AULAS DE BIOQUÍMICA

METABOLISMO DOS GLÚCIDOS (cont.)

Descarboxilação oxidativa do piruvato. Balanço energético.
Regulação do complexo piruvato desidrogenase.
Ciclo de Krebs. Sua natureza anfibólica, reacções anapleróticas e reacções cataplerótica. Pontos de ligação importantes com outras vias metabólicas. Balanço energético. Regulação.
Fosforilação a nível do substrato.
Ciclo do glioxilato (fundamentos).
Material de estudo: diapositivos das aulas e bibliografia recomendada.

Sara Monteiro

Glicólise, neoglucogénese e via das pentoses-fosfato permitem ajustar às necessidades celulares, os teores de NADPH, ATP, ribose-5-P, ácido pirúvico, glucose

Estudámos:

- **Utilização de glúcidos como fonte de energia**
- **Glicólise e formação do ácido pirúvico**
- **Utilização do ácido pirúvico em anaerobiose**
- **A utilização da glucose em reacções de oxidação**

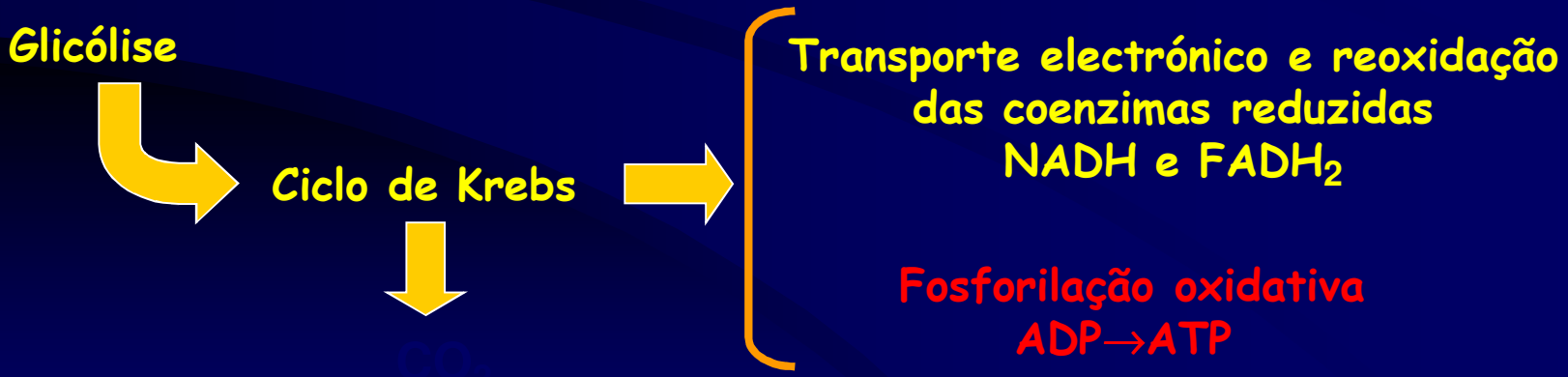
- E em aerobiose?

- E a oxidação da glucose é total?

Em anerobiose não ocorre oxidação total das moléculas orgânicas

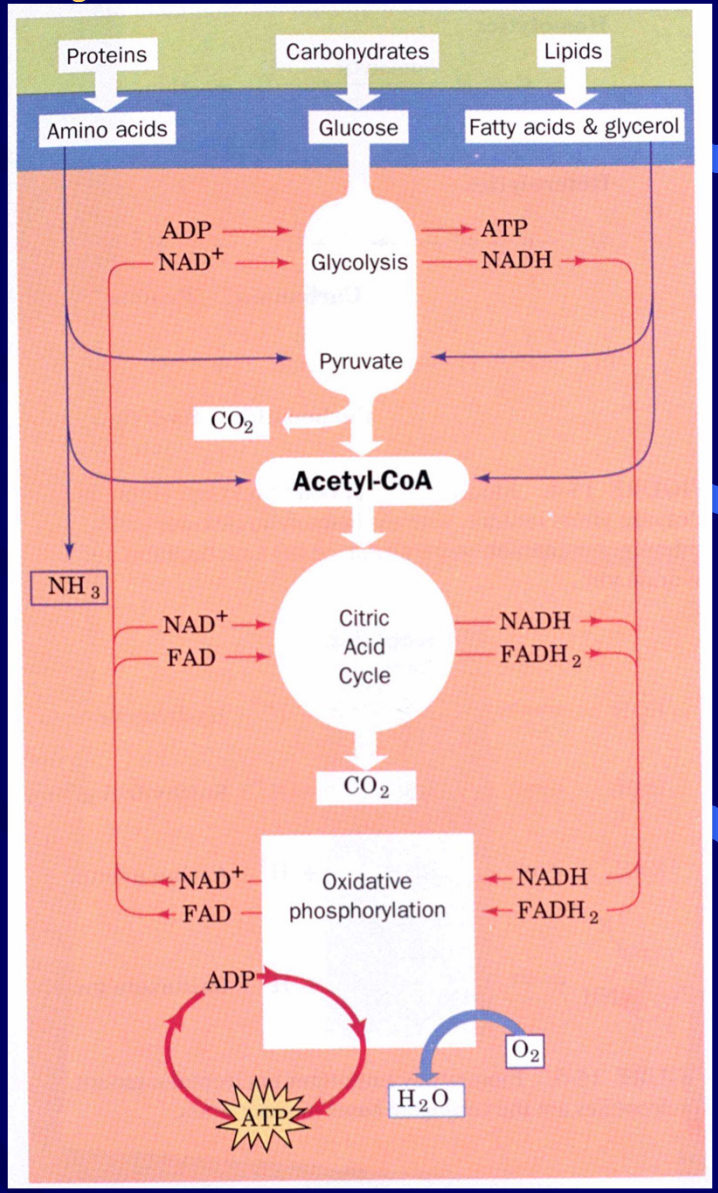
Em aerobiose pode ocorrer oxidação total das moléculas orgânicas com formação de CO_2 e água

- O_2 é o aceitador final dos electrões, formando-se água
- Forma-se CO_2 resultante do carbono existente nos glúcidos
- Grande parte da energia química contida nos glúcidos é "guardada" na forma de ATP

