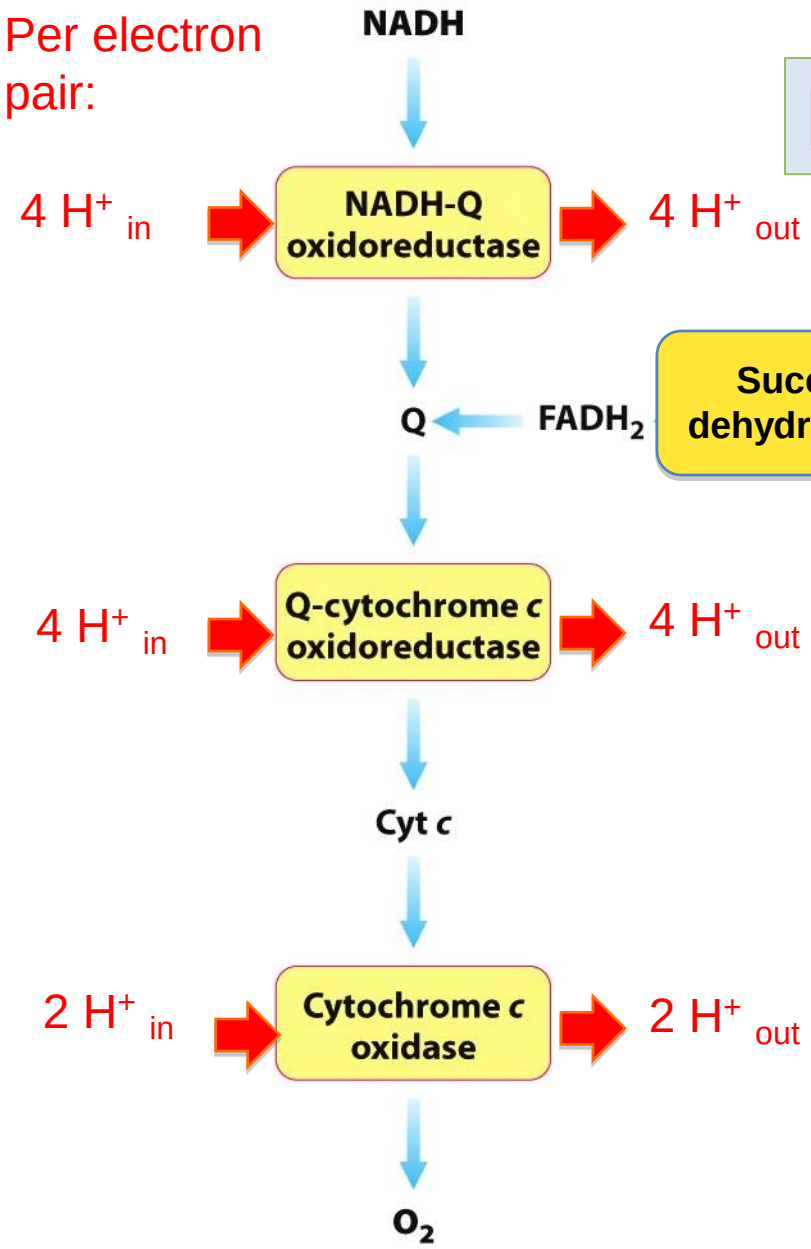
ENERGY METABOLISM

Lecture 2

Electron Transport Chain



ort Course, Second Edition
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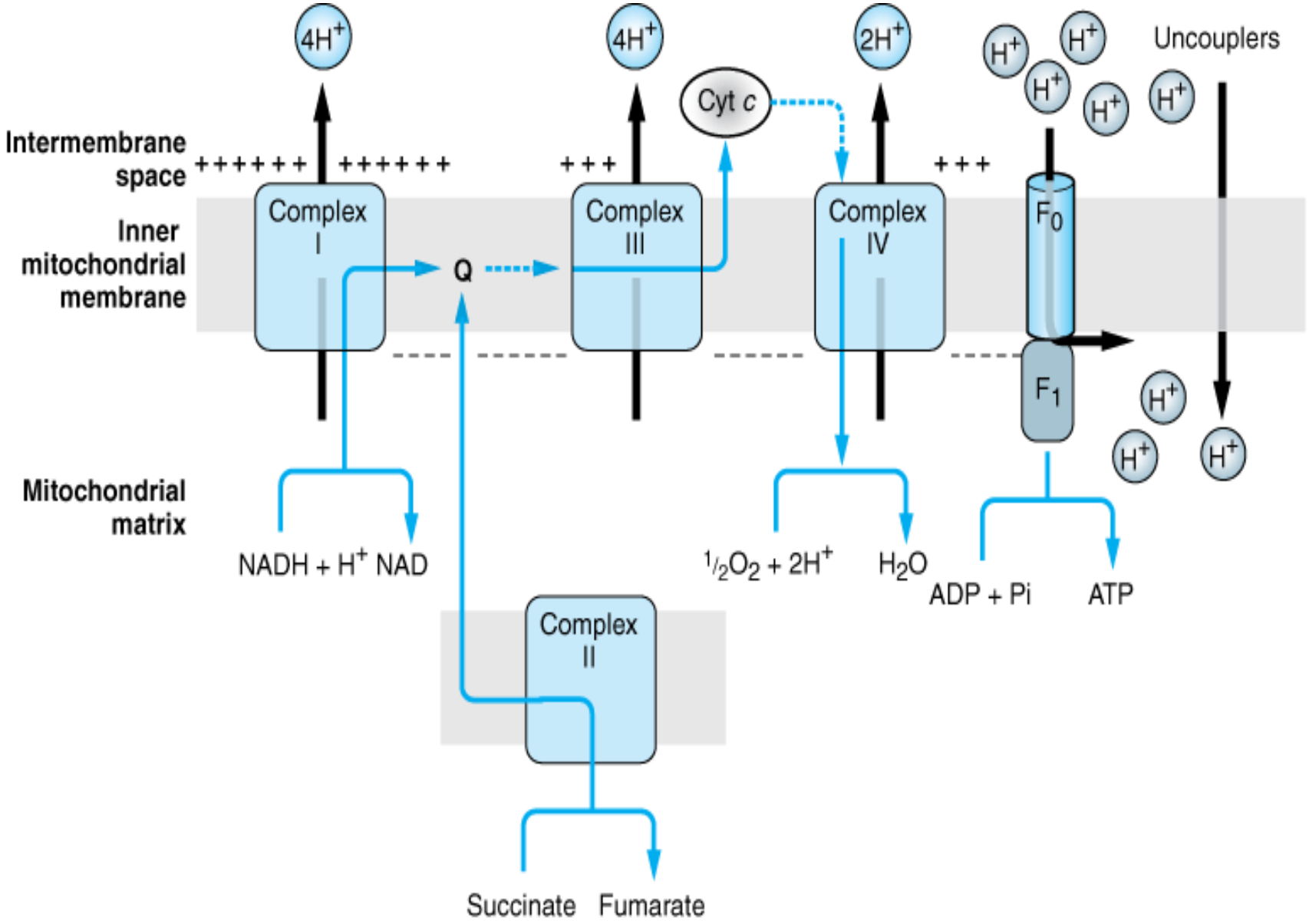
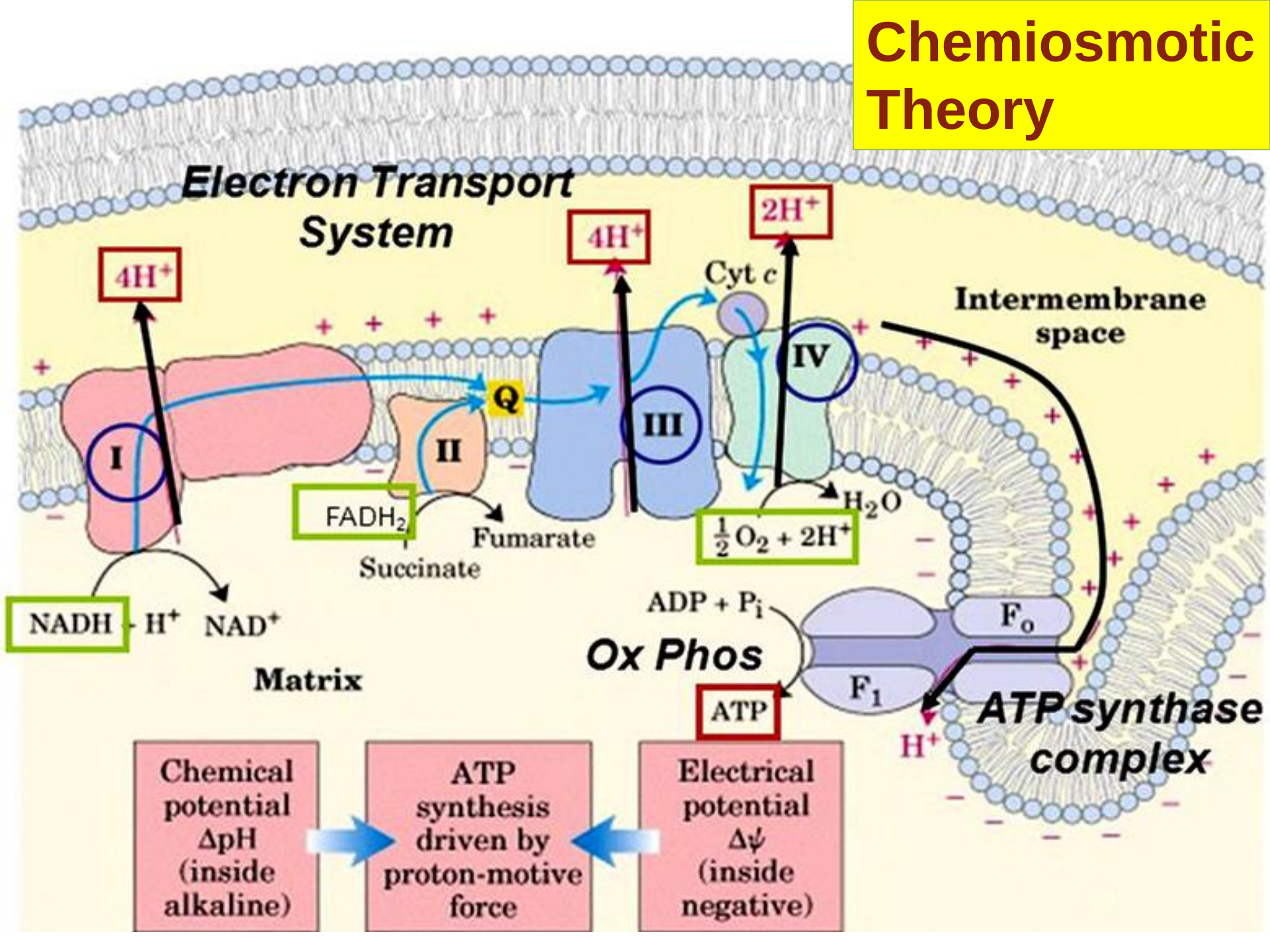
Some carriers pump protons

Chemiosmotic Theory

- Peter Mitchell proposed that electron transport and ATP synthesis are coupled by a proton gradient across the inner mitochondrial membrane
- The transport of electrons through the respiratory chain leads to the pumping of protons from the matrix to the intermembrane space
- The H^+ concentration becomes lower in the matrix, and an electrical field with the matrix side negative is generated
- Mitchell's idea, called the chemiosmotic hypothesis, was that this proton-motive force drives the synthesis of ATP by ATP synthase


4H+
4H+
2H+
Cyt c
Uncouplers
H+
H+
H+
H+
H+
H+
H+
H+
H+
Intermembrane space
++++++ ++++++
+++
+++
Inner mitochondrial membrane
Complex I
Q
Complex III
Complex IV
F0
F1
Mitochondrial matrix
NADH + H+ NAD
1/2O2 + 2H+
H2O
ADP + Pi
ATP
Complex II
Succinate
Fumarate

