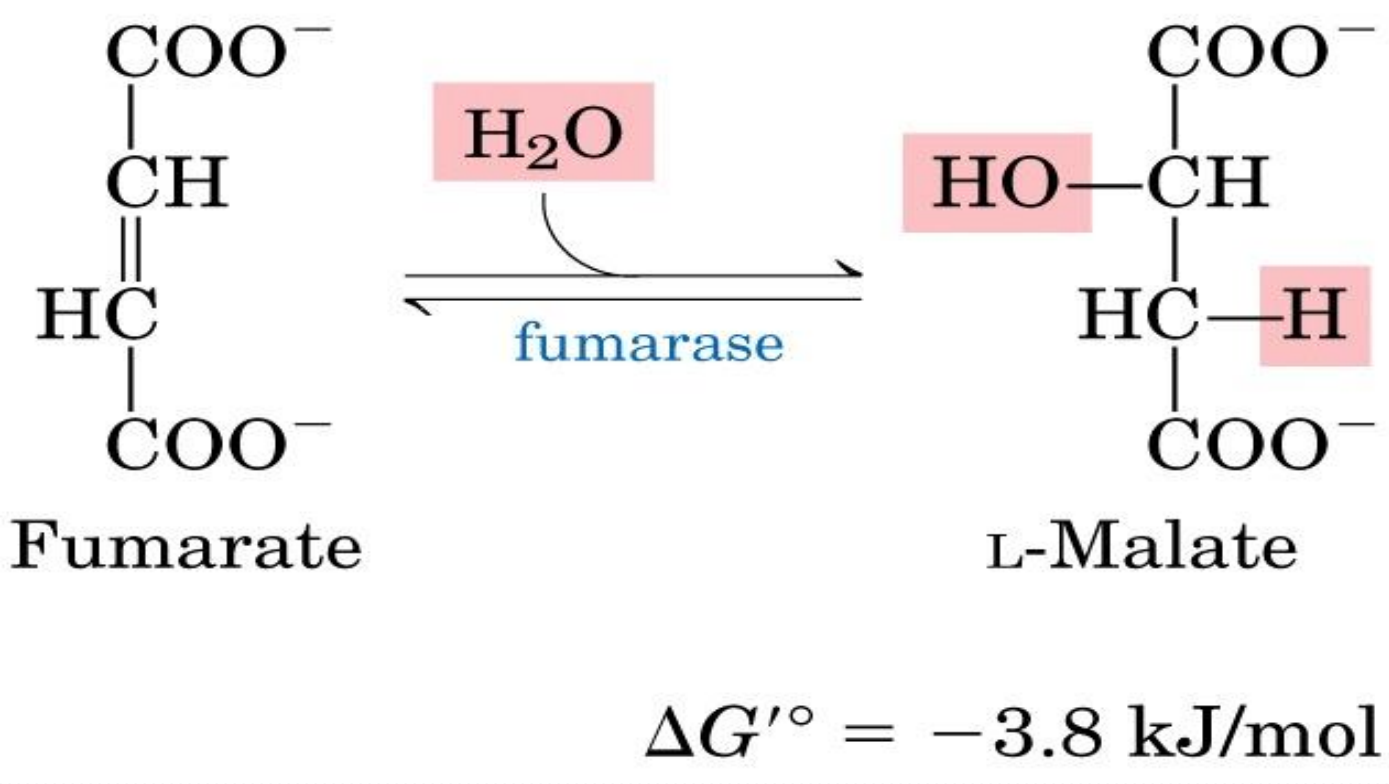


6. Succinate Dehydrogenase: Oxidation of succinate to fumarate. This is the only citric acid cycle enzyme that is tightly bound to the inner mitochondrial membrane. It is an FAD dependent enzyme.

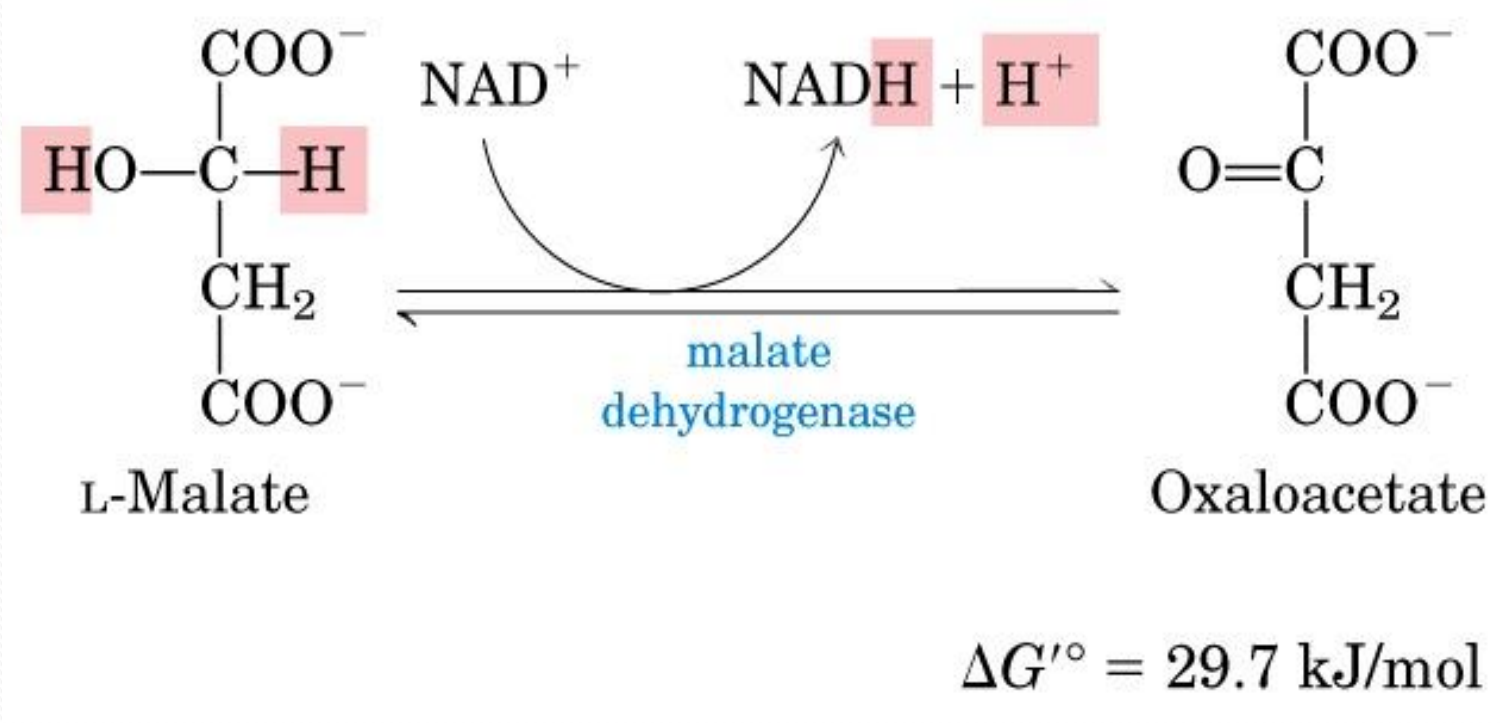
Malonate has similar structure to Succinate, and it competitively inhibits Succinate Dehydrogenase.