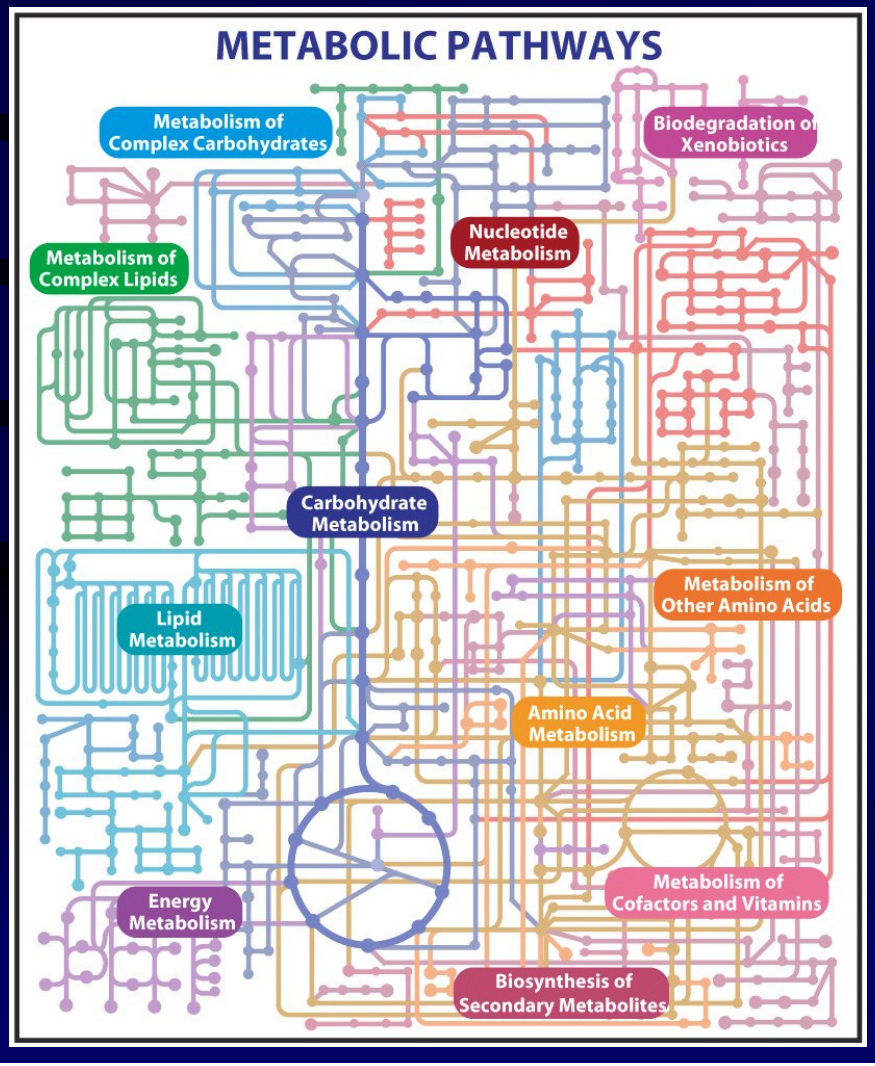


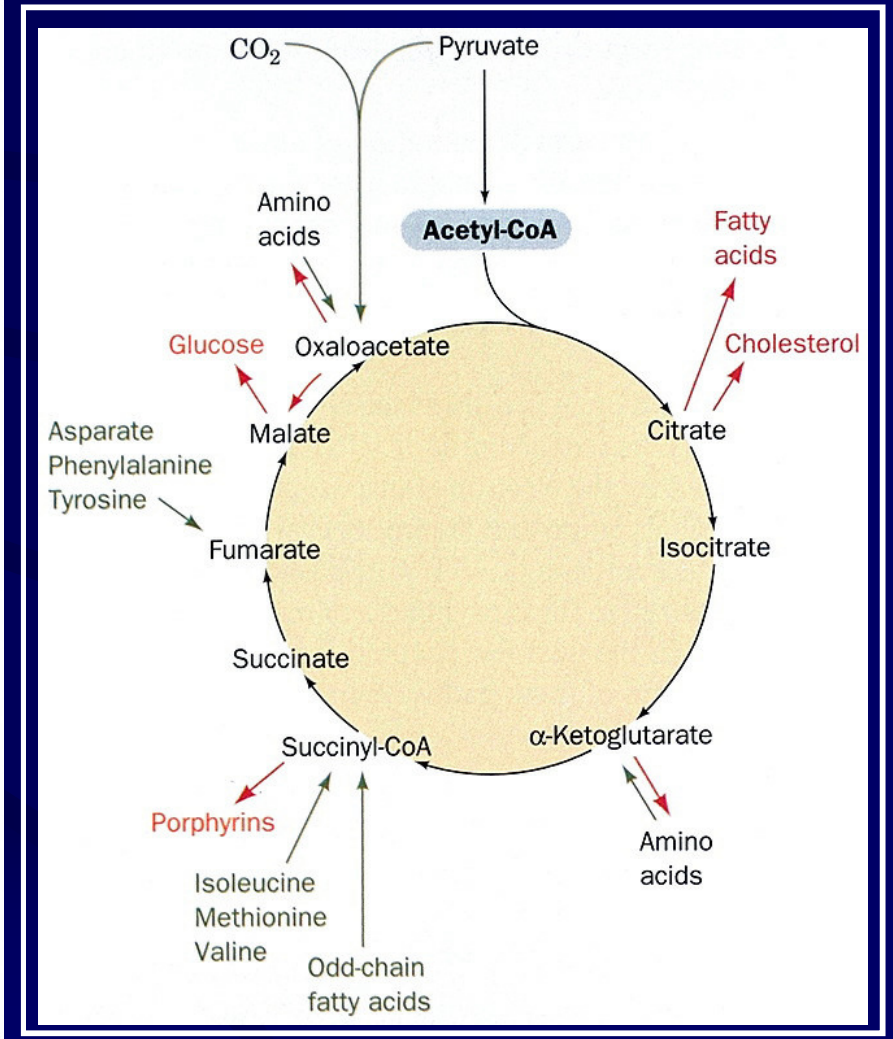
The Citric Acid Cycle

- In aerobic organisms, pyruvate (formed through glycolysis) oxidized to CO_2 and acetyl-CoA using coenzyme A
- Subsequent oxidation of acetyl group carried out using the *citric acid cycle*
- Citric acid cycle is amphibolic
  - Catabolic and anabolic
  - Aerobic catabolism of carbohydrates, lipids, and amino acids merge at citric acid cycle
    - Oxidized acetyl-CoA formed from metabolism of all three
  - Intermediates of citric acid cycle are starting points for many biosynthetic pathways