Chemiosmotic Theory



Chemiosmotic Mechanism

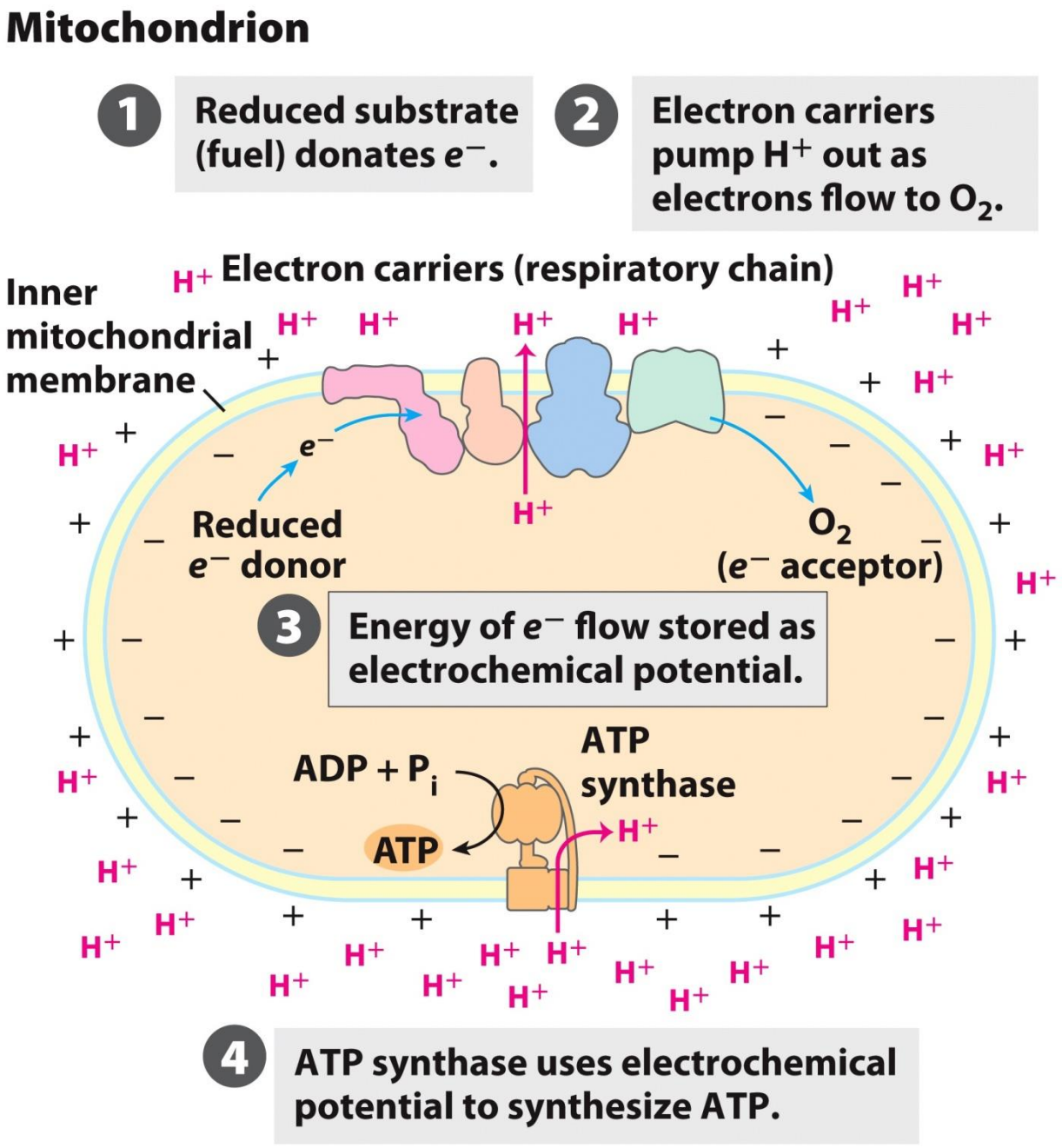
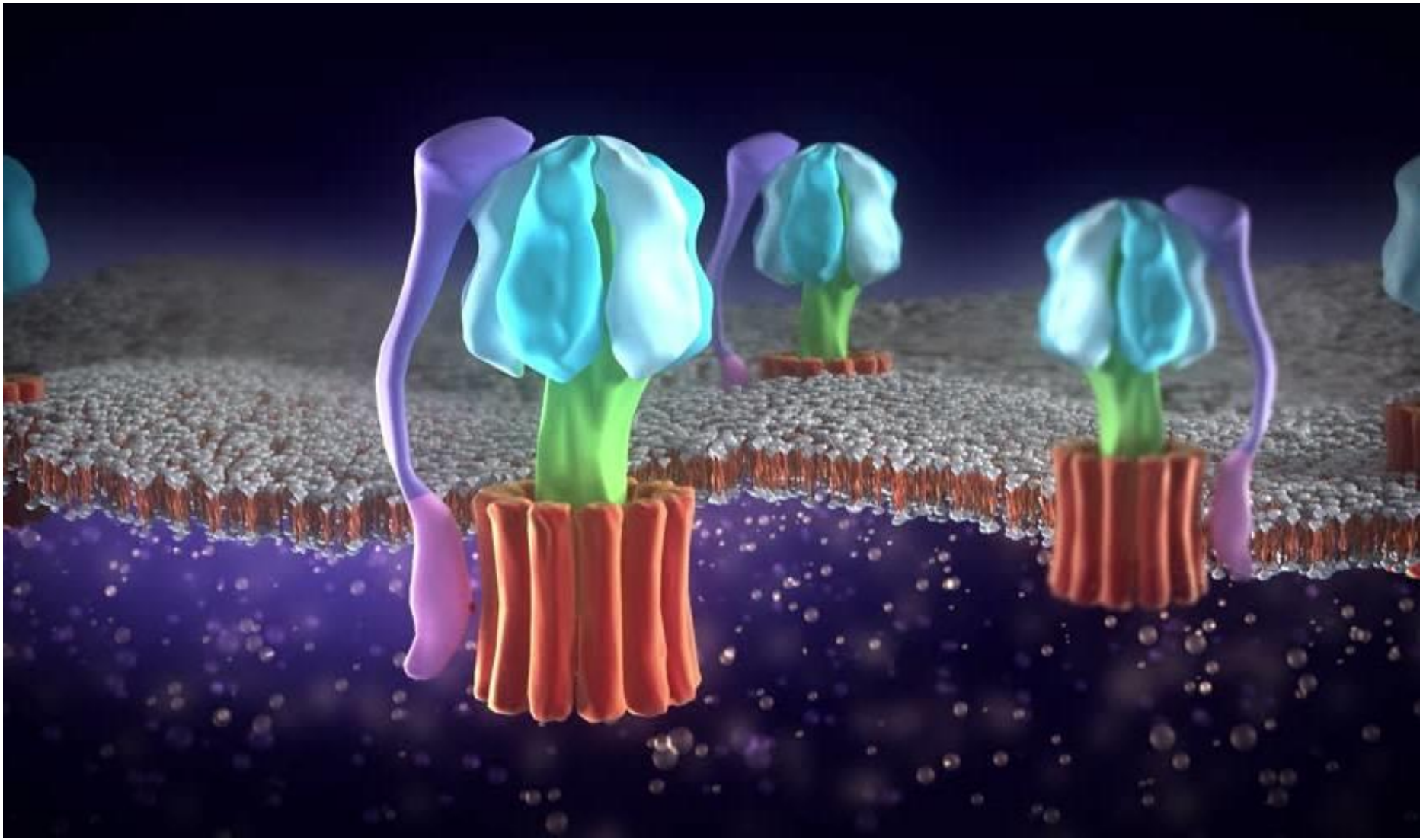


Figure 19-1a
Lehninger Principles of Biochemistry, Sixth Edition

Electron transport chain sets up an H^+ gradient (proton motive force)

Energy of the pmf is harnessed to make ATP



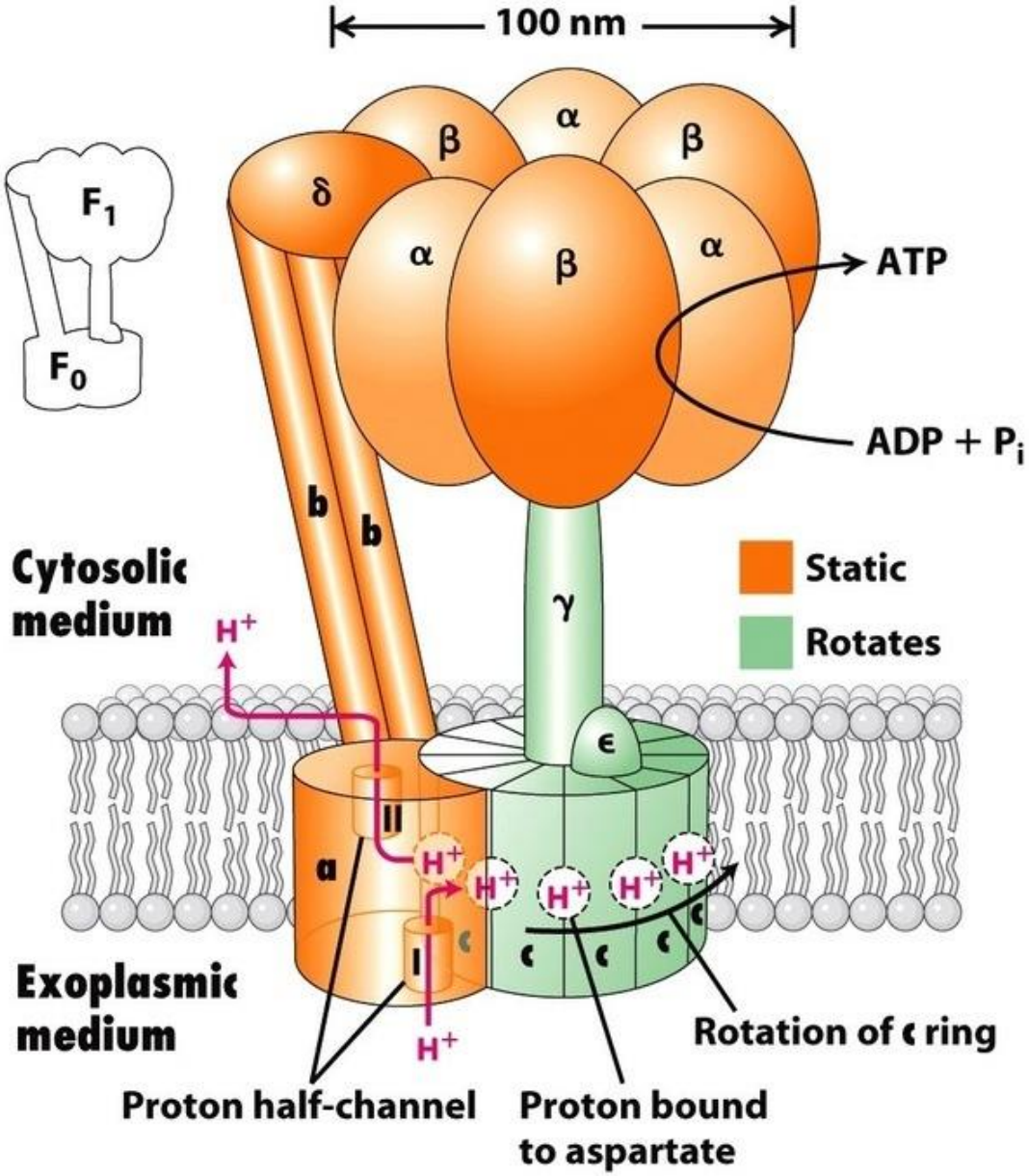


Figure 12-24
Molecular Cell Biology, Sixth Edition

The P/O ratio

is used for the characteristics of energy production in the ETC

The P/O ratio is a measure of the number of high-energy phosphates (*i.e.* amount of ATP) synthesized per atom of oxygen (½O_2) consumed.

The P/O ratio

The P/O ratio for oxidation of metabolites that yield NADH is about 3 and the ratio for those that yield $FADH_2$ is about 2.

In other words, if NADH undergoes oxidation in the ETC, 3 ATP is produced, and if $FADH_2$ - 2 ATP is generated.

Regulation of ETC

Oxidative phosphorylation is regulated by cellular energy needs

Conditions Limiting the Rate of Respiration	
State 1	Availability of ADP and substrate
State 2	Availability of substrate only
State 3	The capacity of respiratory chain itself, when all substrates and components are present in saturating amounts
State 4	Availability of ADP only
State 5	Availability of oxygen only

Regulation of ETC

'Respiratory control' is the dependence of oxygen uptake by mitochondria on the availability of ADP

The mechanism of respiratory control depends on the requirement for ADP and P_i binding to the ATP synthase complex: in the absence of ADP and P_i, protons cannot enter the mitochondrion through this complex, and oxygen consumption markedly decreases, because the proton pumps cannot transport protons.

Regulation of ETC

Activators of ETC:

ADP, P_i, oxygen, and reduced substrates (SH_2)

Regulation of ETC

Numerous toxins can severely impair the electron transport system, the ATP synthase and the translocase that exchanges ATP and ADP across the inner mitochondrial membrane.

The inhibitors of the ETC: sleeping-drugs (barbiturates, amytal, amobarbital, chloropromasin, etc.); antibiotics (antimycin); piericidin; such poisons as cyanine and carbon monoxide (CO).

Specific inhibitors of electron transport chain and ATP-synthase

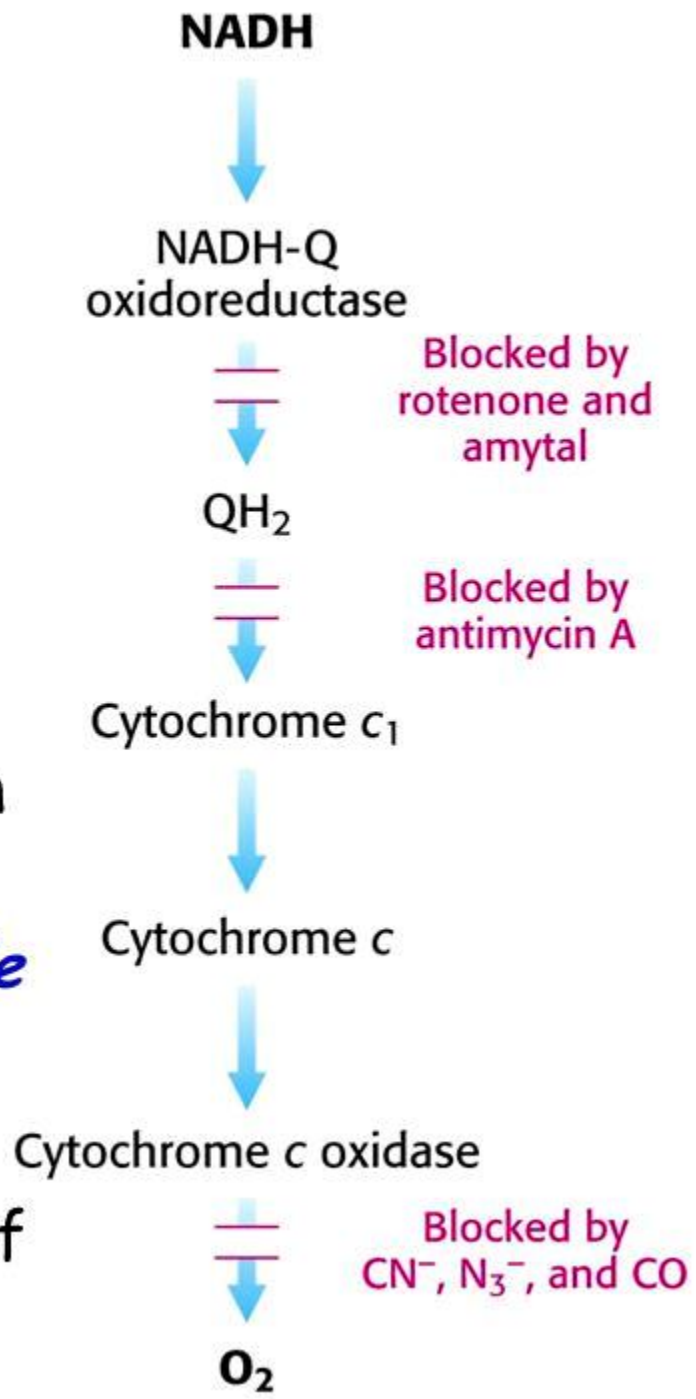
Specific inhibitors of electron transport are invaluable in revealing the sequence of electron carriers.