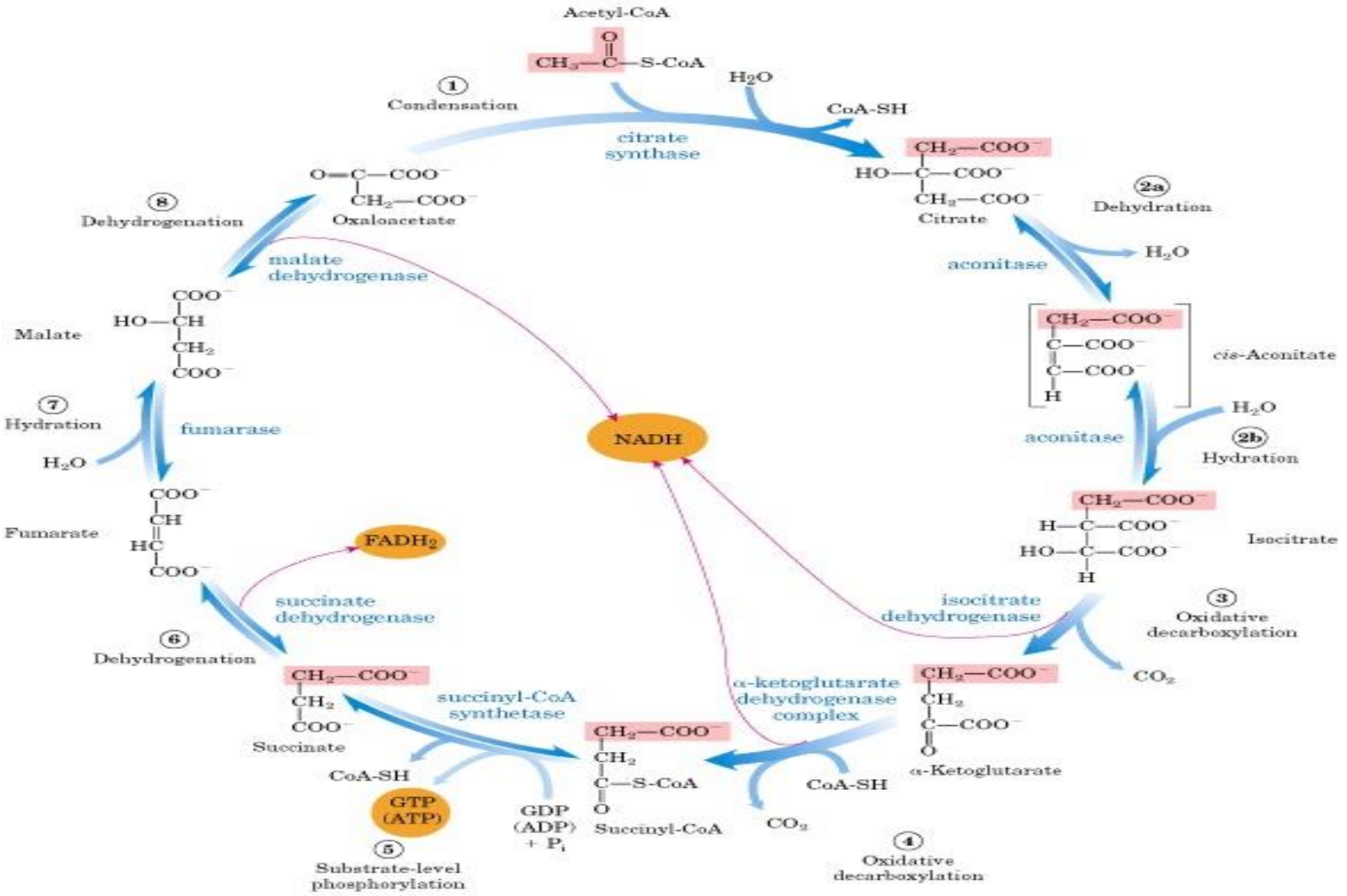


7. Fumarase: **Hydration of Fumarate to malate: It is a highly stereospecific enzyme. cis-Maleate (the cis form of fumarate is not recognized by this enzyme.**



8. L-Malate dehydrogenase: **Oxidation of malate to oxaloacetate: It is an 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.**




Acetyl-CoA
CH3—C(=O)—S-CoA
1
Condensation
H2O
CoA-SH
citrate synthase
CH2—COO⁻
HO—C—COO⁻
CH2—COO⁻
Citrate
2a
Dehydration
H2O
aconitase
CH2—COO⁻
C—COO⁻
C—COO⁻
H
cis-Aconitate
H2O
2b
Hydration
aconitase
CH2—COO⁻
H—C—COO⁻
HO—C—COO⁻
H
Isocitrate
isocitrate dehydrogenase
3
Oxidative decarboxylation
CO2
α-ketoglutarate dehydrogenase complex
CH2—COO⁻
CH2
C—COO⁻
O
α-Ketoglutarate
CoA-SH
CH2—COO⁻
CH2
C—S-CoA
O
Succinyl-CoA
CO2
4
Oxidative decarboxylation
succinyl-CoA synthetase
GTP (ATP)
GDP (ADP) + Pi
5
Substrate-level phosphorylation
CoA-SH
CH2—COO⁻
CH2
COO⁻
Succinate
6
Dehydrogenation
succinate dehydrogenase
FADH2
COO⁻
CH
HC
COO⁻
Fumarate
7
Hydration
H2O
fumarase
COO⁻
HO—CH
CH2
COO⁻
Malate
8
Dehydrogenation
malate dehydrogenase
O=C—COO⁻
CH2—COO⁻
Oxaloacetate
NADH

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+, 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 Ca2+ and ADP. The inhibitor is ATP

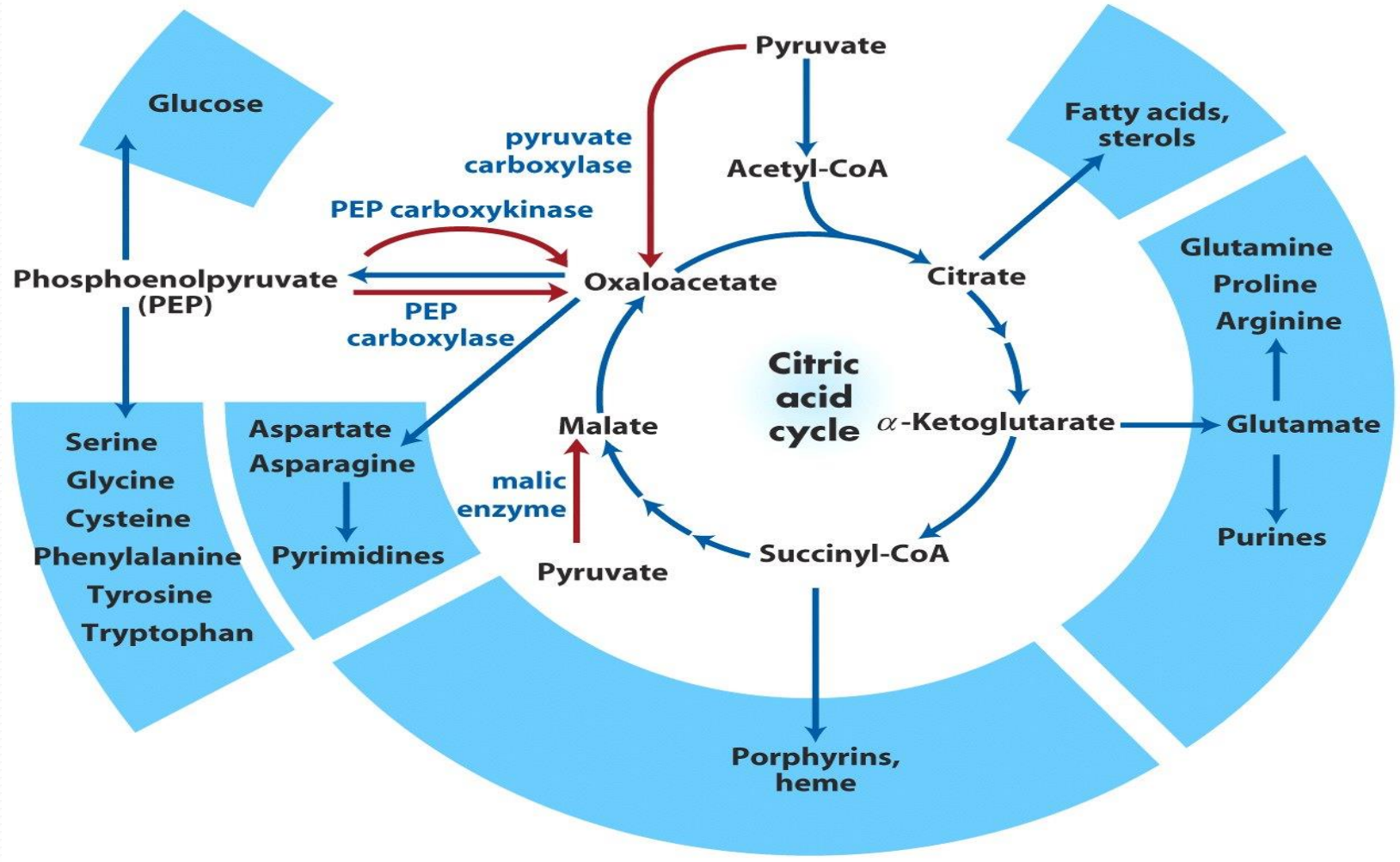
(4)α-KETOGLUTARATE DEHYDROGENASE COMPLEX:

The activator is Ca2+. 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

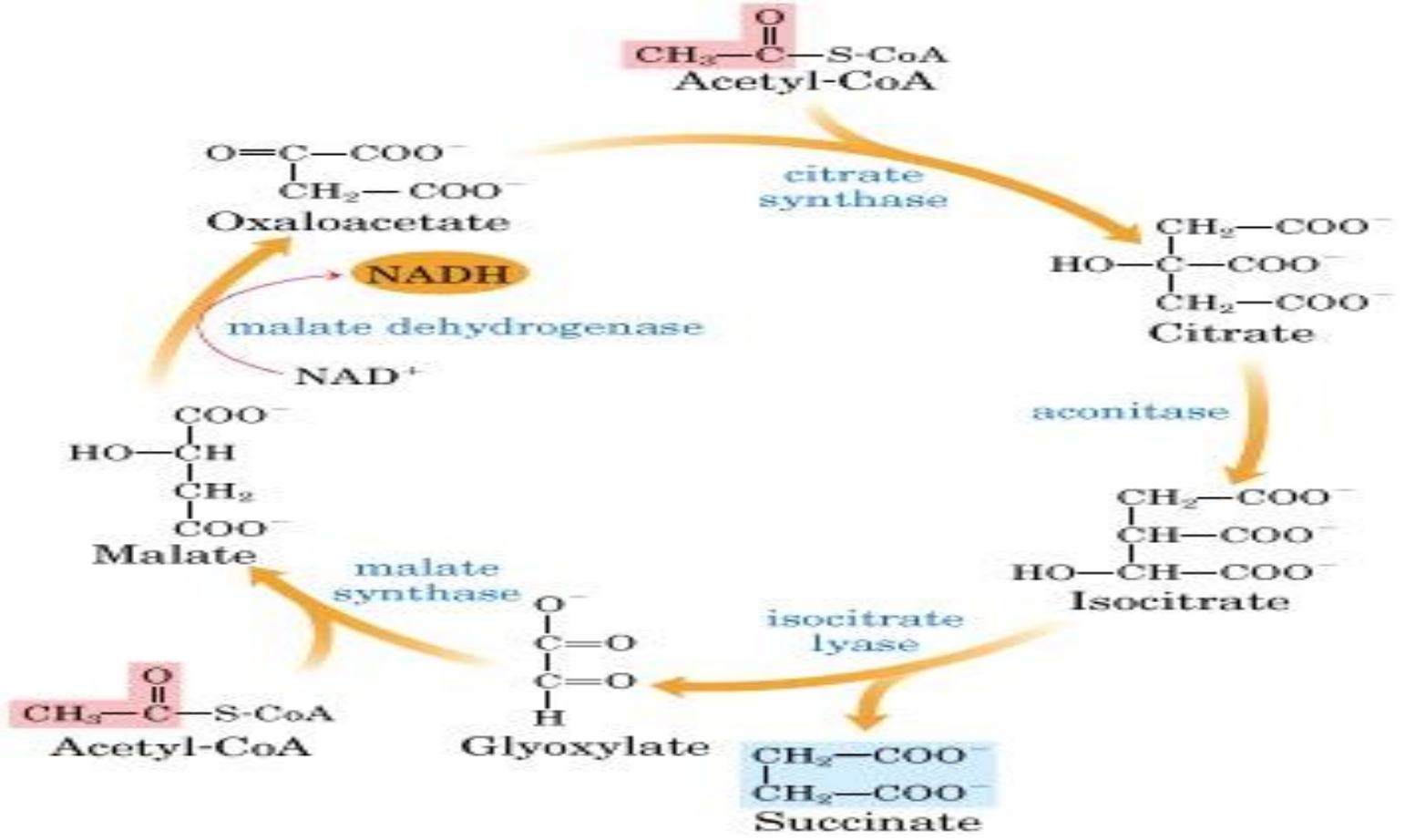
THE AMPHIBOLIC NATURE OF THE T.C.A CYCLE