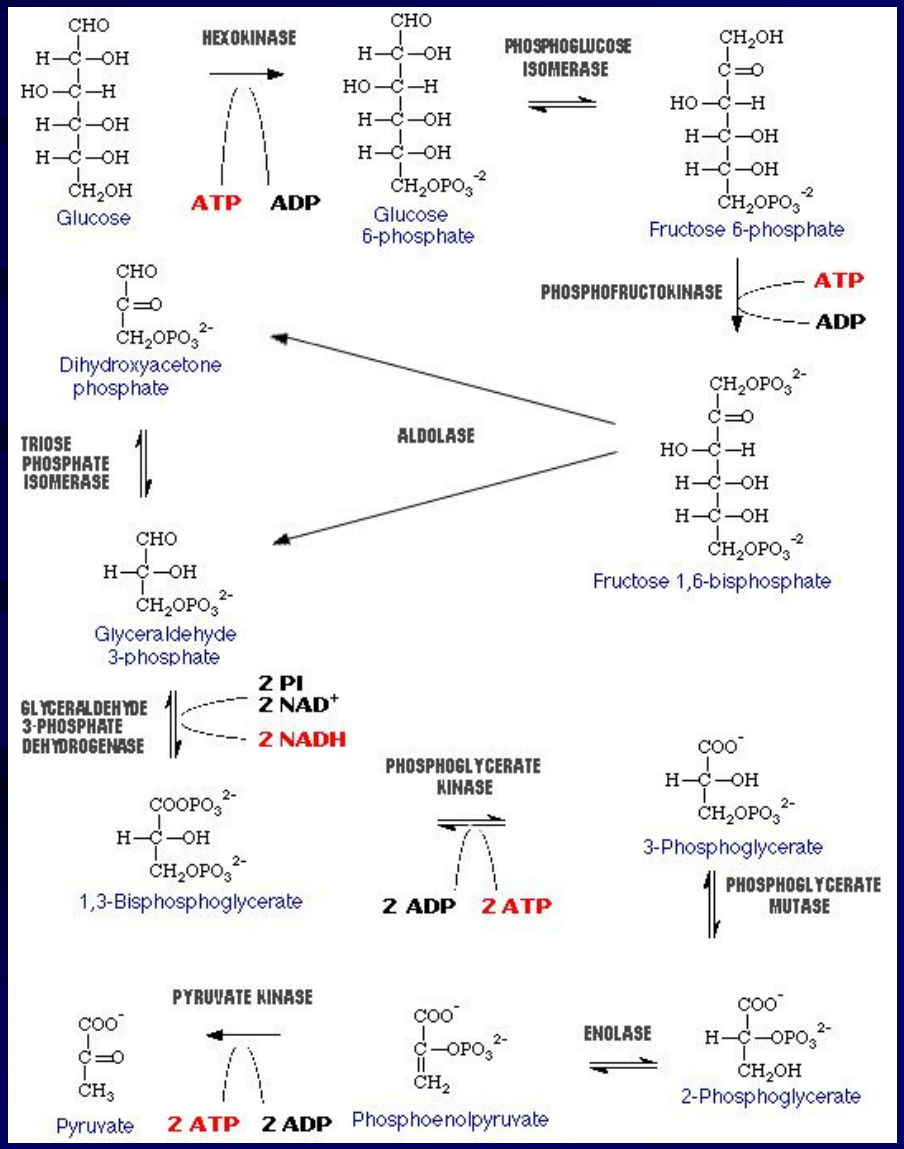


The amphibolic Citric Acid Cycle



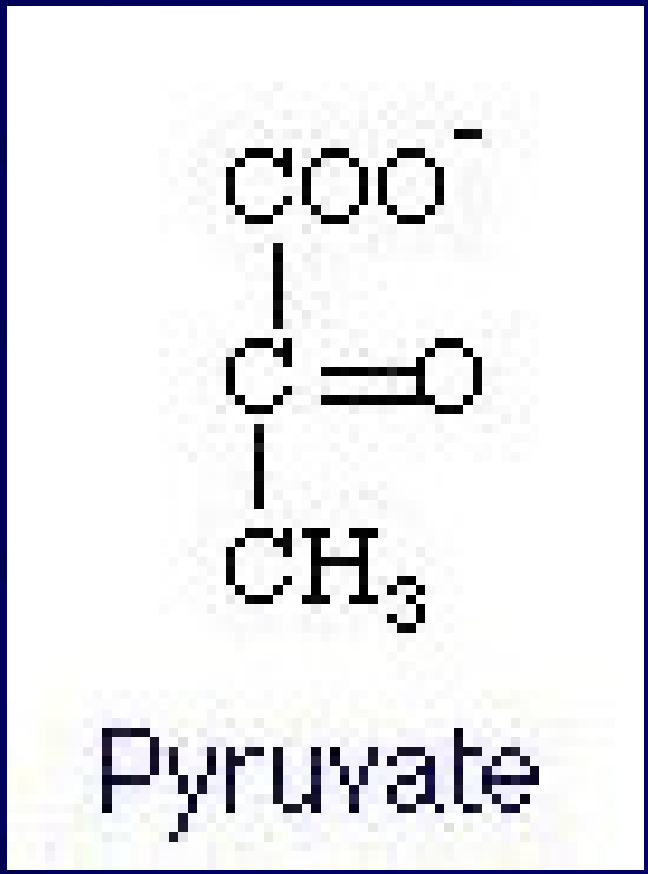


Review of Glycolysis



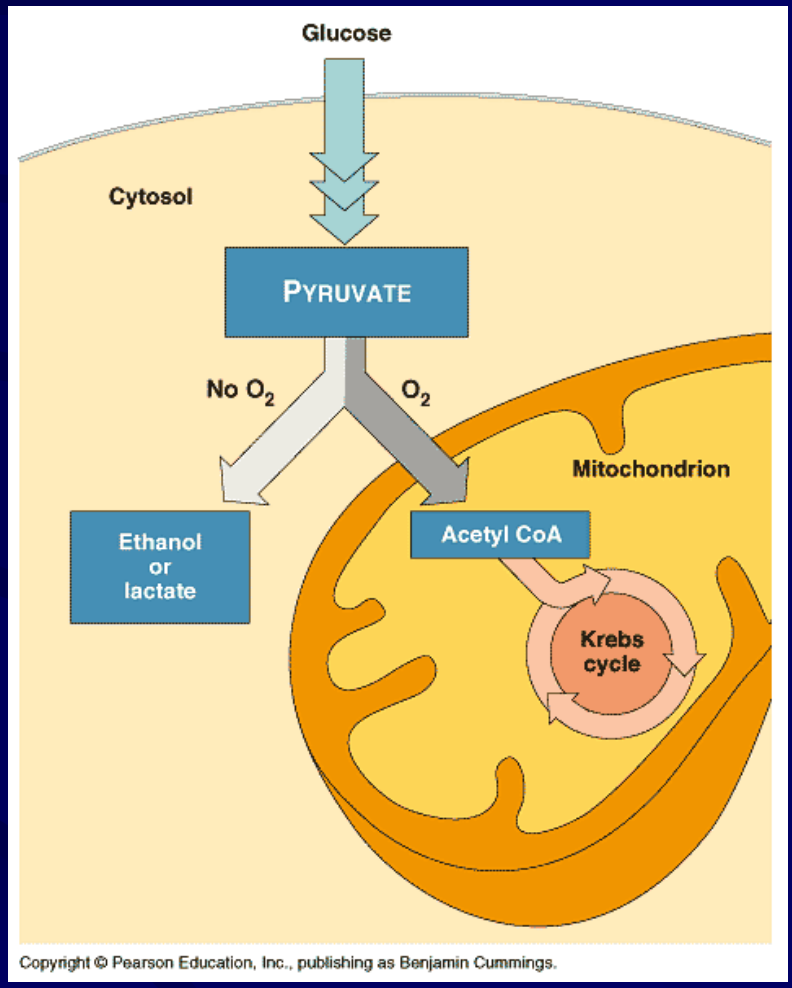
The Fate of Pyruvate

- In the ABSENCE of oxygen - pyruvate gets fermented to oxidize NADH to NAD so that glycolysis (ATP production can continue.

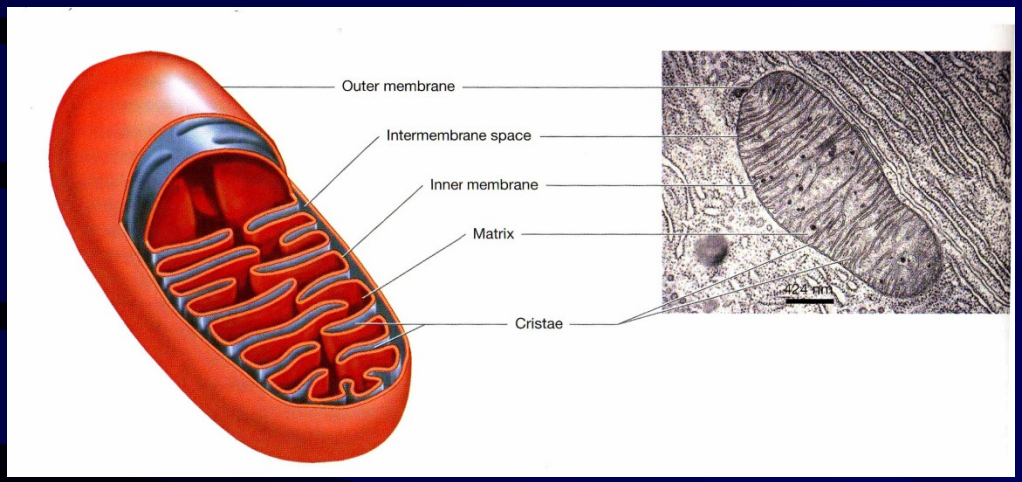


The Fate of Pyruvate

- In the PRESENCE of oxygen - Pyruvate gets turned into Acetyl CoA and then added to other molecules in order to liberate CO_2 (the Calvin Cycle - backwards).