Rotenone and ***amytal*** block electron transfer in **Complex I**.

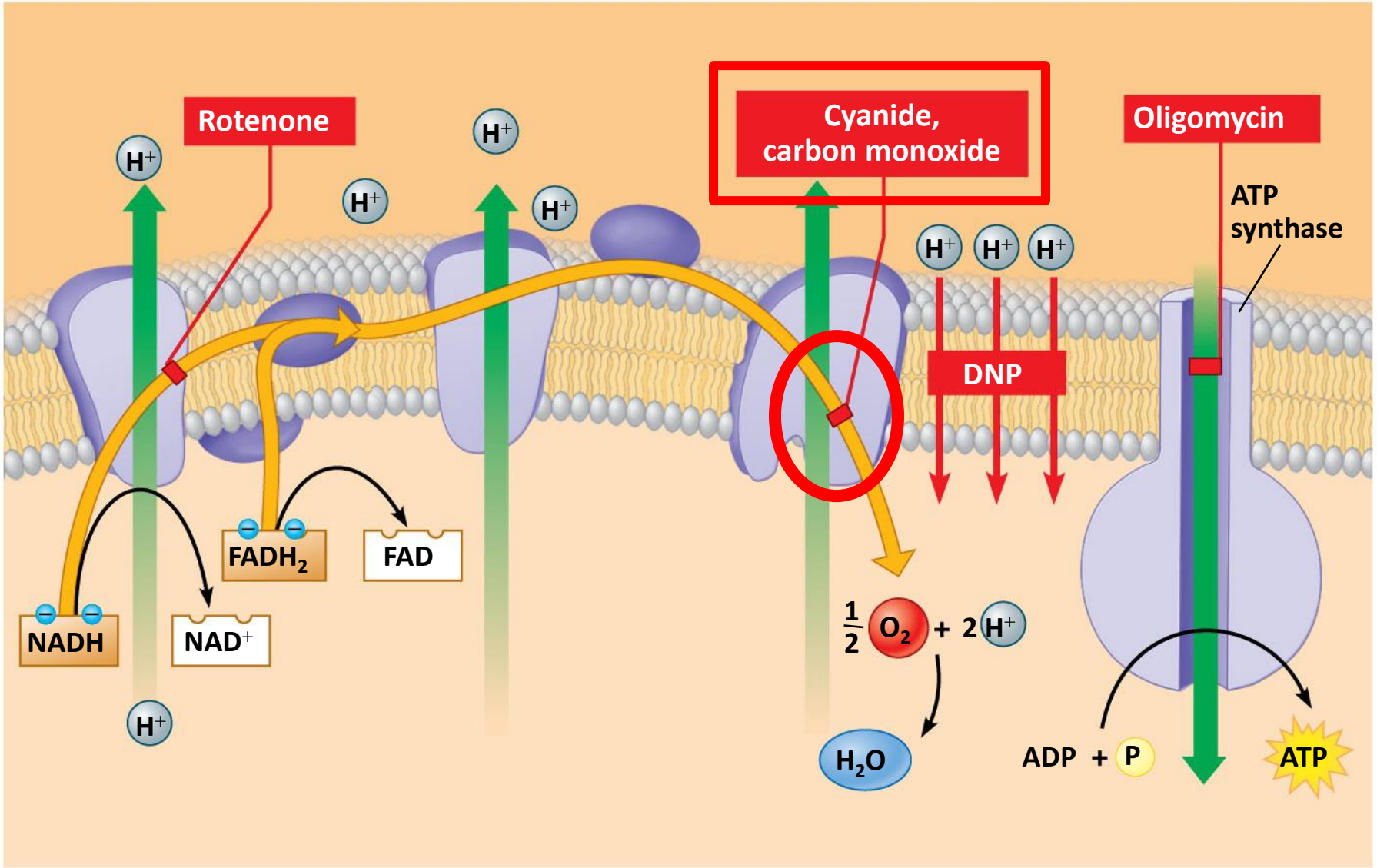
Antimycin A interferes with electron flow thhrough **Complex III**.

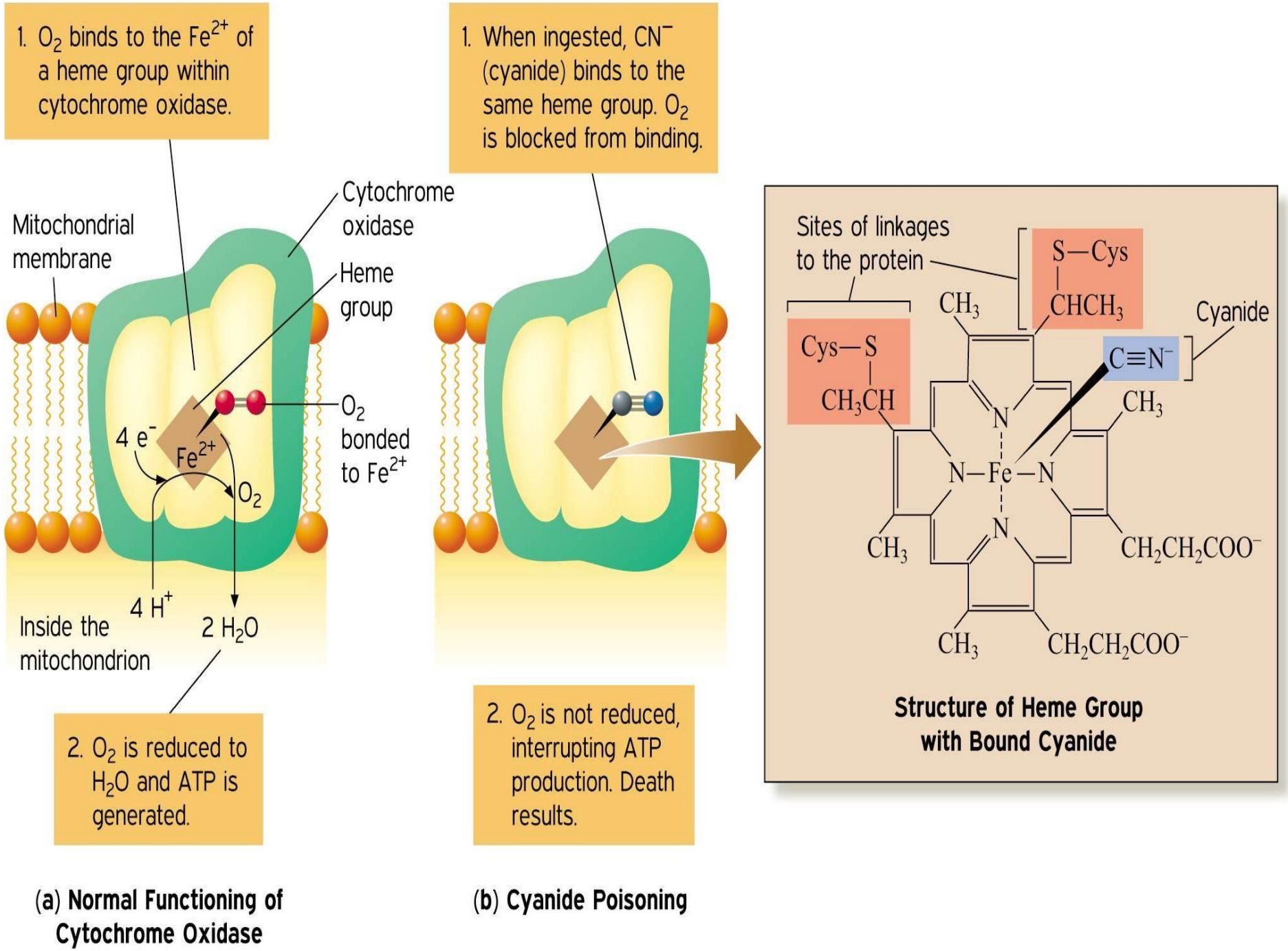
Cyanide, azide, and ***carbon monoxide*** block electron flow in **Complex IV**.

ATP synthase is inhibited by ***oligomycin*** which prevent the influx of protons through ATP synthase.



Inhibitors of cellular respiration




1. O_2 binds to the Fe^{2+} of a heme group within cytochrome oxidase.
Mitochondrial membrane
Cytochrome oxidase
Heme group
O_2 bonded to Fe^{2+}
4 e^-
Fe^{2+}
O_2
4 H^+
2 H_2O
Inside the mitochondrion
2. O_2 is reduced to H_2O and ATP is generated.
(a) Normal Functioning of Cytochrome Oxidase
1. When ingested, CN^- (cyanide) binds to the same heme group. O_2 is blocked from binding.
2. O_2 is not reduced, interrupting ATP production. Death results.
(b) Cyanide Poisoning
Sites of linkages to the protein
S—Cys
CHCH$_3$
Cyanide
Cys—S
CH$_3$CH
C≡N$^-$
CH$_3$
N
N—Fe—N
N
CH$_3$
CH$_2$CH$_2$COO$^-$
CH$_3$
CH$_2$CH$_2$COO$^-$
Structure of Heme Group with Bound Cyanide

Uncouplers (uncoupling agents)

Normally, the ETC and oxidative phosphorylation (i.e. synthesis of ATP) are coupled; when the ETC is functioning, synthesis of ATP takes place.

A portion of energy produced in the ETC is accumulated in ATP, and the other portion of energy is lost as heat.

Mitochondria can become partially uncoupled if the inner membrane loses its structural integrity.

They are said to be 'leaky', because protons can diffuse through the inner membrane without involving ATP synthase.

This occurs if isolated mitochondria are treated with mild detergents that disrupt the inner membrane, or if they have been stored for a period of time.

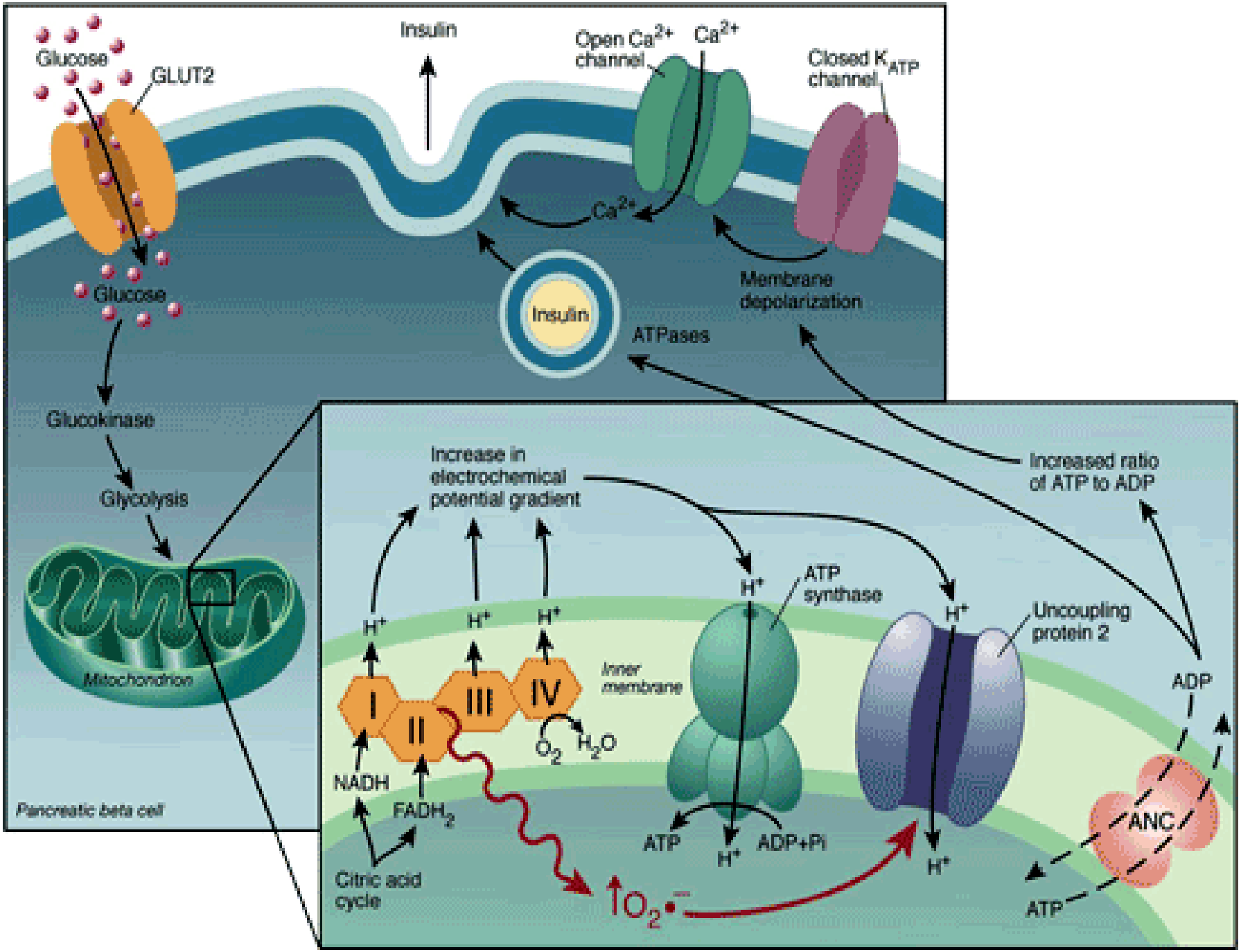
Uncouplers (uncoupling agents)

Uncoupler agents uncouple the ETC and oxidative phosphorylation. As a result, the ETC keeps functioning but ATP production does not occur, and all the energy generated in the ETC is released as heat.