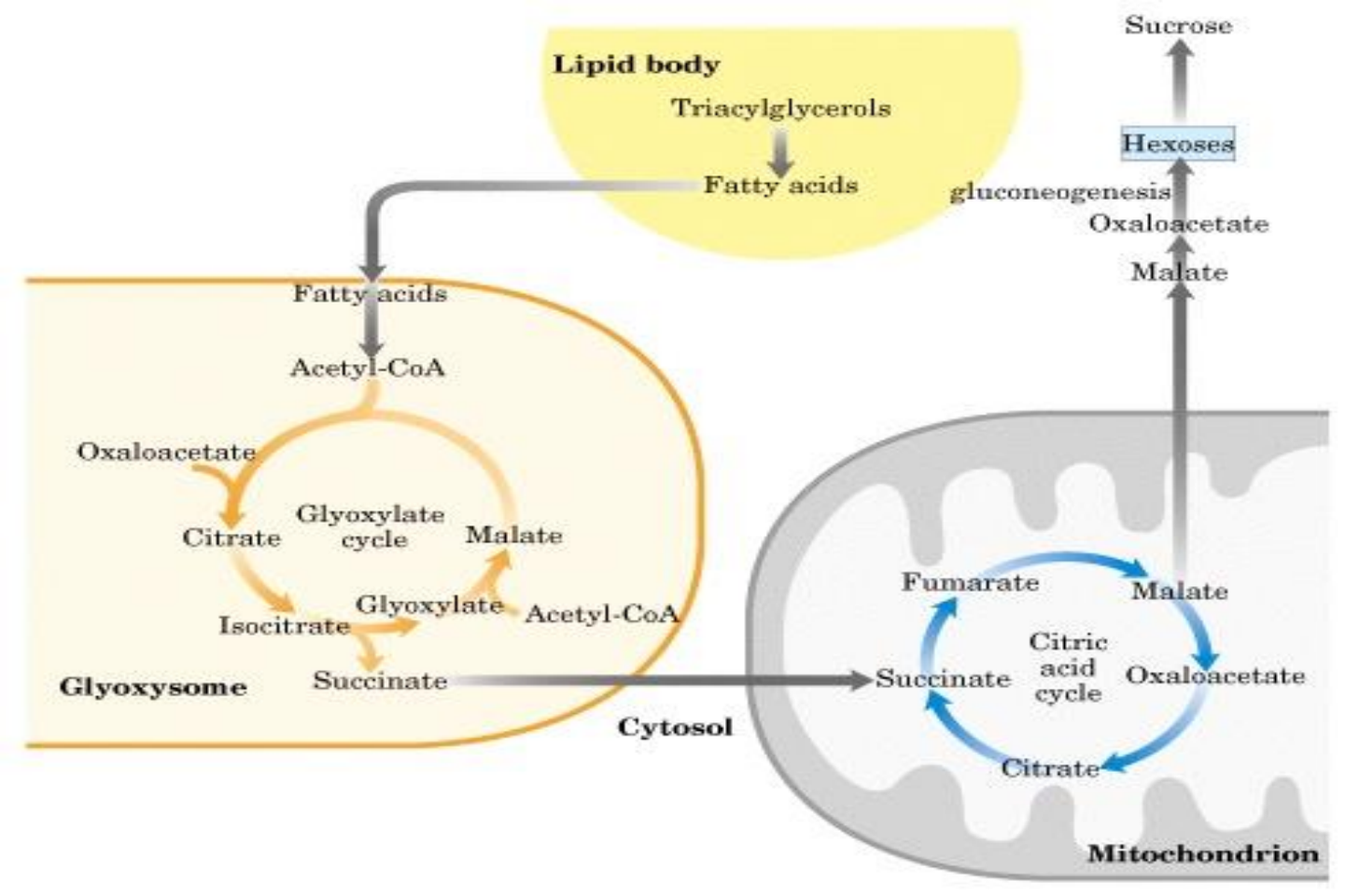
GLYOXYLATE CYCLE

- 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

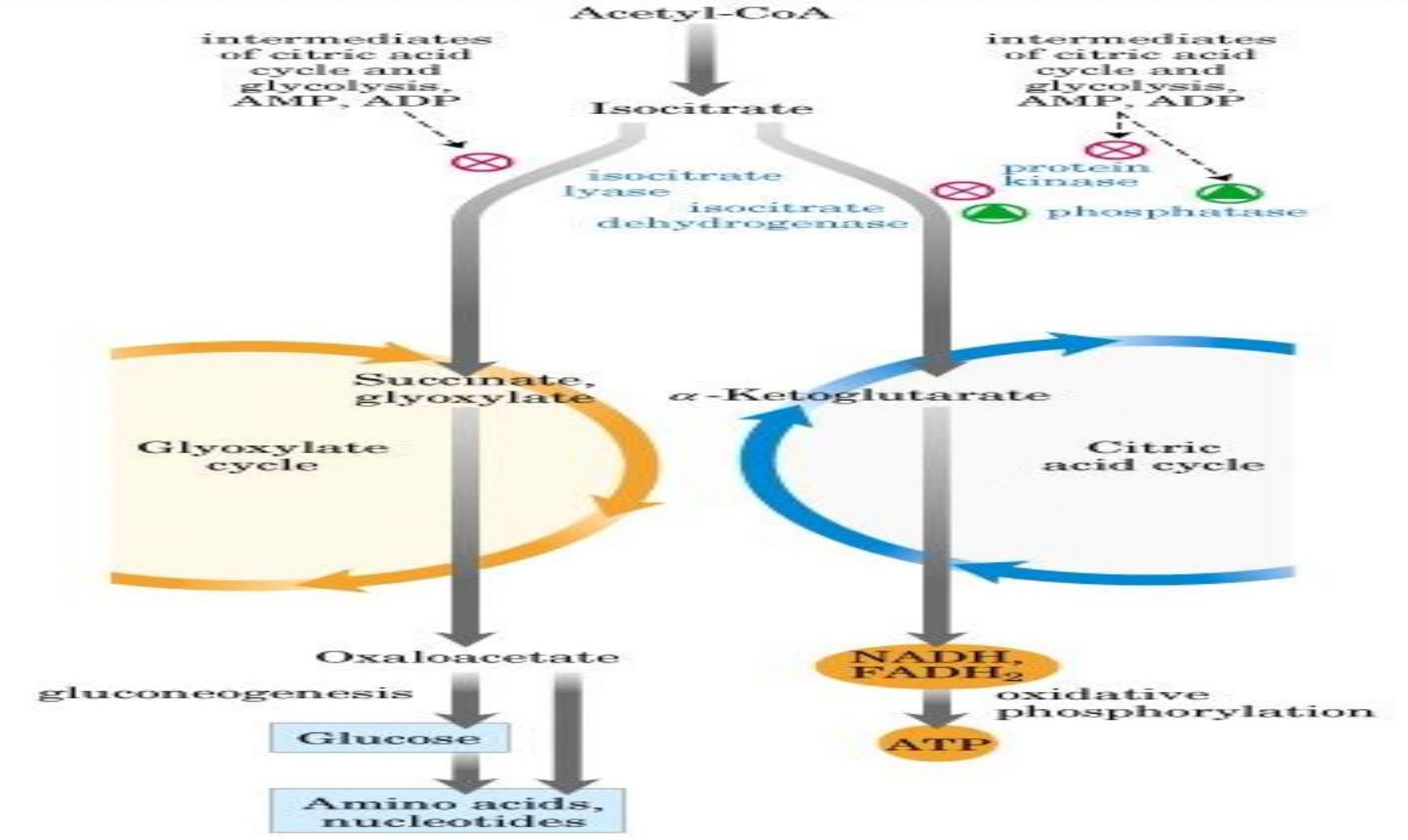
GLYOXYLATE CYCLE



GLYOXYLATE CYCLE CONT'D

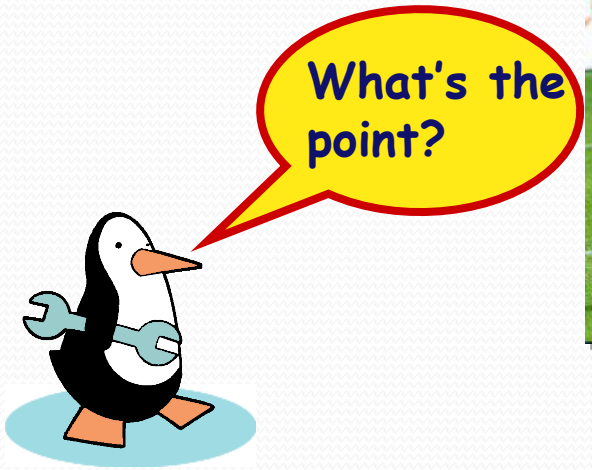


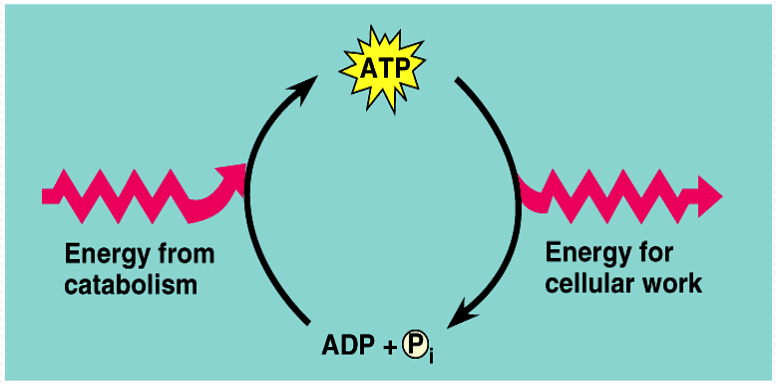
THE T.C.A CYCLE AND GLYOXYLATE CYCLES ARE COORDINATELY REGULATED



ATP accounting so far…

- Glycolysis → 2 ATP
- Kreb'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!

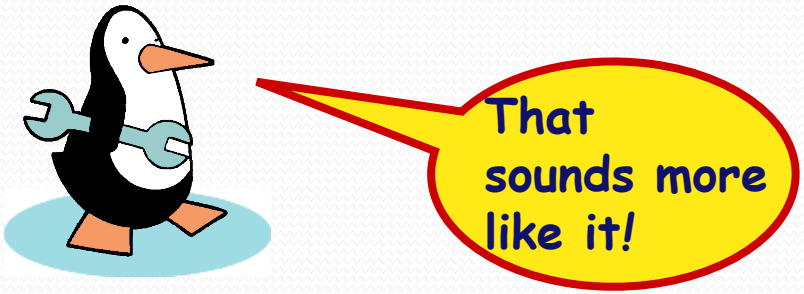




A working muscle recycles over 10 million ATPs per second

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)