The Assault on Pyruvate



- Pyruvate moves easily passed the porous outer mitochondrial membrane and is then transported through the inner membrane where it encouters…

The Pyruvate Dehydrogenase Multienzyme Complex

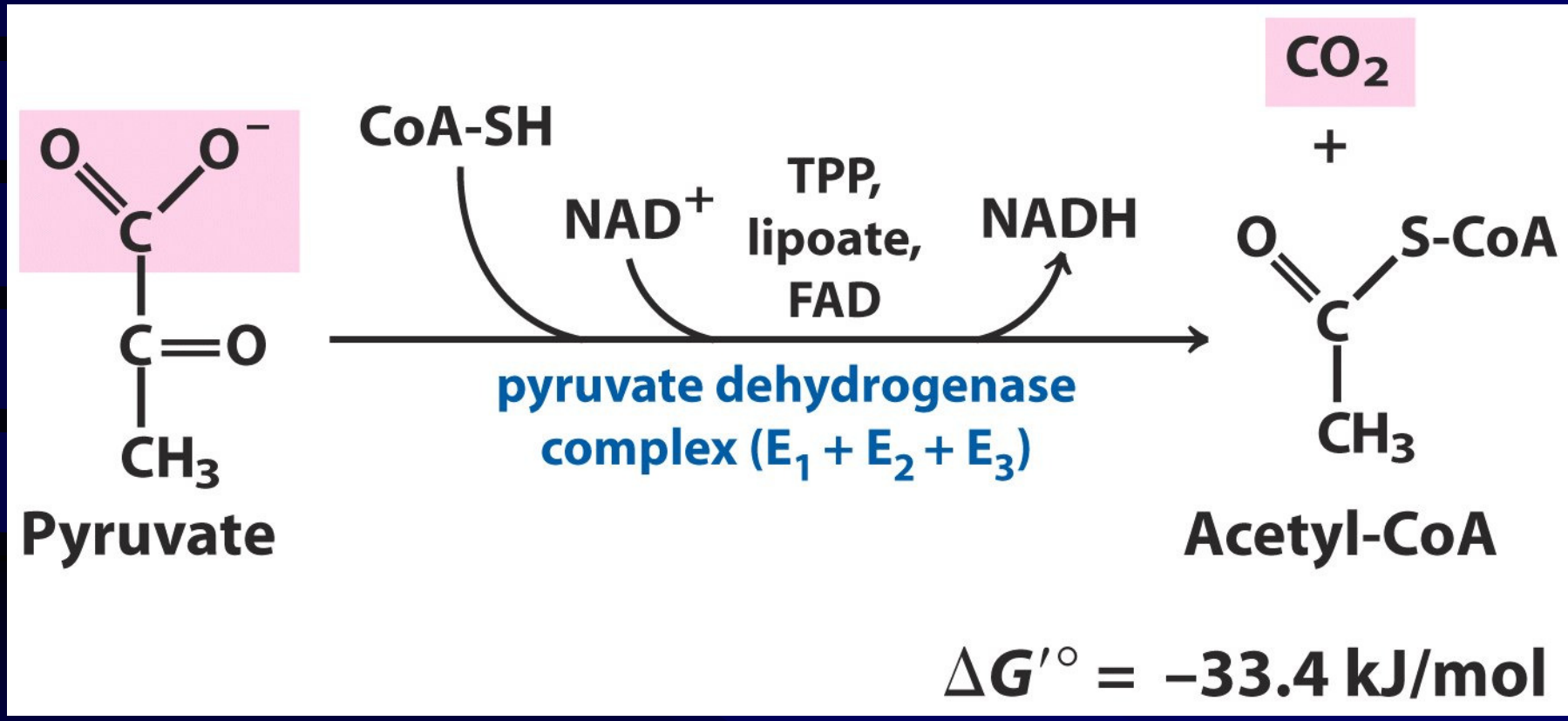
Pyruvate dehydrogenase

- Catalyzes the overall reaction:

  pyruvate + CoA + NAD^+

  $\rightarrow$ acetyl-CoA + CO_2 + NADH

- Sometime referred to as a "multi-vitamin pill", because of all of the cofactors it utilizes.


CO2
+
CoA-SH
NAD+
TPP,
lipoate,
FAD
NADH
S-CoA
pyruvate dehydrogenase
complex (E1 + E2 + E3)
Pyruvate
Acetyl-CoA
ΔG′° = –33.4 kJ/mol

Pyruvate dehydrogenase complex:

E_1 pyruvate dehydrogenase

Es E_2 dihydrolipoyl transacetylase

E_3 dihydrolipoyl dehydrogenase

thiamine pyrophosphate, TPP (VB_1)

cofactors
- HSCoA (pantothenic acid)
- lipoic Acid
- NAD^+ (Vpp)
- FAD (VB_2)

Back to Pyruvate Dehydrogenase

- This is a complex enzyme. It is composed of multiple copies of 3 different polypeptides:
- **E1: pyruvate dehydrogenase**

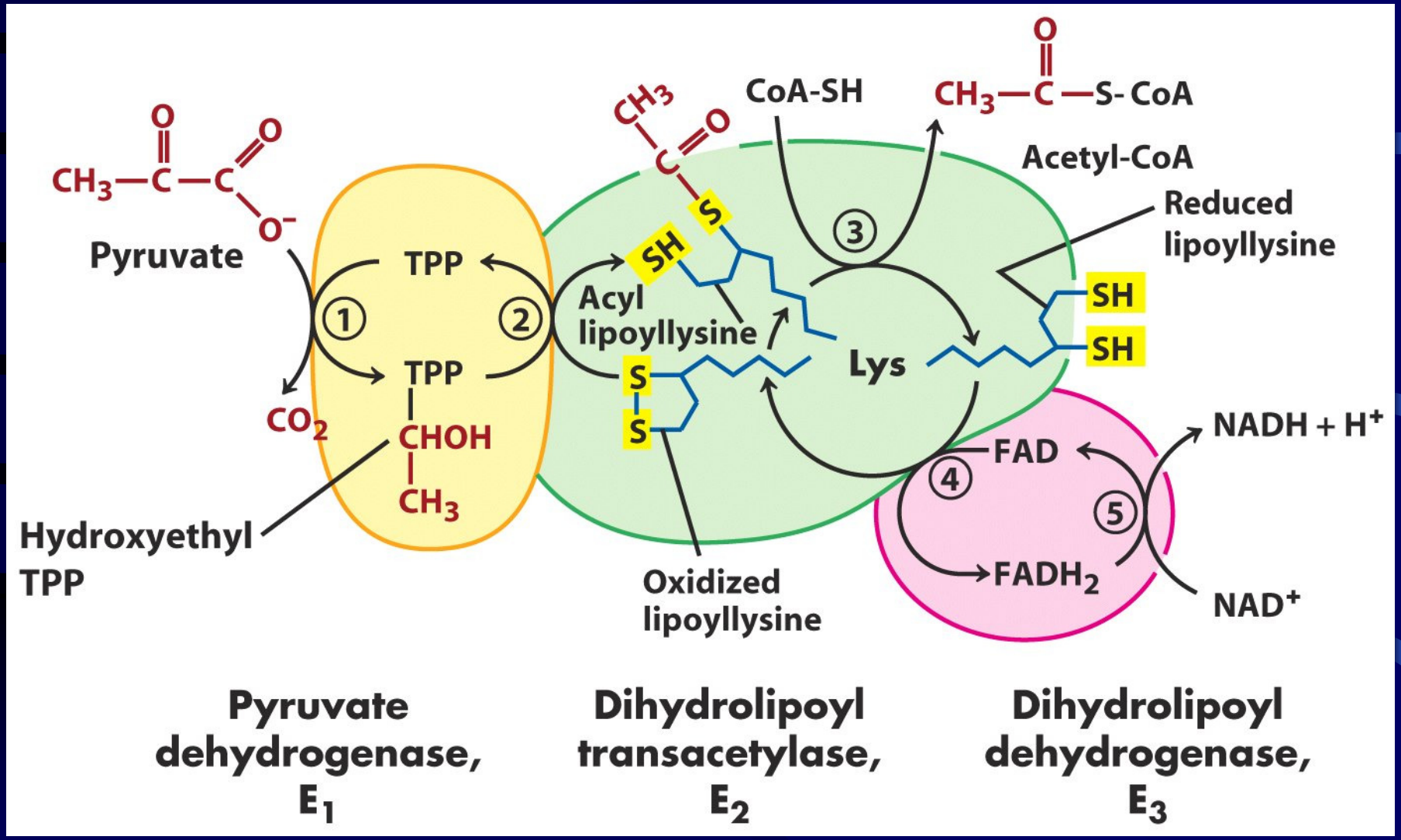
Contains TPP at active site and catalyzes