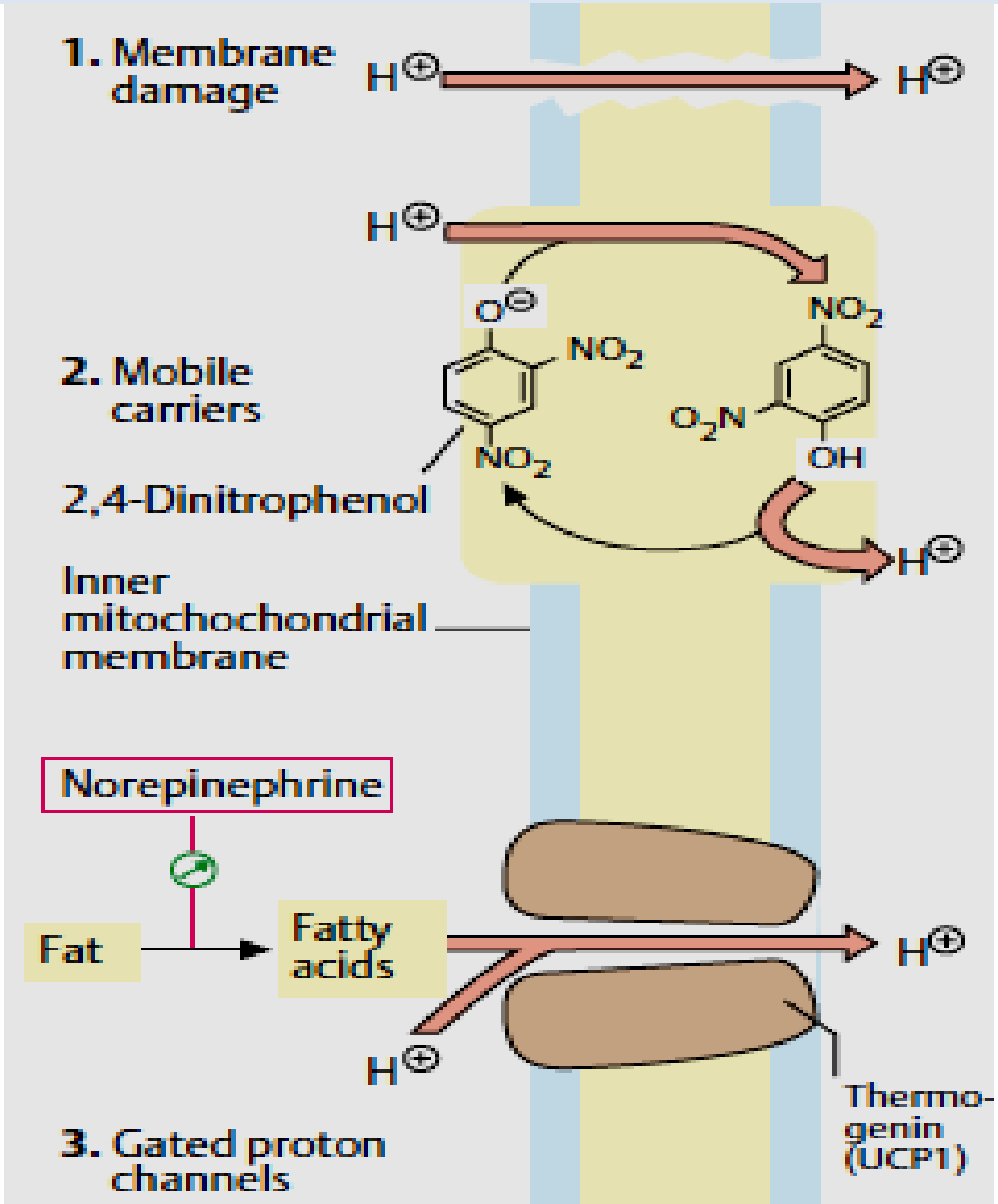
Symptoms of the uncoupler action are hyperthermia and muscle weakness.

Uncouplers (uncoupling agents)

Uncouplers of oxidative phosphorylation dissipate the proton gradient by transporting protons back into mitochondria, bypassing the ATP synthase. Uncouplers are typically hydrophobic compounds.


Glucose
GLUT2
Glucose
Glucokinase
Glycolysis
Mitochondrion
Pancreatic beta cell
Insulin
Open Ca^{2+} channel
Ca^{2+}
Closed K_{ATP} channel
Ca^{2+}
Insulin
Membrane depolarization
ATPases
Increase in electrochemical potential gradient
Increased ratio of ATP to ADP
H^+
H^+
H^+
I
II
III
IV
Inner membrane
O_2
H_2O
NADH
$FADH_2$
Citric acid cycle
H^+
ATP synthase
ATP
H^+
ADP+Pi
H^+
Uncoupling protein 2
H^+
$\uparrow O_2 \bullet^-$
ADP
ANC
ATP

Uncouplers (uncoupling agents)



Uncoupling proteins

The first discovered was uncoupling protein-1 (UCP1), formerly known as thermogenin, which is found exclusively in brown adipose tissue. The sole function of UCP1 is to provide body heat during cold stress. It accomplishes this by uncoupling the proton gradient, thereby generating heat (thermogenesis) instead of ATP.

Uncoupling proteins

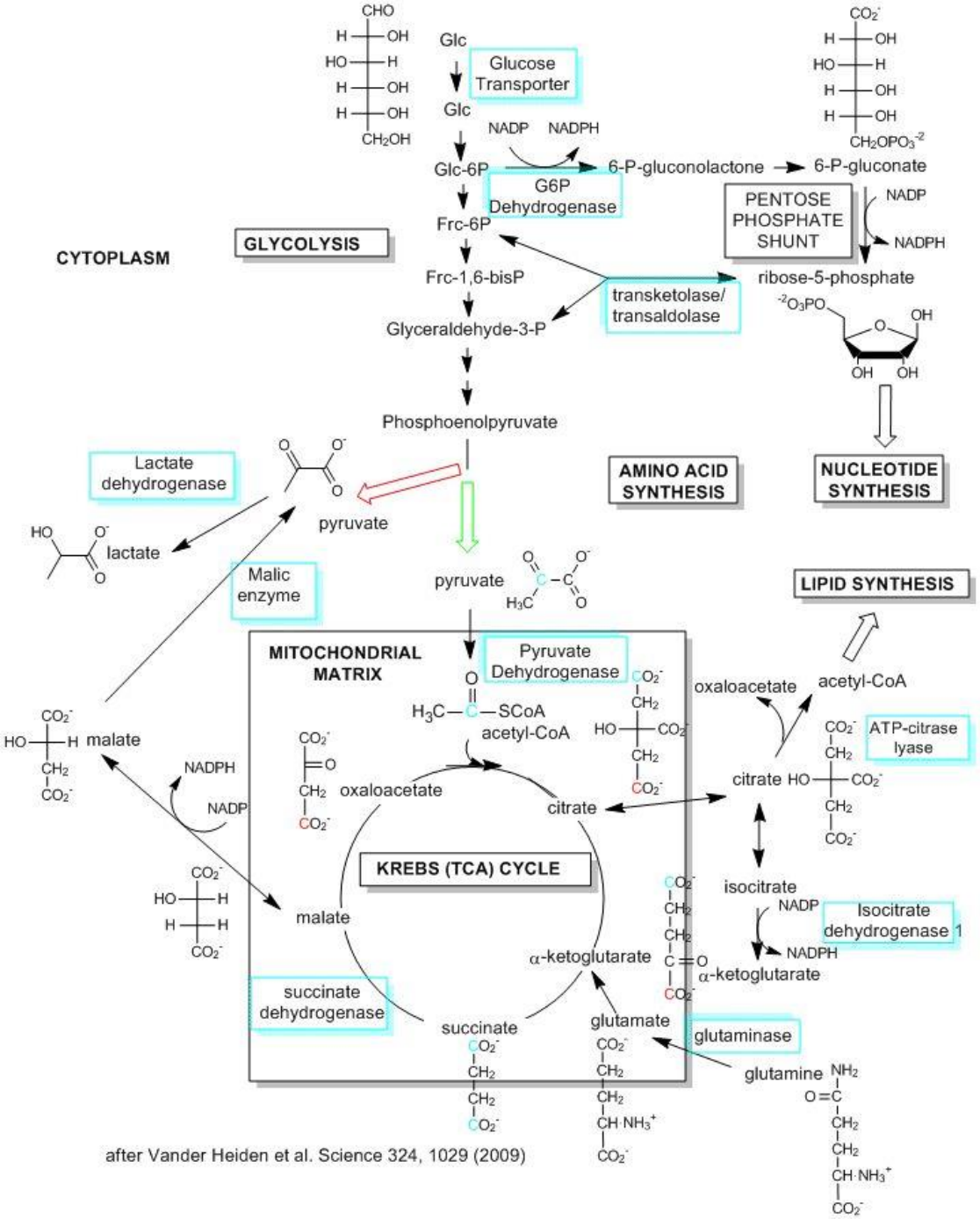
4 additional uncoupling proteins are expressed by the human genome, UCP2, UCP3, UCP4 and UCP5. UCP2 is expressed ubiquitously, UCP3 is mainly expressed in skeletal muscle, and UCP4 and UCP5 are expressed in the brain.

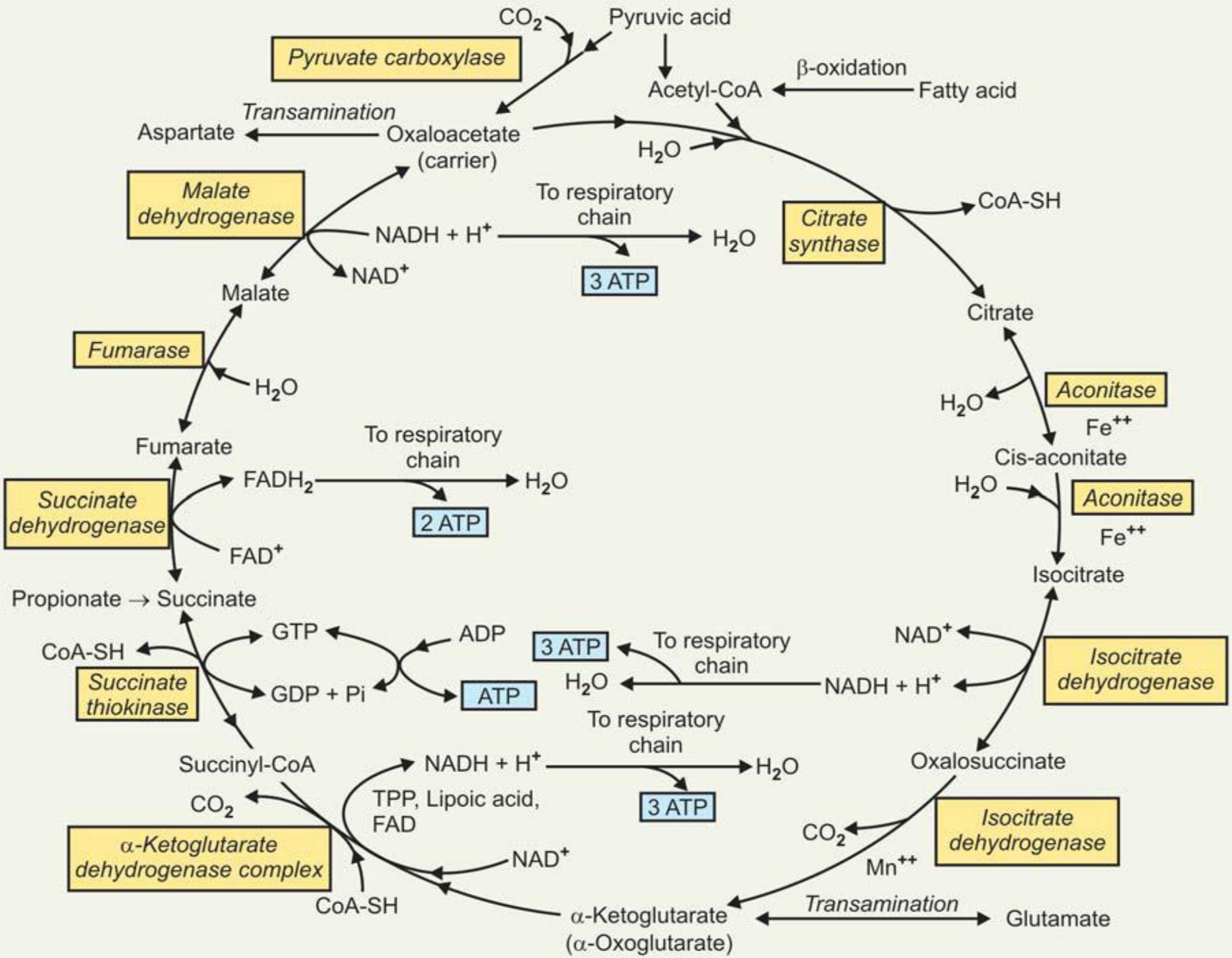
The thyroid hormone has been shown to stimulate thermogenesis by promoting the synthesis of UCP3 in skeletal muscle.