THE ELECTRON TRANSPORT CHAIN

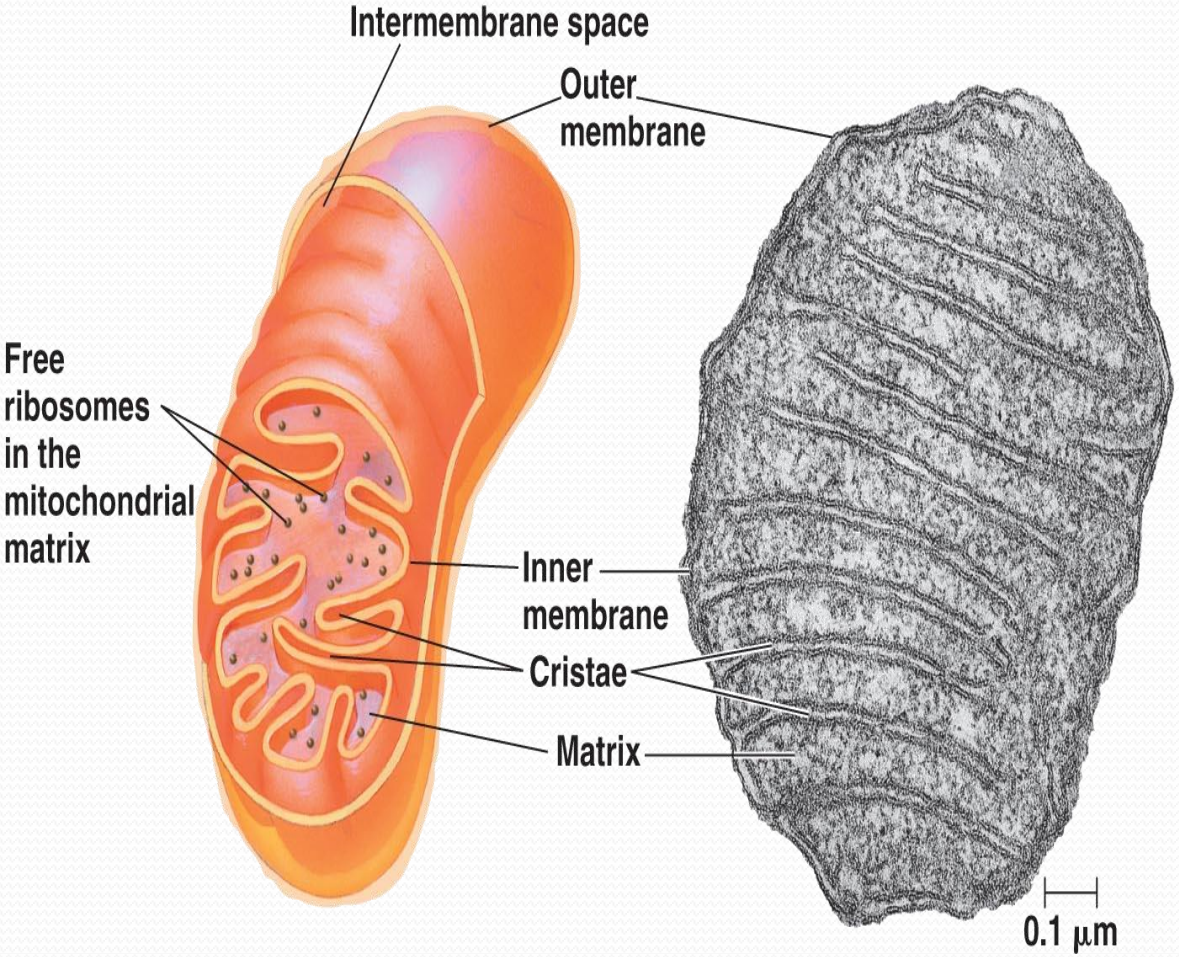
- The 12 electron pairs involved in glucose oxidation are not transferred directly to O_2.
- Rather, as we have seen, they are transferred to the coenzymes NAD+ and FAD to form 10NADH and $2FADH_2$
- In the reactions catalyzed by the glycolytic enzyme glyceraldeyhde-3- phosphate dehydrogenase, pyruvate dehydrogenase and the citric acid enzymes :isocitrate dehydrogenase, α-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

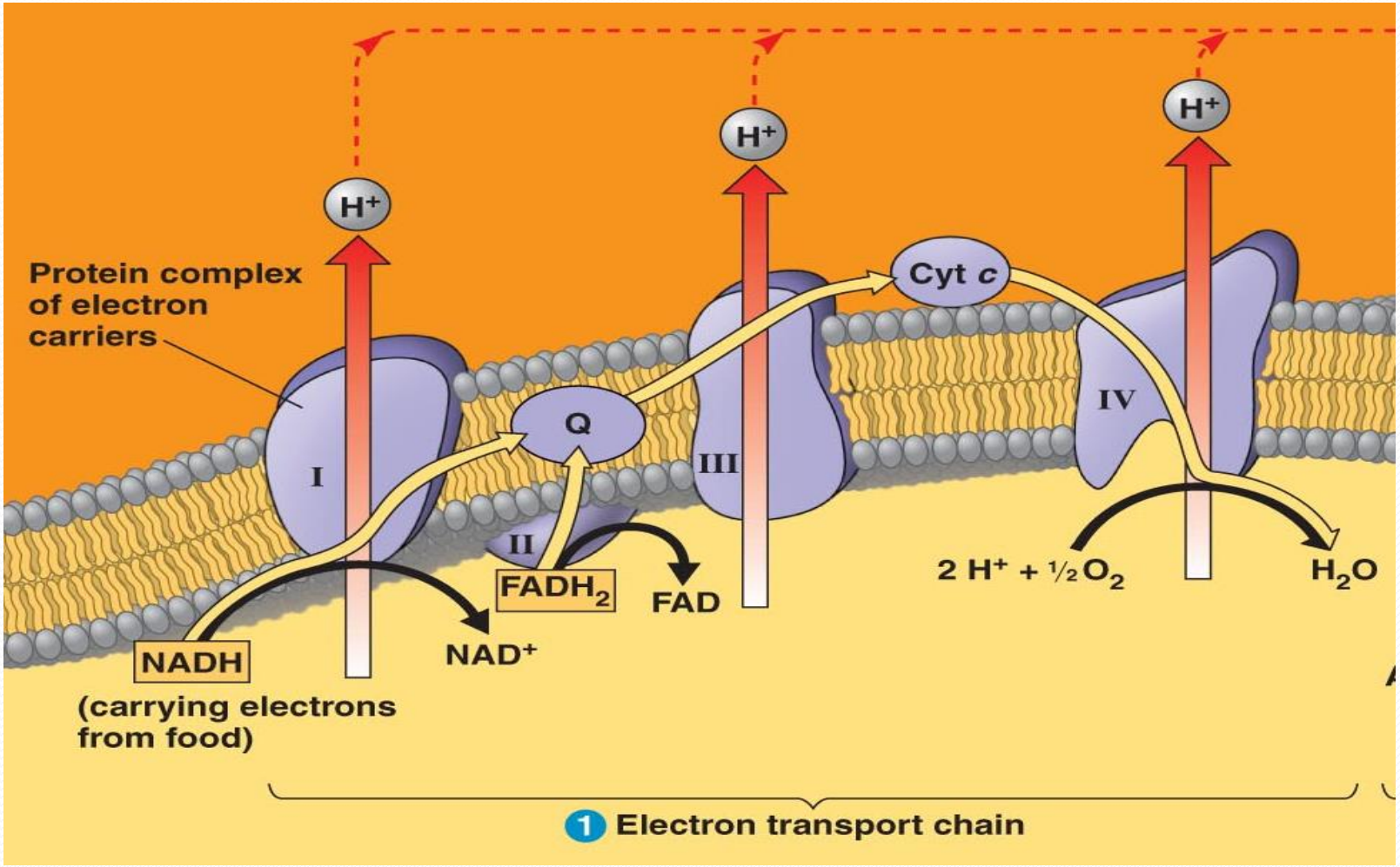
- Double membrane
  - outer membrane
  - inner membrane
    - highly folded cristae
    - enzymes & transport proteins
  - intermembrane space
    - fluid-filled space between membranes