pyruvate + TPP(E1)

$\rightarrow$ CO_2 + hydroxyethyl-TPP(E1)

hydroxyethyl-TPP(E1) + lipoate(E2)

$\rightarrow$ TPP(E1) + acetyl-lipoate(E2)


Pyruvate
TPP
TPP
CHOH
CH3
CO2
Hydroxyethyl TPP
CH3
C
O
S
CoA-SH
CH3—C—S- CoA
Acetyl-CoA
Reduced lipoyllysine
SH
SH
Acyl lipoyllysine
Lys
S
S
Oxidized lipoyllysine
FAD
FADH2
NADH + H+
NAD+
1
2
3
4
5
Pyruvate dehydrogenase, E1
Dihydrolipoyl transacetylase, E2
Dihydrolipoyl dehydrogenase, E3

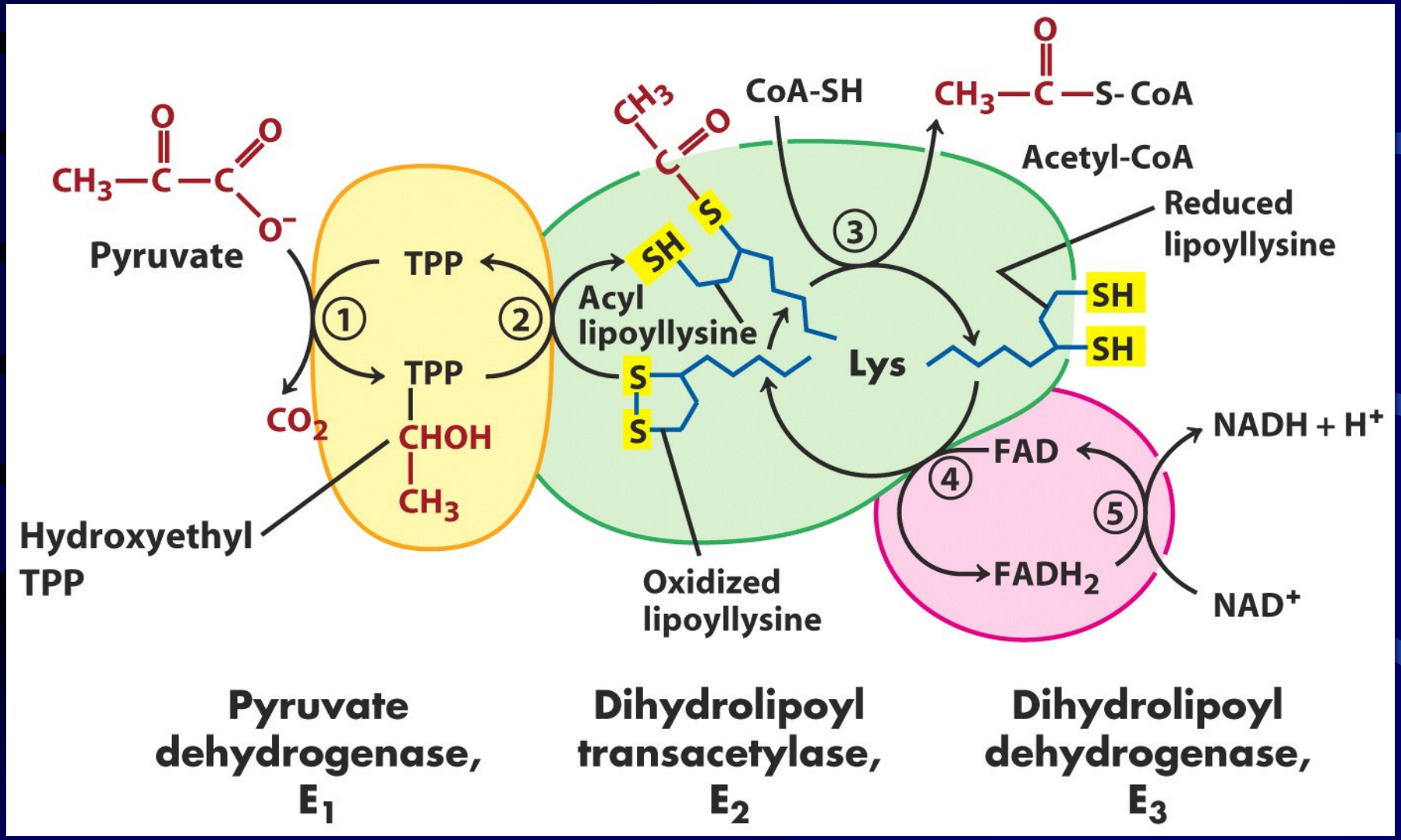
Back to Pyruvate Dehydrogenase

- This is a complex enzyme. It is composed of multiple copies of 3 different polypeptides:
- **E2: dihydrolipoyl transacetylase**

Each polypeptide contains 3 covalently-linked lipoic acids. It catalyzes:

acetyl-lipoate(E2) + CoA-SH

$\rightarrow$ acetyl-CoA + dihydrolipoate(E2)


Pyruvate
TPP
TPP
CHOH
CH3
CO2
Hydroxyethyl TPP
CH3
C
O
S
CoA-SH
CH3—C—S- CoA
Acetyl-CoA
Reduced lipoyllysine
SH
SH
Acyl lipoyllysine
Lys
S
S
Oxidized lipoyllysine
FAD
FADH2
NADH + H+
NAD+
1
2
3
4
5
Pyruvate dehydrogenase, E1
Dihydrolipoyl transacetylase, E2
Dihydrolipoyl dehydrogenase, E3

Back to Pyruvate Dehydrogenase

- This is a complex enzyme. It is composed of multiple copies of 3 different polypeptides:
- **E3: dihydrolipoyl dehydrogenase**