The tricarboxylic acid cycle (the Krebs' cycle)

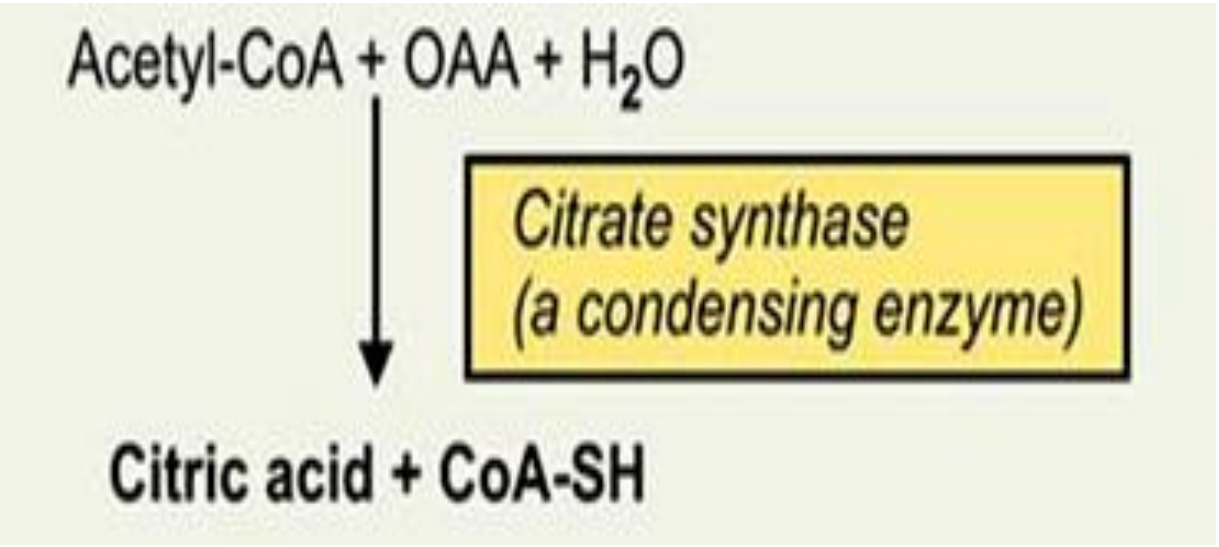
The tricarboxylic acid cycle is a common pathway for metabolism of all fuels.
It oxidatively strips electrons from fat, carbohydrate and protein fuels, producing the majority of the reduced coenzymes that are used for the generation of ATP in the electron transport chain.

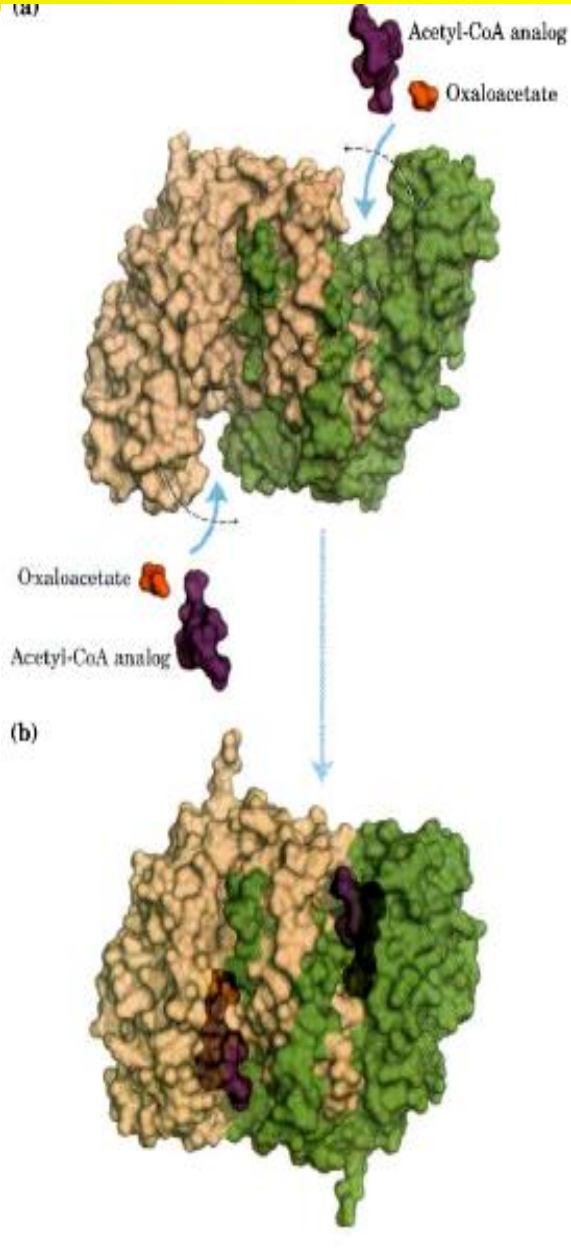
Krebs Hans Adolf (1900-1981)


Glc
Glucose Transporter
Glc
NADP
NADPH
Glc-6P
6-P-gluconolactone
6-P-gluconate
G6P Dehydrogenase
PENTOSE PHOSPHATE SHUNT
NADP
NADPH
Frc-6P
CYTOPLASM
GLYCOLYSIS
Frc-1,6-bisP
transketolase/ transaldolase
ribose-5-phosphate
Glyceraldehyde-3-P
Phosphoenolpyruvate
Lactate dehydrogenase
pyruvate
lactate
AMINO ACID SYNTHESIS
NUCLEOTIDE SYNTHESIS
Malic enzyme
pyruvate
LIPID SYNTHESIS
MITOCHONDRIAL MATRIX
Pyruvate Dehydrogenase
acetyl-CoA
oxaloacetate
acetyl-CoA
ATP-citrase lyase
malate
NADPH
NADP
oxaloacetate
citrate
citrate
KREBS (TCA) CYCLE
isocitrate
NADP
NADPH
Isocitrate dehydrogenase 1
malate
α-ketoglutarate
α-ketoglutarate
succinate dehydrogenase
succinate
glutamate
glutaminase
glutamine
after Vander Heiden et al. Science 324, 1029 (2009)


CO_2
Pyruvic acid
Pyruvate carboxylase
β-oxidation
Acetyl-CoA
Fatty acid
Transamination
Aspartate
Oxaloacetate
(carrier)
H_2O
Malate dehydrogenase
To respiratory chain
CoA-SH
Citrate synthase
$NADH + H^+$
H_2O
NAD^+
3 ATP
Malate
Citrate
Fumarase
H_2O
H_2O
Aconitase
Fe^{++}
Fumarate
To respiratory chain
Cis-aconitate
$FADH_2$
H_2O
H_2O
Aconitase
Succinate dehydrogenase
2 ATP
Fe^{++}
FAD^+
Isocitrate
Propionate → Succinate
GTP
ADP
3 ATP
To respiratory chain
NAD^+
CoA-SH
Succinate thiokinase
GDP + Pi
ATP
H_2O
$NADH + H^+$
Isocitrate dehydrogenase
To respiratory chain
Succinyl-CoA
$NADH + H^+$
H_2O
Oxalosuccinate
CO_2
TPP, Lipoic acid, FAD
3 ATP
CO_2
Isocitrate dehydrogenase
α-Ketoglutarate dehydrogenase complex
NAD^+
Mn^{++}
CoA-SH
Transamination
α-Ketoglutarate
(α-Oxoglutarate)
Glutamate

Formation of Citric Acid from Acetyl-CoA and Oxaloacetic acid (OAA)





An *irreversible and an exergonic reaction.*

- Acetyl group of acetyl-CoA is transferred to OAA, no oxidation or decarboxylation is involved.
- A molecule of H_2O is required to hydrolyse the "high energy" bond between the acetyl group and CoA, the energy released is used for citrate condensation.
- CoA-SH released is reutilised

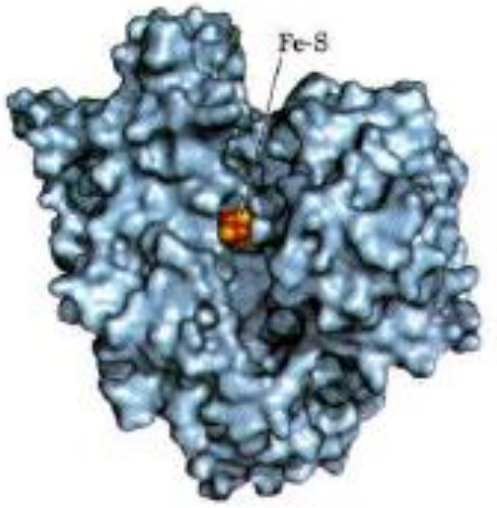
Formation of cis-aconitic acid and isocitric acid from citric acid

Citric acid is converted to isocitric acid by the enzyme ***aconitase.***

***This conversion takes place in* two steps:**

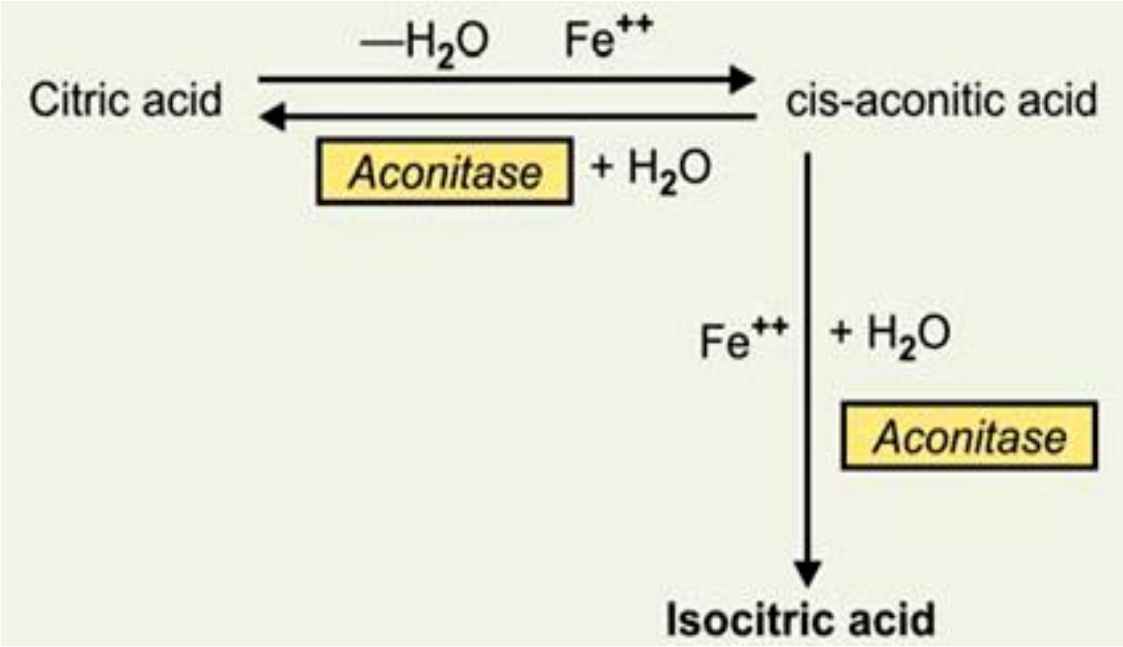
• Formation of cis-aconitic acid from citric acid as a result of ***dehydration, and***

• Formation of isocitric acid from cis-aconitic acid as a result of ***rehydration.***

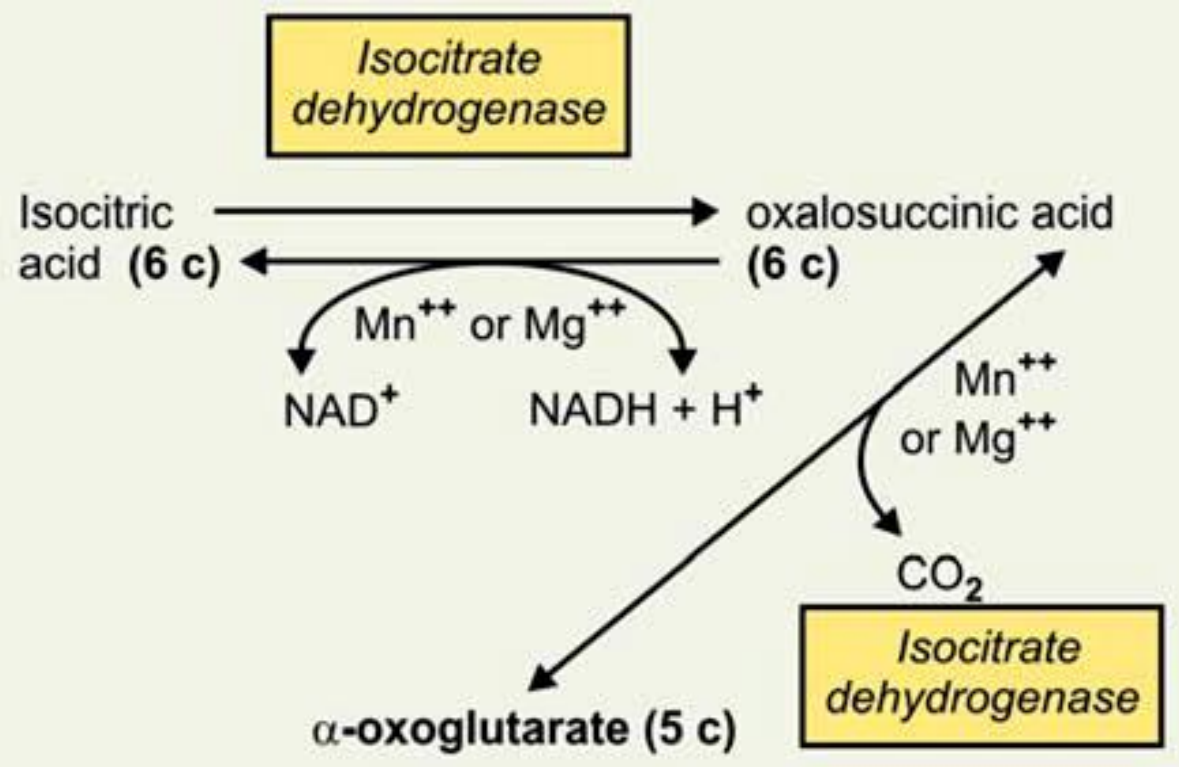


Both processes are catalysed by the same enzyme

ACONITASE which requires Fe^{++}.