SEQUENCE OF ELECTRON TRANSPORT

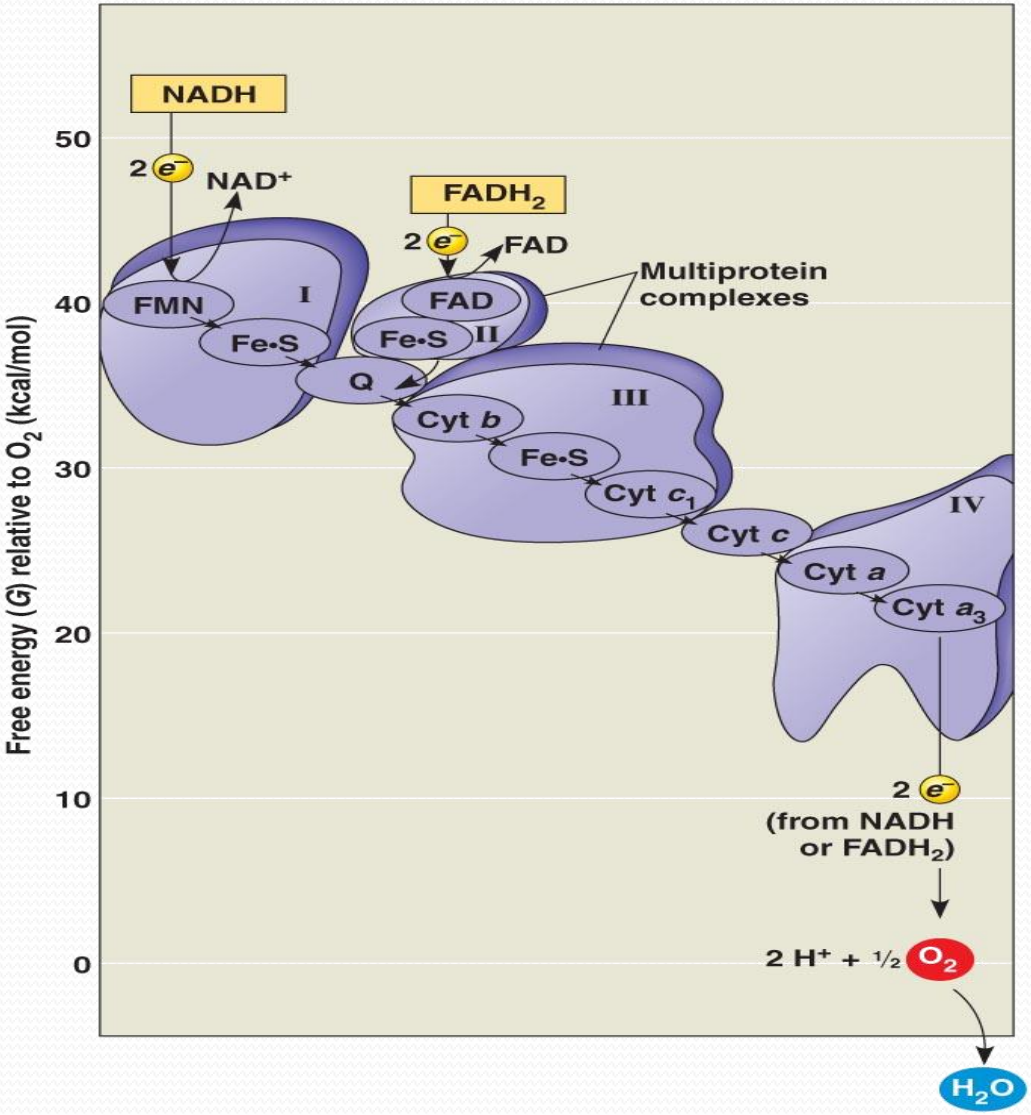
- The free energy necessary to generate ATP is extracted from the oxidation of NADH and $FADH_2$ by the electron-transport chain, a series of four protein complexes through which electrons pass from lower to higher standard reduction potentials.
- Electrons are carried from complexes I and II to complex III by coenzyme Q(CoQ or Ubiquinone) and from complex III to complex IV by the peripheral membrane protein Cytochrome c

ELECTRON TRANSPORT CHAIN



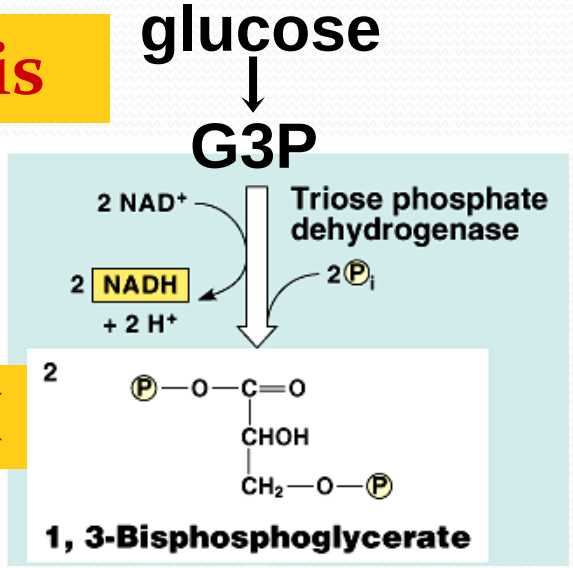
COMPONENTS OF THE ELECTRON TRANSPORT CHAIN

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

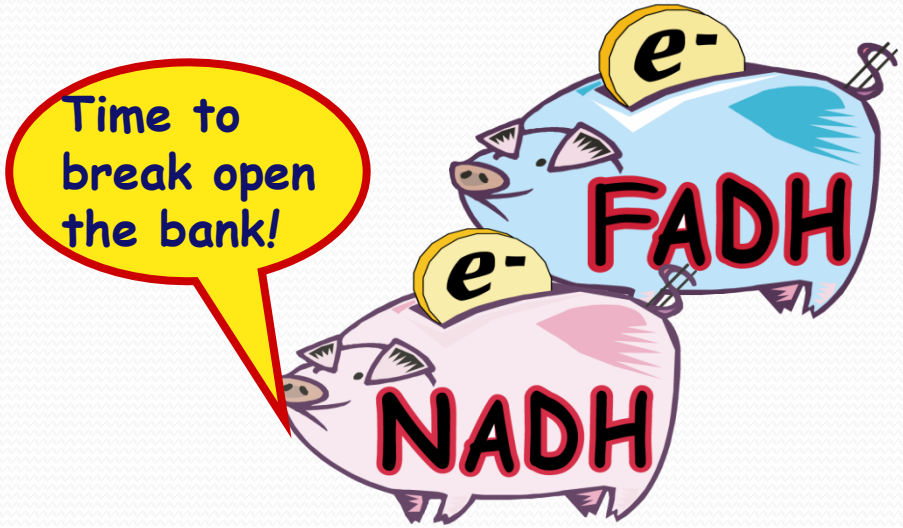


REMEMBER THE ELECTRON CARRIERS?