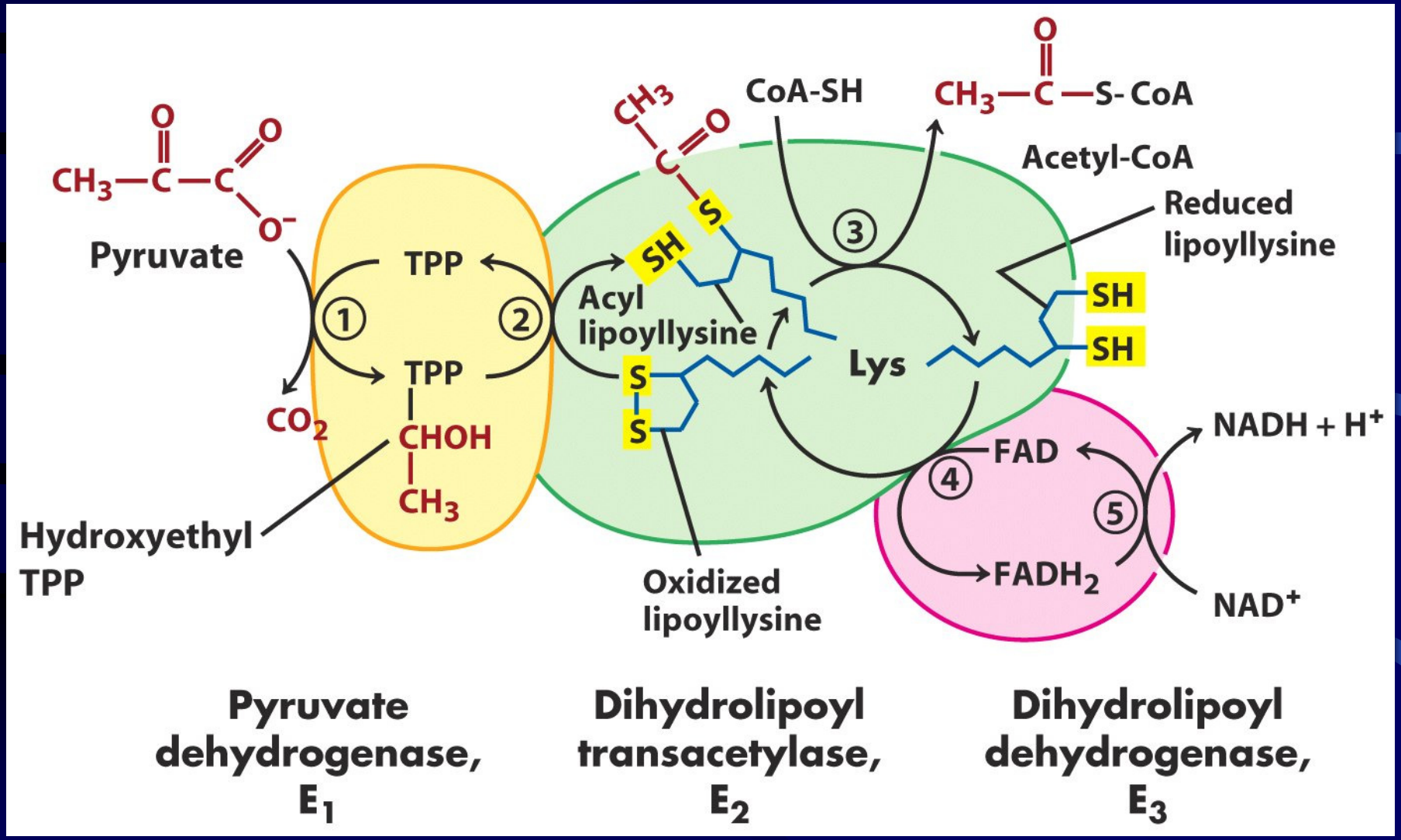
Contains FAD at active site and catalyzes:

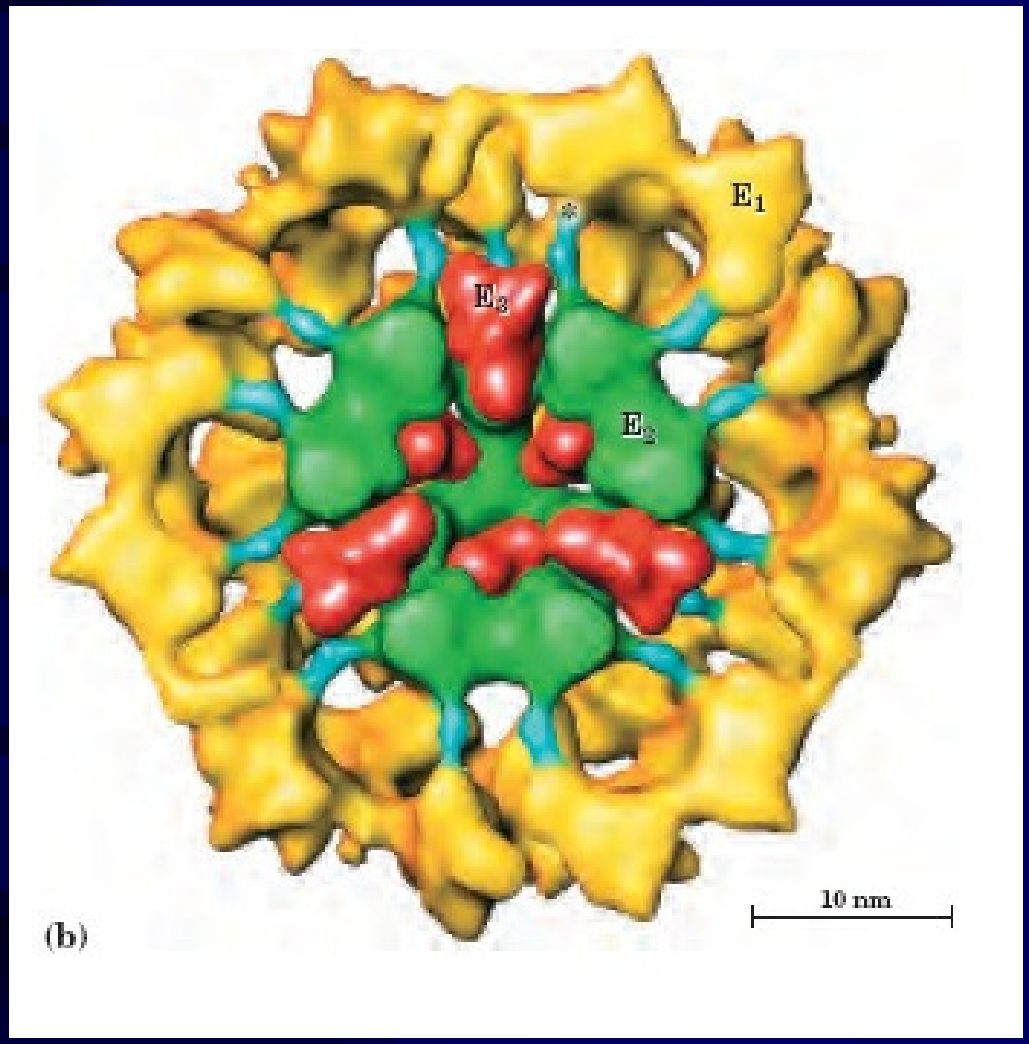
dihydrolipoate(E2) + FAD(E3)

$\rightarrow$ lipoate(E2) + $FADH_2$(E3)

$FADH_2$(E3) + NAD^+

$\rightarrow$ FAD(E3) + NADH + H^+


Pyruvate
TPP
TPP
CHOH
CH3
CO2
Hydroxyethyl TPP
CH3
C
O
S
CoA-SH
CH3—C—S- CoA
Acetyl-CoA
Reduced lipoyllysine
SH
SH
Acyl lipoyllysine
Lys
S
S
Oxidized lipoyllysine
FAD
FADH2
NADH + H+
NAD+
1
2
3
4
5
Pyruvate dehydrogenase, E1
Dihydrolipoyl transacetylase, E2
Dihydrolipoyl dehydrogenase, E3



The structure of pyruvate dehydrogenase complex

Pyruvate Dehydrogenase

Logic of the Pyr DH reaction:

- Thiamine allows the oxidative α-decarboxylation of pyruvate to occur, but it cannot serve as the electron acceptor.
- Neither can Coenzyme A, although it will serve as the acceptor of the acetyl moiety.
- Lipoic acid serves as the go-between, ferrying the acetyl group from TPP to CoA, but it keeps the 2 electrons.
- The electrons are transferred to NADH via the flavin.
- Thus, each cofactor has an essential role to play in this complicated dance, suited to its chemical properties.