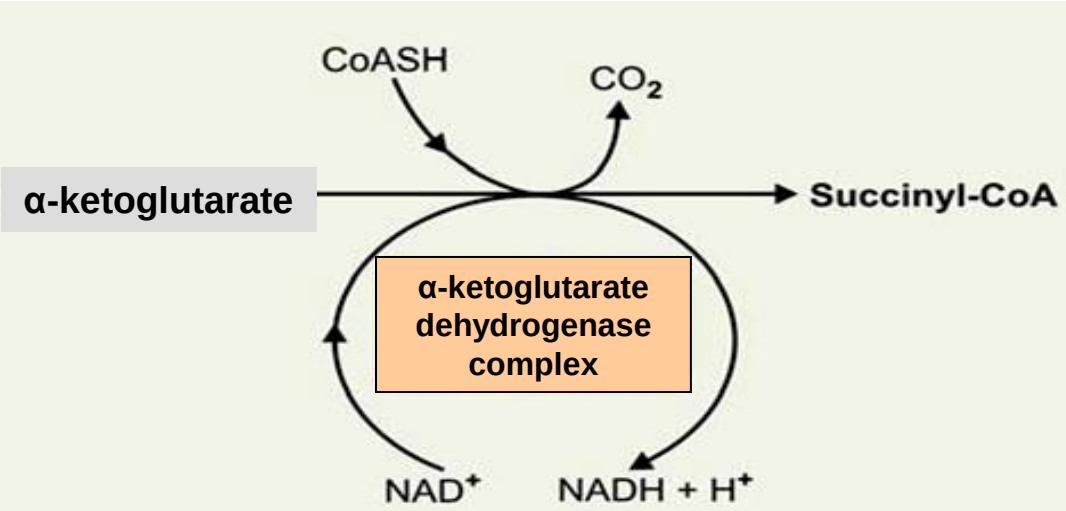


Formation of oxalosuccinic acid and α-ketoglutarate from isocitric acid



These two reactions are catalyzed by a single enzyme.
It is believed that oxalosuccinate is not a free intermediate but rather exists bound to the enzyme.

Oxidative decarboxylation of α-ketoglutarate to succinyl-CoA



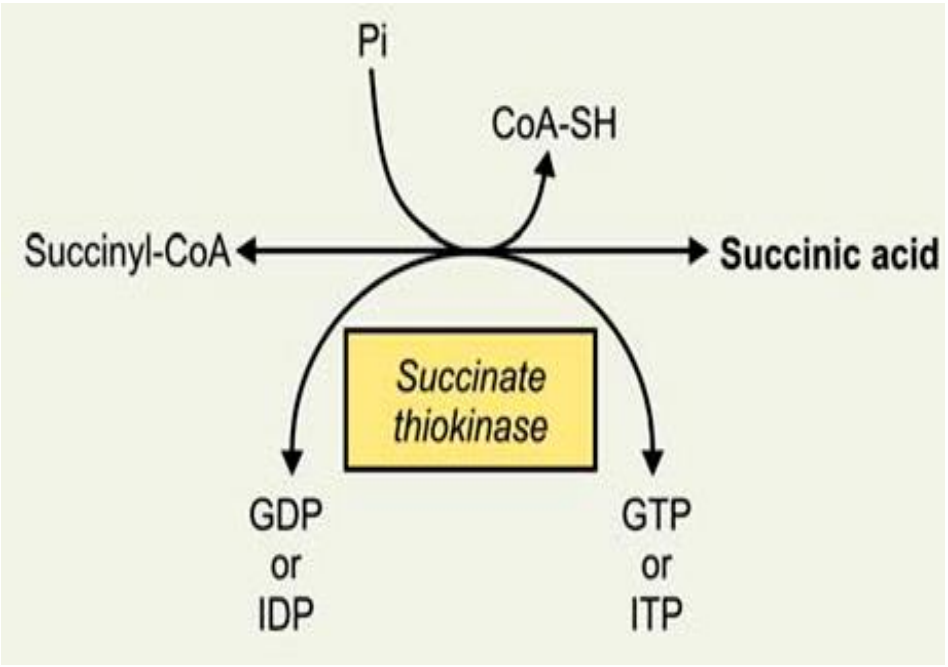
Enzyme is α-ketoglutarate dehydrogenase complex. It requires next coenzymes and cofactors: **TDP, Lipoic acid, CoASH, FAD, NAD^+ and Mg^{++}.**

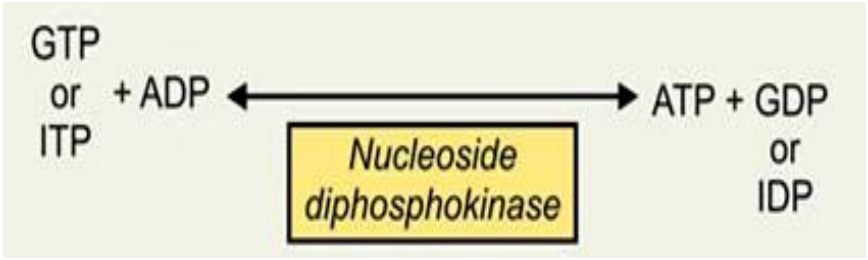
The reaction is ***irreversible.***

The release of free energy from oxidative decarboxylation of α-ketoglutarate is sufficient to generate a high energy bond in addition to the formation of NADH.

The succinyl-CoA syhthetase reaction

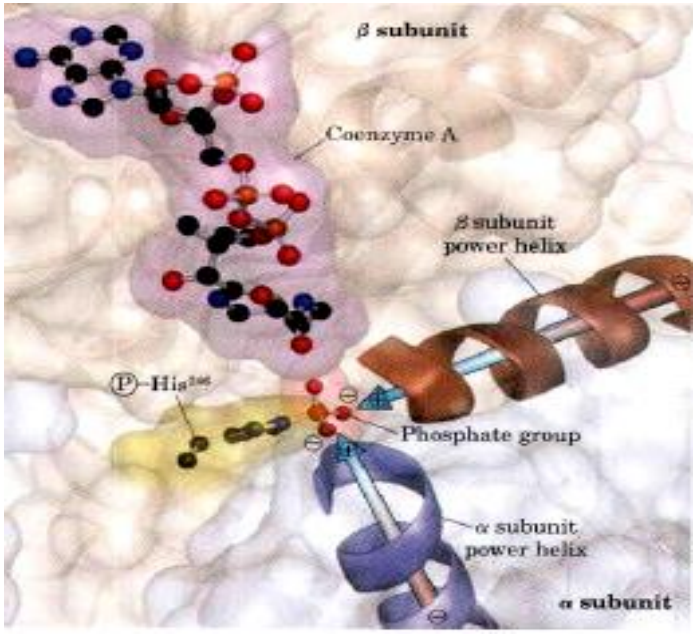
Enzyme catalysing this reaction is *succinate thiokinase (also called as succinyl-CoA synthetase).*



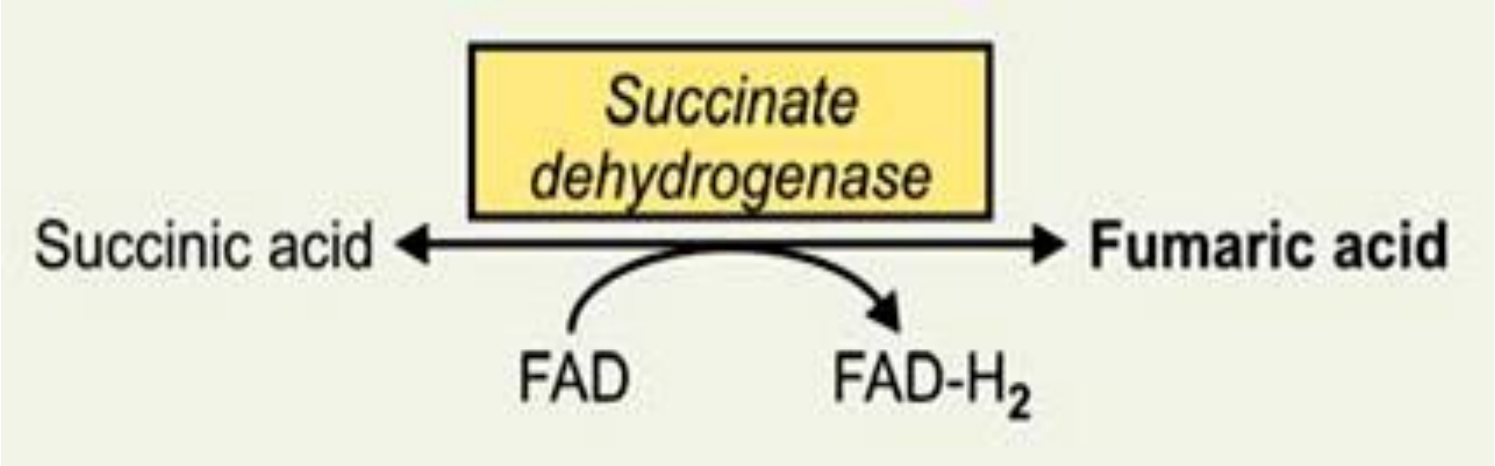


Reaction requires GDP, which is converted in presence of Pi to GTP.
In presence of enzyme *nucleoside diphosphate kinase,* ATP is produced from GTP.

Thus, **ATP is produced at substrate level without** participation of electron transport chain. ***This is the only example of substrate level phosphorylation in TCA cycle.***



Oxidation of succinic acid to fumaric acid

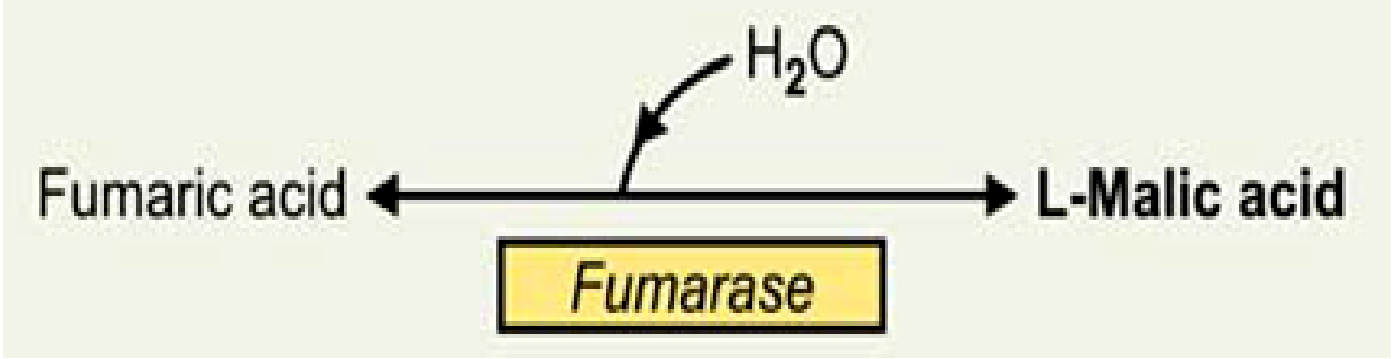


It is a dehydrogenation reaction catalysed by the enzyme *succinate dehydrogenase, hydrogen acceptor is FAD.*

The enzyme is Ferri-flavoprotein, containing FAD and Iron-sulphur.

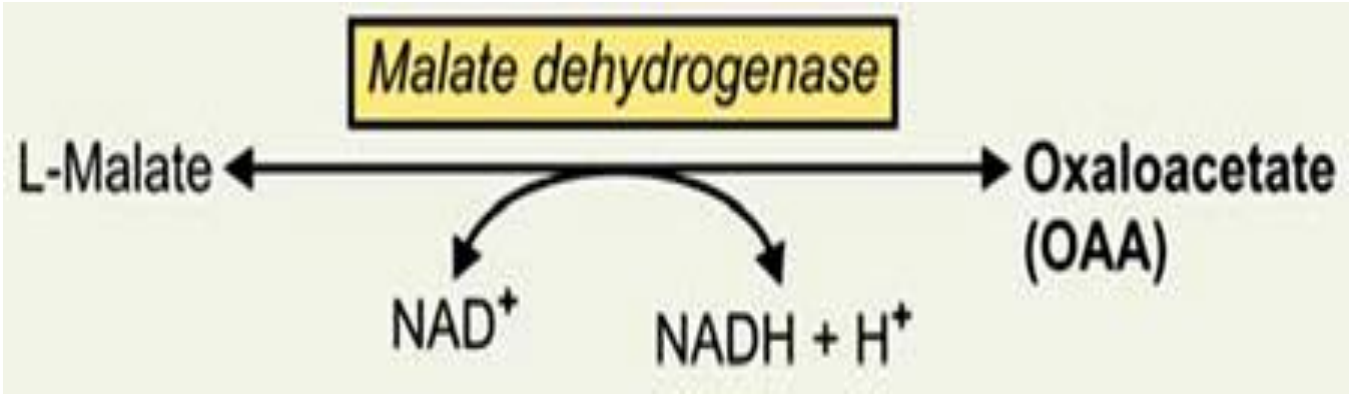
In contrast to other enzymes of TCA cycle, this enzyme is bound to the inner surface of the inner mitochondrial membrane.