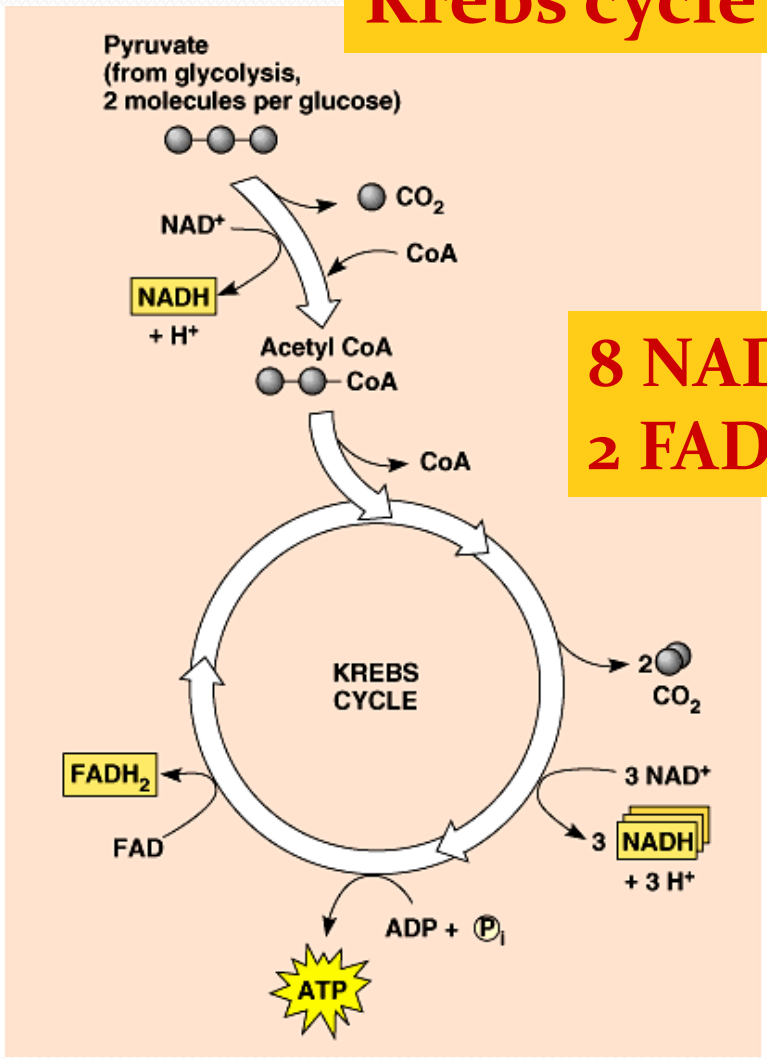
Glycolysis



2 NADH

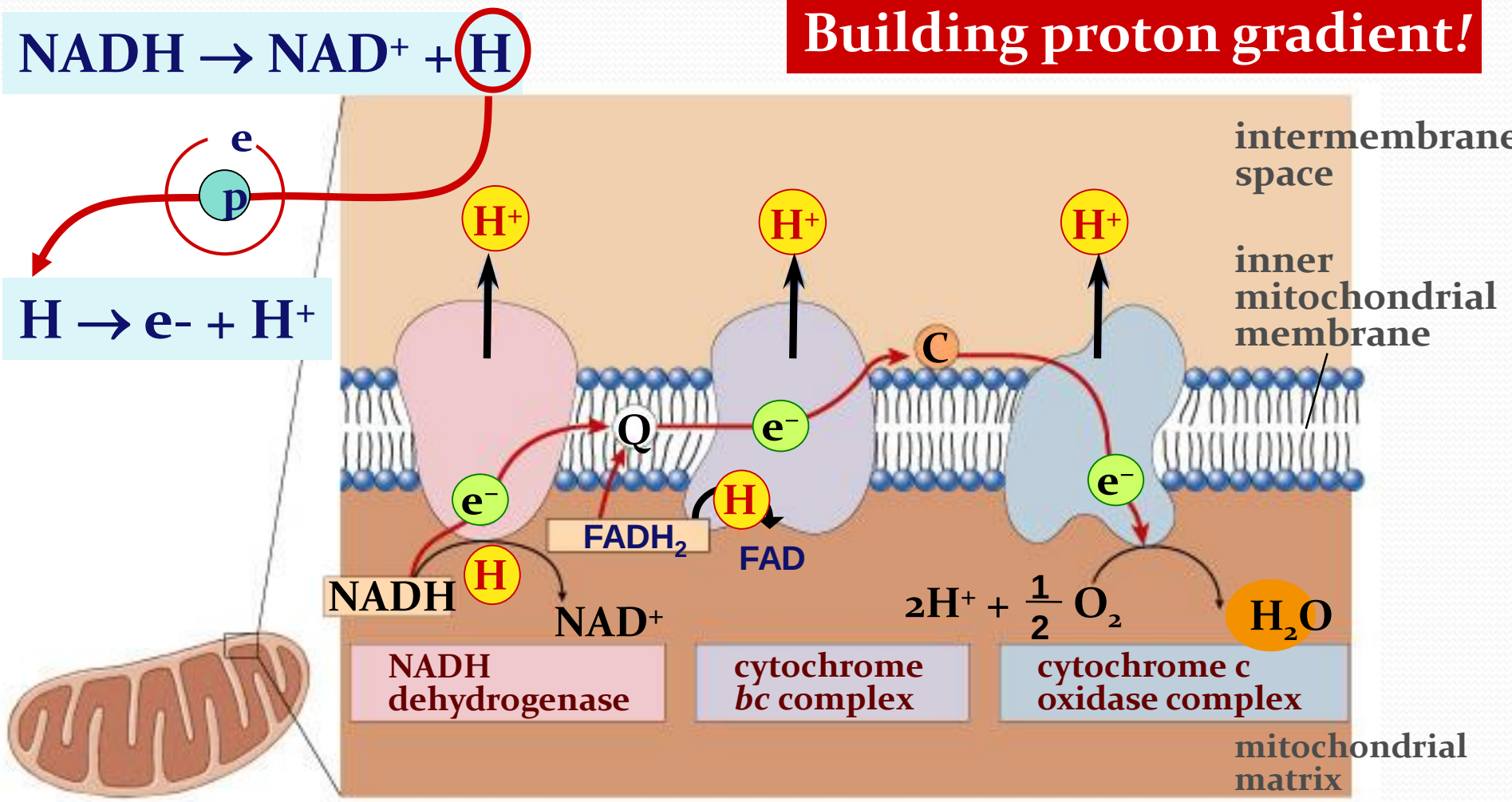


Krebs cycle



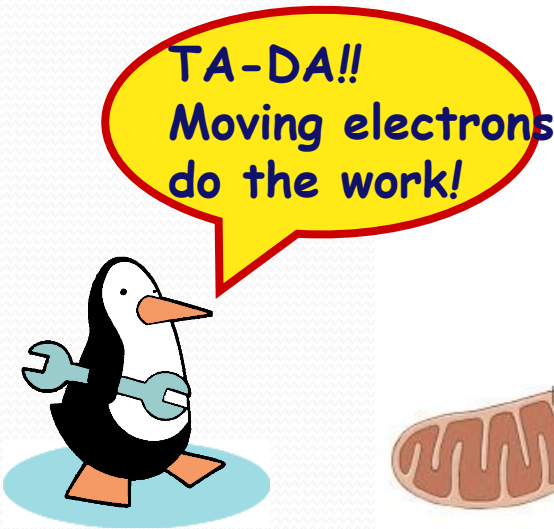
8 NADH
2 $FADH_2$

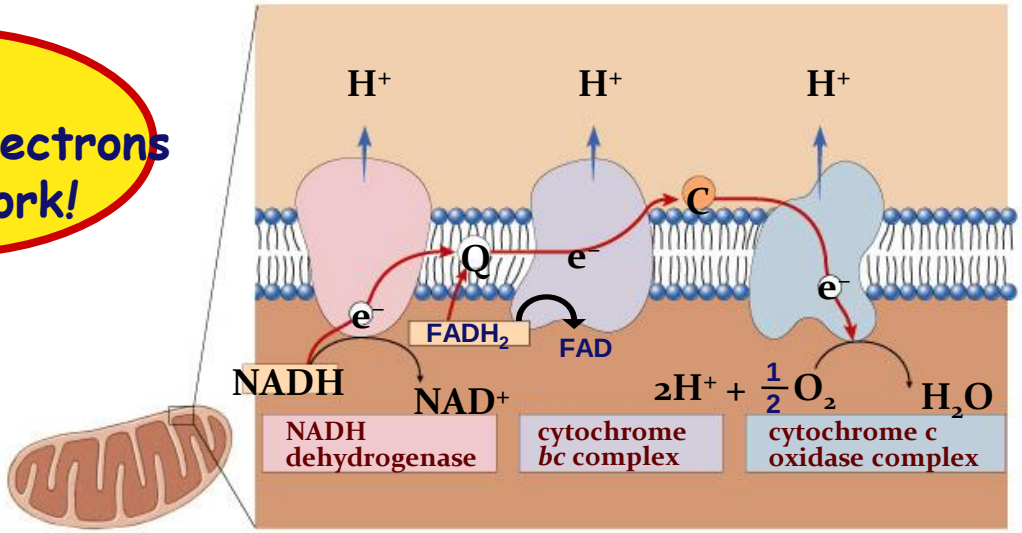
ELECTRON TRANSPORT CHAIN

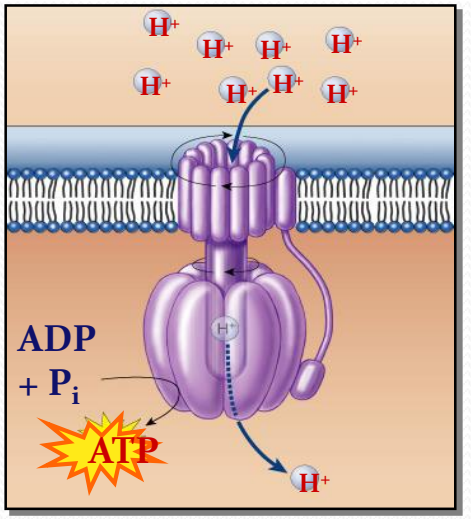


STRIPPING H FROM ELECTRON CARRIERS

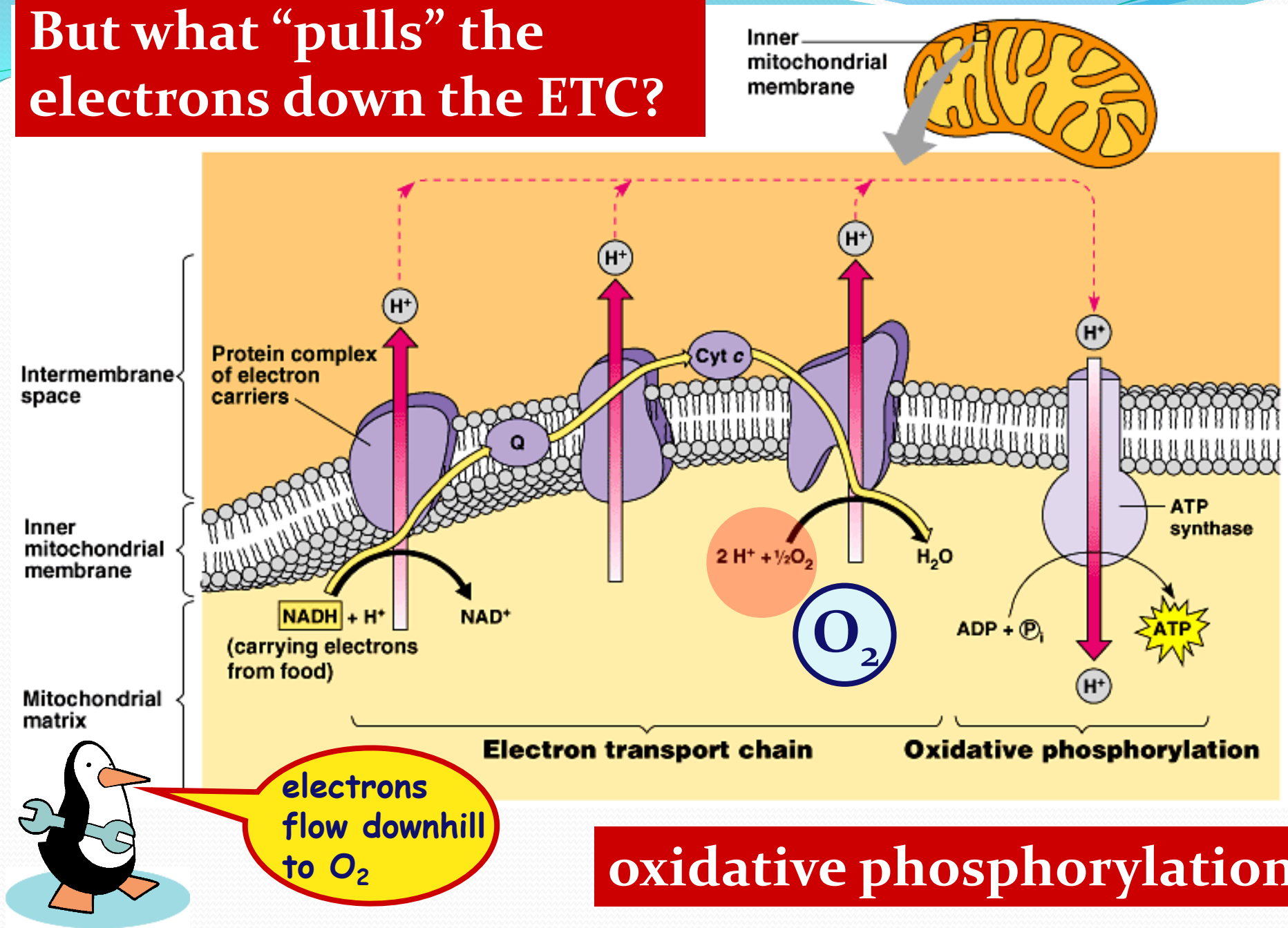
- NADH passes electrons to ETC
  - H cleaved off NADH & $FADH_2$
  - electrons stripped from H atoms → H^+ (protons)
  - electrons passed from one electron carrier to next in mitochondrial membrane (ETC)
  - transport proteins in membrane pump H^+ (protons) across inner membrane to intermembrane space



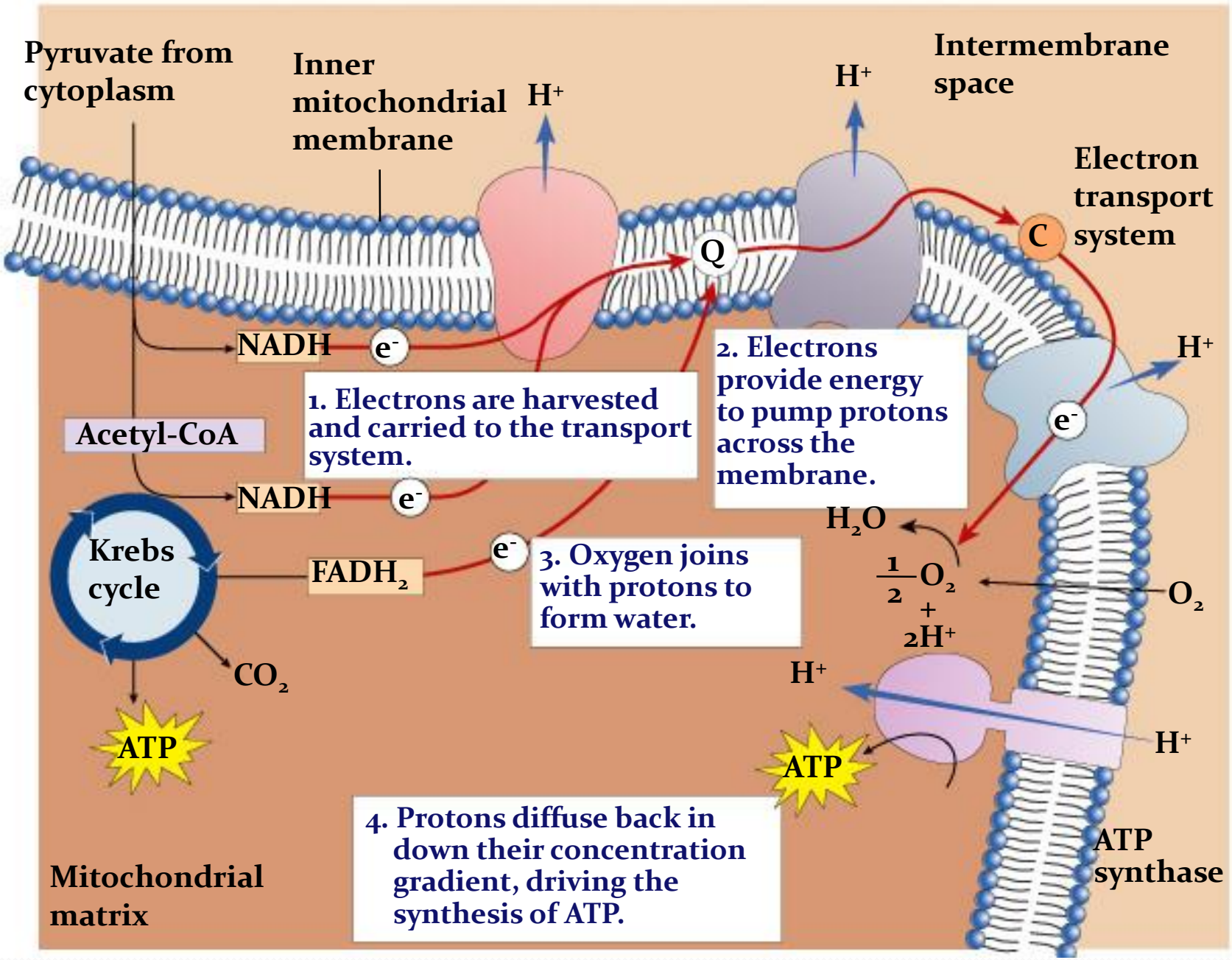




But what "pulls" the electrons down the ETC?



oxidative phosphorylation


Pyruvate from cytoplasm
Inner mitochondrial membrane
H+
H+
Intermembrane space
Electron transport system
C
Q
NADH
e-
1. Electrons are harvested and carried to the transport system.
2. Electrons provide energy to pump protons across the membrane.
H+
e-
Acetyl-CoA
NADH
e-
Krebs cycle
FADH2
e-
3. Oxygen joins with protons to form water.
H2O
1/2 O2
+
2H+
O2
CO2
H+
ATP
ATP
H+
4. Protons diffuse back in down their concentration gradient, driving the synthesis of ATP.
Mitochondrial matrix
ATP synthase

Summary of cellular respiration

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + \sim ATP$$

- Where did the glucose come from?
- Where did the O_2 come from?
- Where did the CO_2 come from?
- Where did the CO_2 go?
- Where did the H_2O come from?
- Where did the ATP come from?
- What else is produced that is not listed in this equation?
- Why do we breathe?