Pyurvate dehydrogenase complex

A large, highly integrated complex of three kinds of enzymes

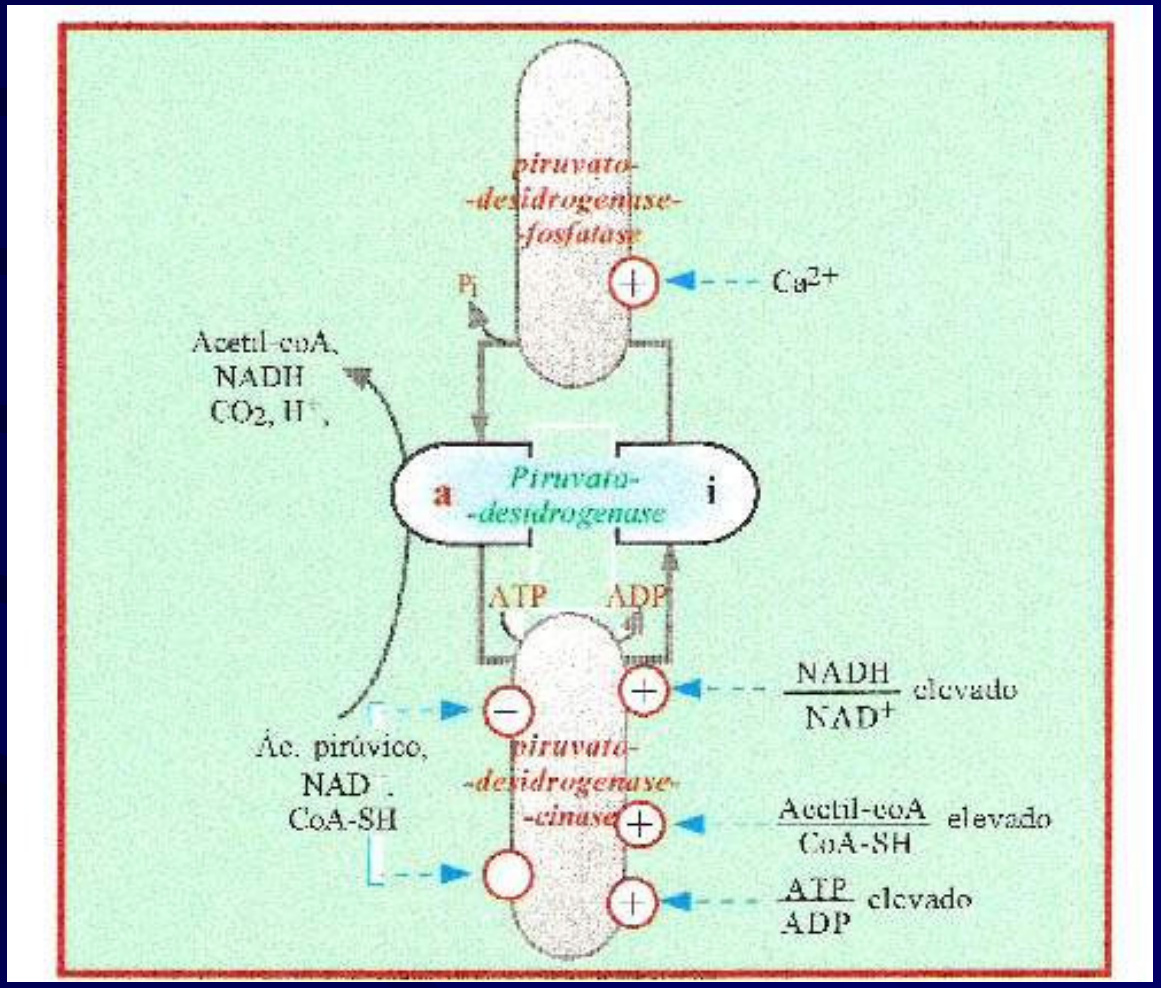
Pyruvate + CoA + NAD^+ $\Rightarrow$ acetyl CoA + CO_2 + NADH

TABLE 17.1 **Pyruvate dehydrogenase complex of *E. coli***

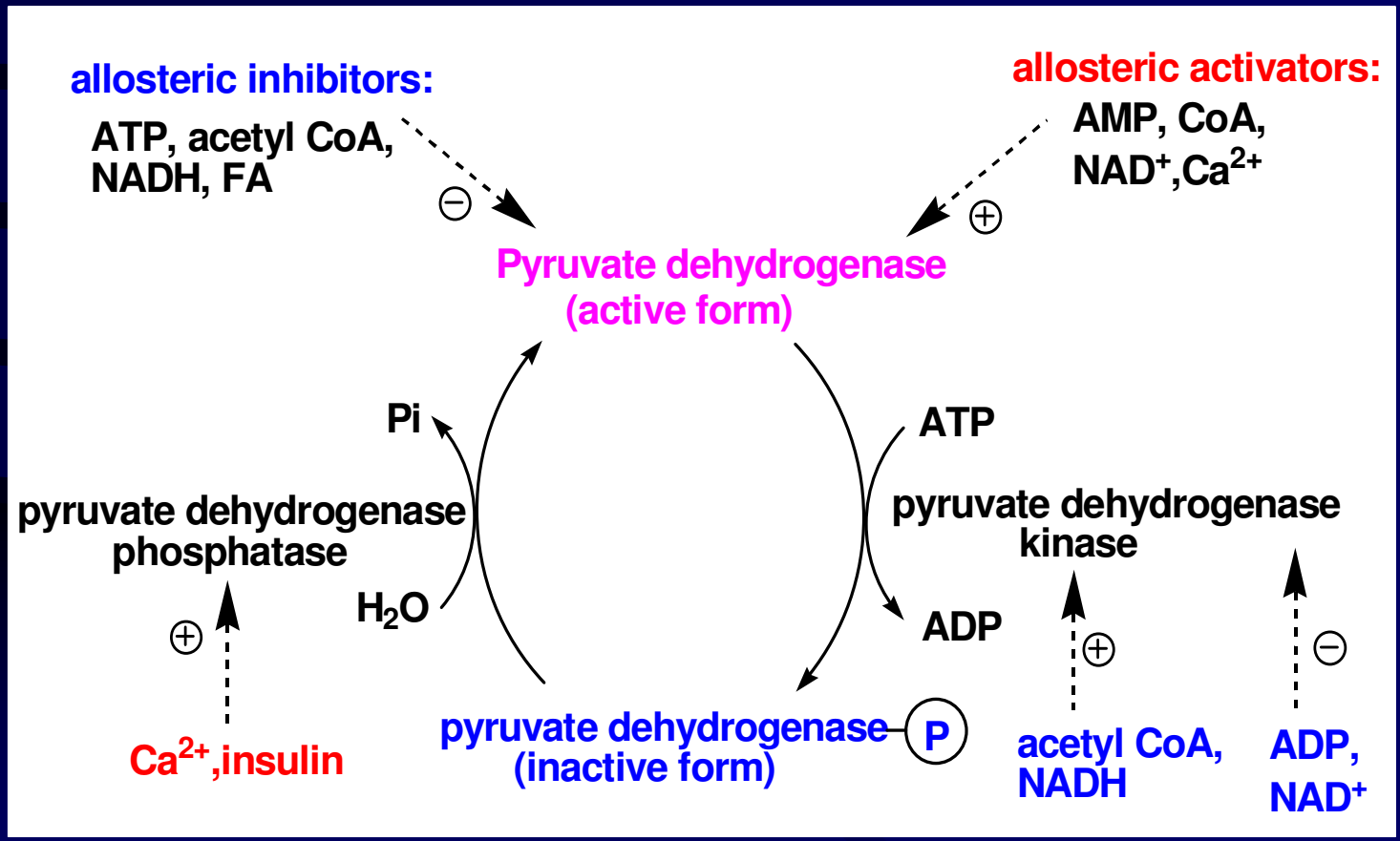
Enzyme	Abbreviation	Number of chains	Prosthetic group	Reaction catalyzed
Pyruvate dehydrogenase component	E_1	24	TPP	Oxidative decarboxylation of pyruvate
Dihydrolipoyl transacetylase	E_2	24	Lipoamide	Transfer of the acetyl group to CoA
Dihydrolipoyl dehydrogenase	E_3	12	FAD	Regeneration of the oxidized form of lipoamide