Formation of Malic Acid from Fumaric Acid



Oxidation of malic acid to oxaloacetate (OAA):

***The* reaction is catalyzed by the enzyme *Malate dehydrogenase* which requires NAD^+ as H-acceptor**.



PROCESS: CITRIC ACID CYCLE

All 8 reactions of the citric acid cycle occur in the mitochondrial matrix, outside the cristae

The two red carbons enter the cycle via acetyl CoA

Acetyl CoA

Citrate

Isocitrate

CO_2

NADH

NAD+

H_2O

HS—CoA

In each turn of the cycle, the two blue carbons are converted to CO_2

α-Ketoglutarate

CO_2

NAD+

NADH

HS—CoA

Oxaloacetate

The CITRIC ACID CYCLE

runs twice for each glucose precursor

NADH

NAD+

Succinyl CoA

HS—CoA

GTP

GDP

ATP

or

ADP

Malate

In the next cycle, this red carbon becomes a blue carbon

H_2O

FAD

Fumarate

Succinate

$FADH_2$

Each reaction is catalyzed by a different enzyme

ROLE OF VITAMINS IN TCA CYCLE

Five vitamins are associated with TCA cycle essential for yielding energy

- **Riboflavin (B_2)**: In the form of flavin adenine dinuleotide (FAD)- a cofactor for succinate dehydrogenase enzyme.
- **Niacin (PP)**: in the form of nicotinamide adenine dinucleotide (NAD) the electron acceptor for isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, and malate dehydrogenase.
- **Thiamine (B_1)**: as "thiamine diphosphate"- required as coenzyme for decarboxylation in the α-ketoglutarate dehydrogenase reaction.
- **Lipoic acid**: it is required as coenzyme for α-ketoglutarate dehydrogenase reaction.
- **Pantothenic acid (B_5)**: as part of coenzyme A, the cofactor attached to "active" carboxylic acid residues such as acetyl CoA and succinyl-CoA.

The functions of the TCA cycle

1. Catabolic

TCA cycle is the final common pathway for the oxidation of carbohydrates, lipids, and proteins because glucose, fatty acids and most amino acids are metabolized to acetyl-CoA or intermediates of the cycle

The functions of the TCA cycle

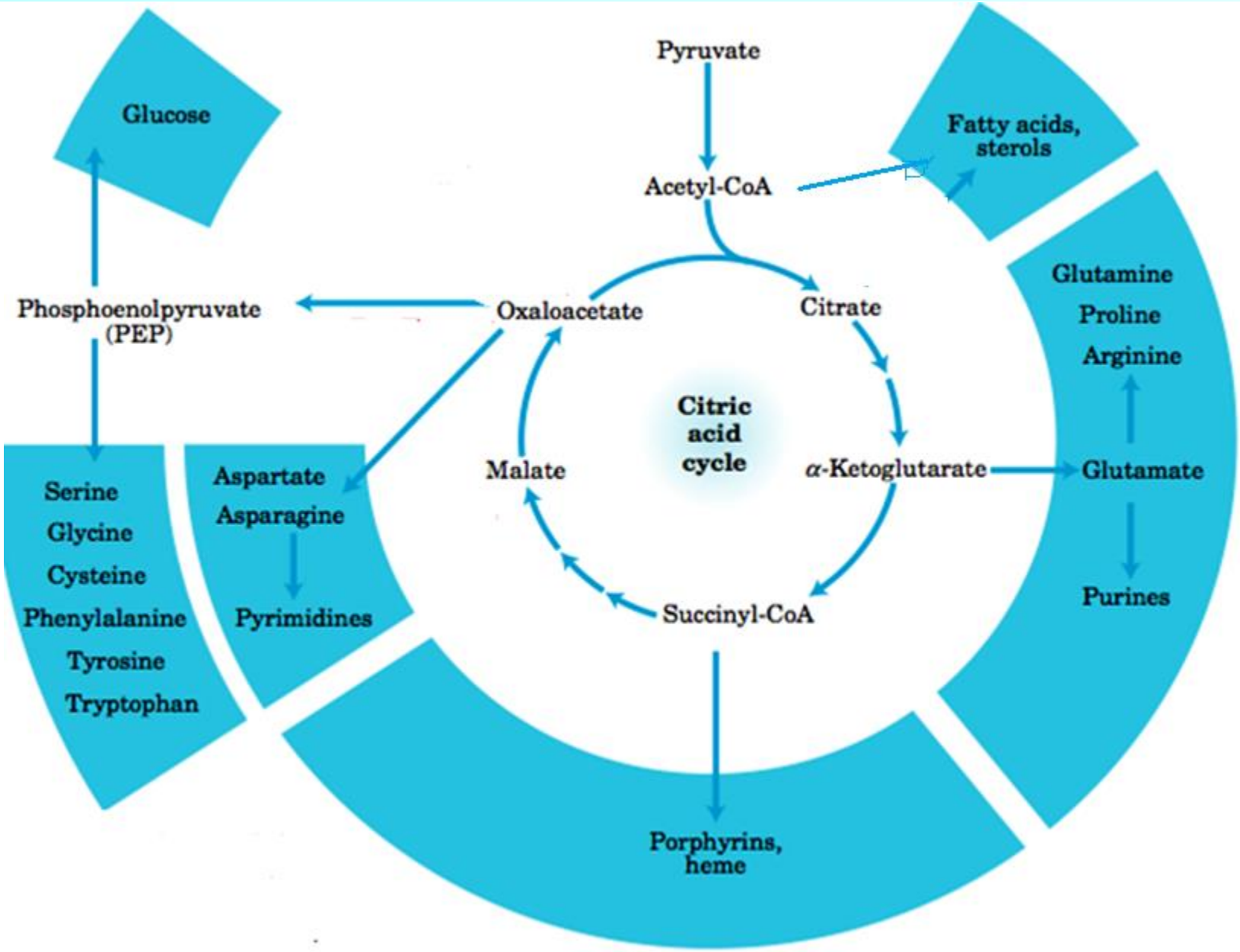
2. Anabolic function

metabolites of the cycle may serve

as substrates

in a variety of biosynthetic reactions

Anabolic (synthetic) function of TCA



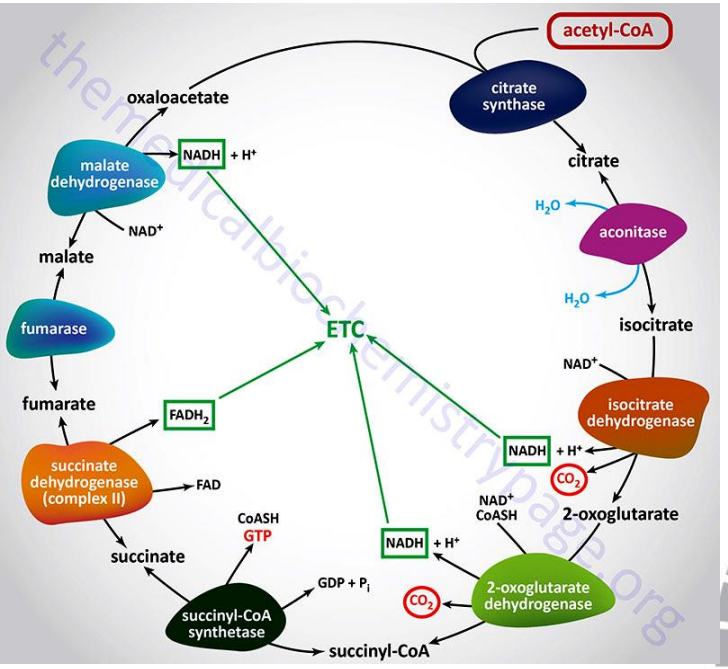
The functions of the TCA cycle

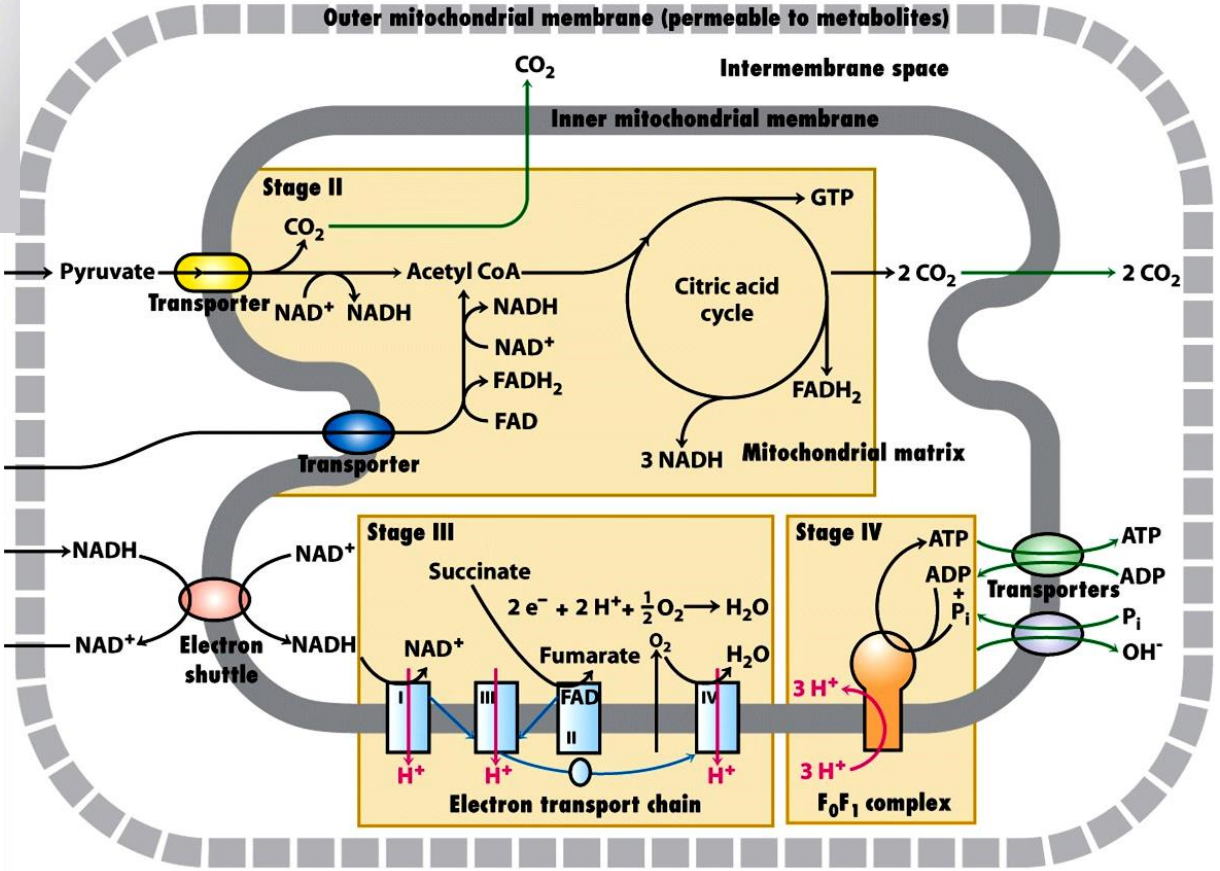
3. Energy production

the function of the cycle is the harvesting of electrons from carbon fuels

generates approximately 12 molecules of ATP per turn of the cycle

Relation of TCA cycle whith ETC





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Energy yield of TCA cycle

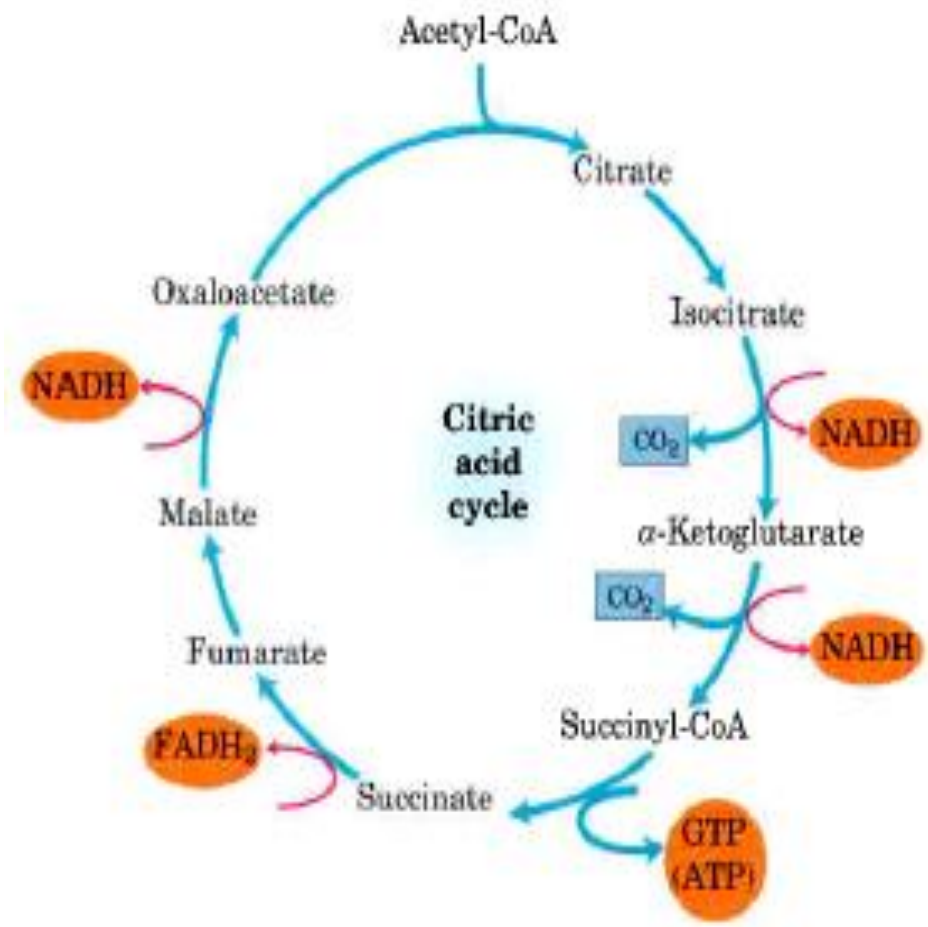
1. **Isocitrate dehydrogenase**
NADH → NAD+ **3 ATP**

2. α-Ketoglutarate dehydrogenase complex
NADH → NAD+ **3 ATP**

3. Substrate level phosphorylation succinate thiokinase
GTP → ATP **1 ATP**

4. Succinate dehydrogenase
$FADH_2$ → FAD **2 ATP**

5. Malate dehydrogenase
NADH → NAD+ **3 ATP**



Total 12 ATP

The functions of the TCA cycle

4. Integrating function

all types of metabolism (carbohydrate, lipid and amino acid metabolism) can be interrelated through the cycle by conversion of one types of substrate into others

TCA cycle has dual role: catabolic, and anabolic

Catabolic role: The two carbon compound acetyl-CoA produced from metabolism of carbohydrates, lipids and proteins are oxidized in this cycle to produce CO_2, H_2O and energy as ATP.

Anabolic (synthetic role): Intermediates of the cycle are utilized for synthesis of various compounds.

Citric Acid Cycle is
a amphibolic process

Regulation of TCA Cycle

1. **As the primary function of TCA cycle is to provide** energy, respiratory control via the ETC and oxidative phosphorylation exerts the main control.
2. **In addition to this, several** enzymes of TCA cycle are also important in the regulation.

There are several levels of control of the TCA cycle.

The overall activity of the cycle depends on the availability of NAD^+ for the dehydrogenase reactions. This, in turn, is linked to the rate of NADH consumption by the electron transport system, which ultimately depends on the rate of ATP utilization and production of ADP by metabolism.

Thus, as ATP is used for metabolic work, ADP is produced, then NADH is consumed by the electron transport system for ATP production, and NAD^+ is produced. The TCA cycle is activated, fuels are consumed, and more NADH is produced so that more ATP may be made.

Three Key enzymes are:

- ***Citrate synthase***
- ***Isocitrate dehydrogenase***
- **α-*ketoglutarate dehydrogenase***

These enzymes are responsive to the energy status as expressed by the ATP/ADP ratio and $NADH/NAD^+$ ratio

Regulation of TCA

- **Major sites for regulation:**
- ***Citrate synthase***

A: ADP

I: ATP, long-chain acyl-CoA

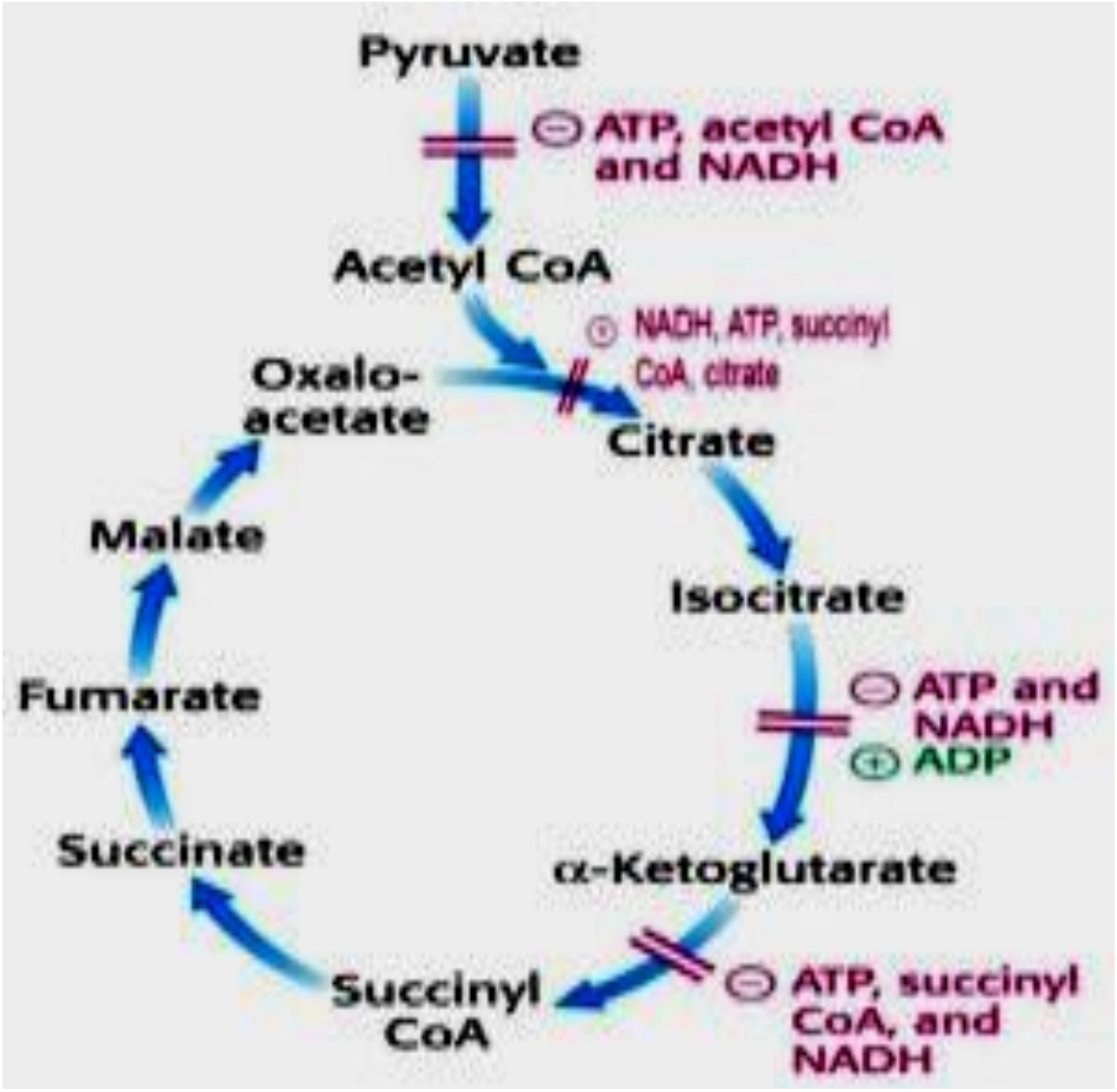
- ***Isocitrate dehydrogenase***

A: ADP

I: ATP, NADH

- ***α-ketoglutarate dehydrogenase***

I: ATP, NADH


Pyruvate
⊖ ATP, acetyl CoA and NADH
Acetyl CoA
⊕ NADH, ATP, succinyl CoA, citrate
Oxalo-acetate
Citrate
Isocitrate
⊖ ATP and NADH
⊕ ADP
α-Ketoglutarate
⊖ ATP, succinyl CoA, and NADH
Succinyl CoA
Succinate
Fumarate
Malate

In addition to above, *succinate dehydrogenase enzyme* is inhibited by OAA and the availability of OAA is controlled by *malate dehydrogenase, which depends on* NADH/NAD^+ ratio.

Oxaloacetate is required for entry of acetyl-CoA into the TCA cycle but, at times, the availability of oxaloacetate appears to regulate the activity of the cycle. This occurs especially during fasting when levels of ATP and NADH, derived from fat metabolism, are increased in the mitochondrion.

The increase in NADH shifts the malate:oxaloacetate equilibrium toward malate, directing TCA cycle intermediates toward malate, which is exported to the cytosol for gluconeogenesis. Acetyl-CoA derived from fat metabolism is directed toward synthesis of ketone bodies because of the lack of oxaloacetate.

ANAPLEROTIC ('FILLING UP') REACTIONS

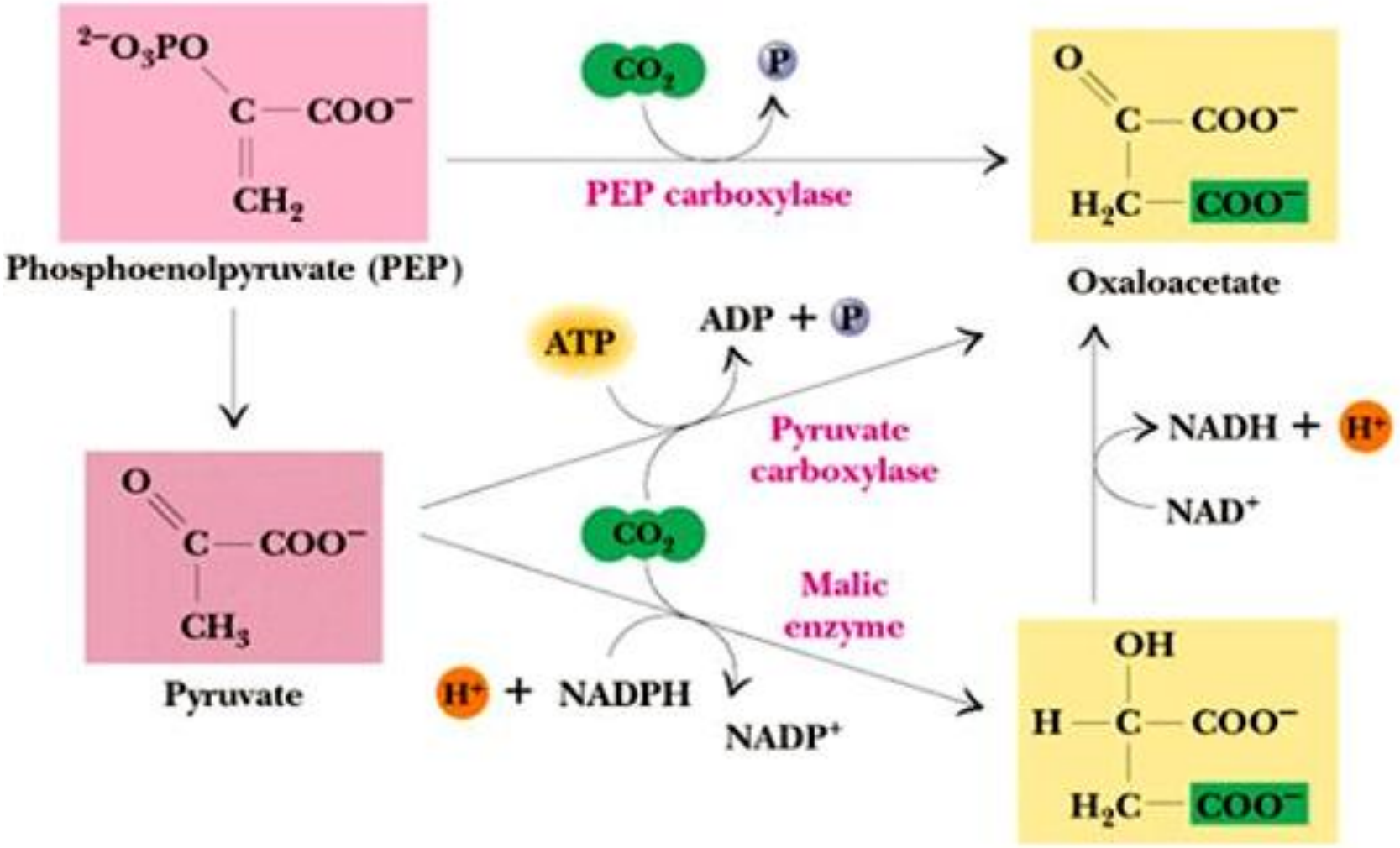
TCA cycle intermediates participate in biosynthetic processes, which all require anaplerosis.

For example, removal of succinyl-CoA for heme biosynthesis could gradually deplete mitochondrial oxaloacetate.

The TCA cycle would cease to function if the intermediates were not replenished, because acetyl-CoA cannot yield a net synthesis of TCA cycle intermediates.

Anaplerotic reactions provide the TCA cycle with intermediates other than acetyl-CoA to maintain activity of the cycle.

- Pyruvate + HCO_3^- + ATP $\leftrightarrow$ Oxaloacetate + ADP +P_i
- Phosphoenolpyruvate + CO_2 + GDP $\leftrightarrow$ Oxaloacetate + GTP
- Phosphoenolpyruvate + HCO_3^- $\leftrightarrow$ Oxaloacetate + P_i (plants, yeast, bacteria)
- Pyruvate + HCO_3^- + NAD(P)H $\leftrightarrow$ malate + $NAD(P)^+$


$^{2-}O_3PO$
C
COO^-
CH_2
Phosphoenolpyruvate (PEP)
CO_2
P
PEP carboxylase
O
C
COO^-
H_2C
COO^-
Oxaloacetate
ATP
ADP + P
Pyruvate carboxylase
NADH + H^+
NAD^+
O
C
COO^-
CH_3
Pyruvate
CO_2
Malic enzyme
H^+ + NADPH
$NADP^+$
OH
H
C
COO^-
H_2C
COO^-

- **Pyruvate carboxylase converts pyruvate to malate, a precursor of oxaloacetate, which is required for initiation of the cycle.**
- **Malic enzyme in the cytoplasm also converts pyruvate to malate, which can enter the mitochondrion as a substrate for the TCA cycle.**
- **α-Ketoglutarate can be produced through an aminotransferase reaction from glutamate, as well as by the glutamate dehydrogenase reaction.**
- **Several other 'glucogenic' amino acids may also serve as sources of pyruvate or TCA cycle intermediates, guaranteeing that the cycle is never stalled because of a lack of intermediates.**