Groups travel from one active site to another, connected by tethers to the core of the structure

Regulação da descarboxilação do ácido piruvico nas células:
(intracelular)



Regulação da descarboxilação do ácido piruvico nas células: (intracelular)



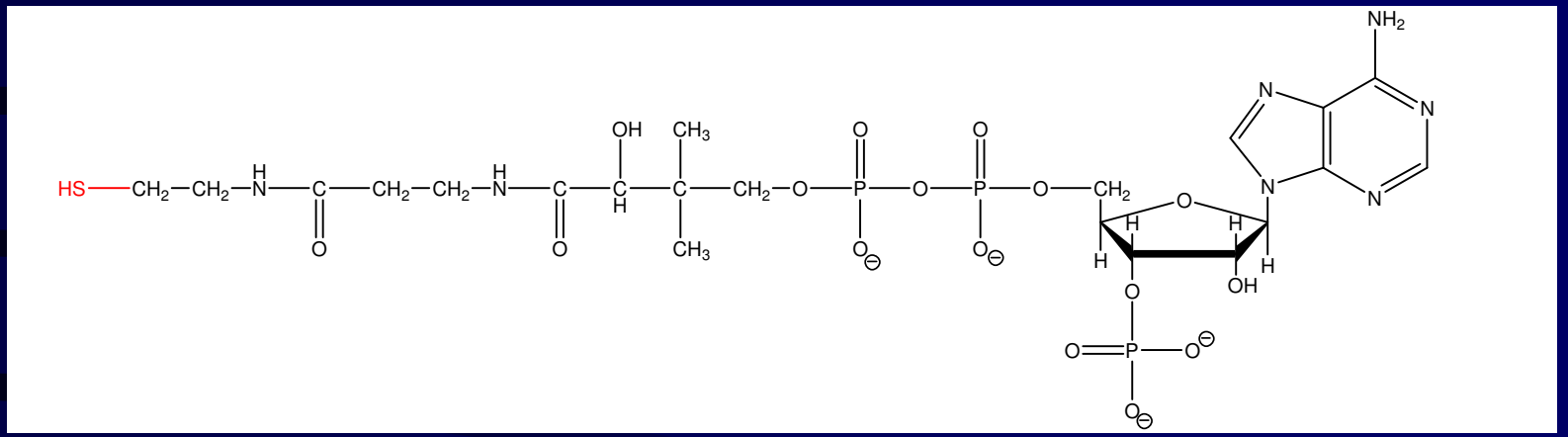
Rendimento energético:

1 piruvato ⇒ 1 acetil-coA ⇒ 1 NADH ⇒ 2,5 ATP

1 Glucose ⇒ 2 piruvato ⇒ 2 acetil-coA ⇒ 2 NADH ⇒ 5 ATP

- E o acetil-coA formado ainda pode ser oxidado?

Synthesis of acetyl-CoA

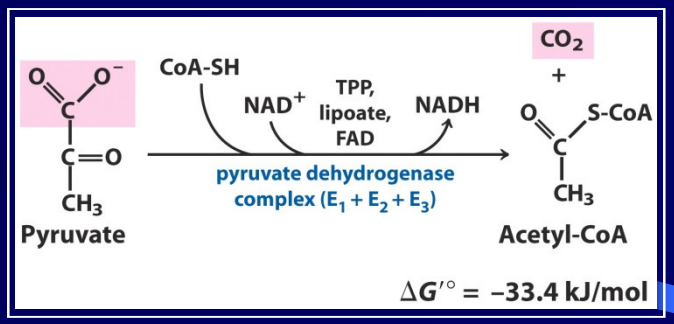


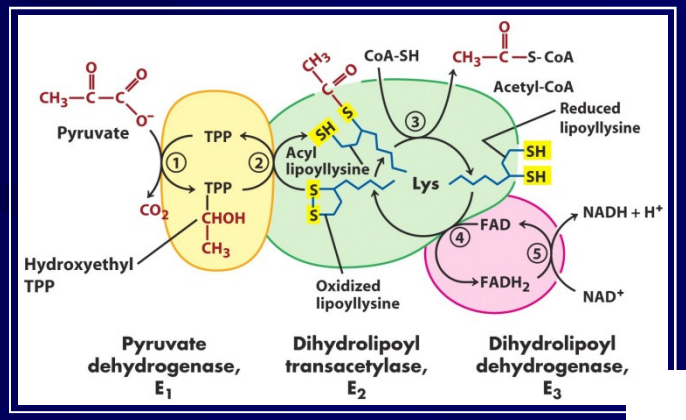
- Not part of cycle, but must occur first
- First step: entry of pyruvate into the mitochondrian
  - In aerobic cells, all enzymes of the citric acid cycle are located within the mitochondrion
  - Mitochondrion enclosed by a double membrane
  - Pyruvate passes through outer membrane via aqueous channels formed by transmembrane proteins (porins)
  - Pyruvate translocase is a protein embedded in the inner mitochondrial membrane which transports pyruvate from the intermembrane space to the mitochondrial matrix (interior space of the mitochondrion)

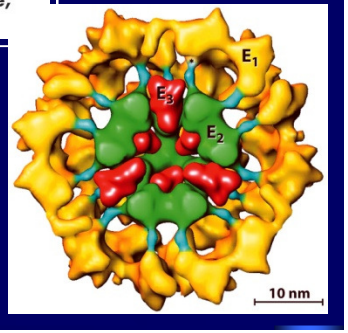
Conversion of pyruvate to acetyl-CoA

- Oxidative decarboxylation
  - Series of 5 reactions
  - Irreversible
  - Mechanism is highly complicated
- Catalyzed by complex of enzymes and cofactors
  - Pyruvate dehydrogenase complex
  - Multi-enzyme structure located in mitochondrial matrix
  - Contains multiple copies of three non-covalently associated enzymes and five coenzymes
  - E1 and E3 surround core of 24-60 E_2 chains (# chains depends on type of cell)
- Overall reaction:

$$CH_3C(O)CO_2^- + NAD^+ + CoASH \rightarrow CH_3C(O)\text{-}SCoA + NADH + CO_2$$







Glucose

↓

Ác. fosfoenolpirúvico

↓

$H_3C-\overset{\overset{O}{\|}}{C}-COOH$

ác. pirúvico

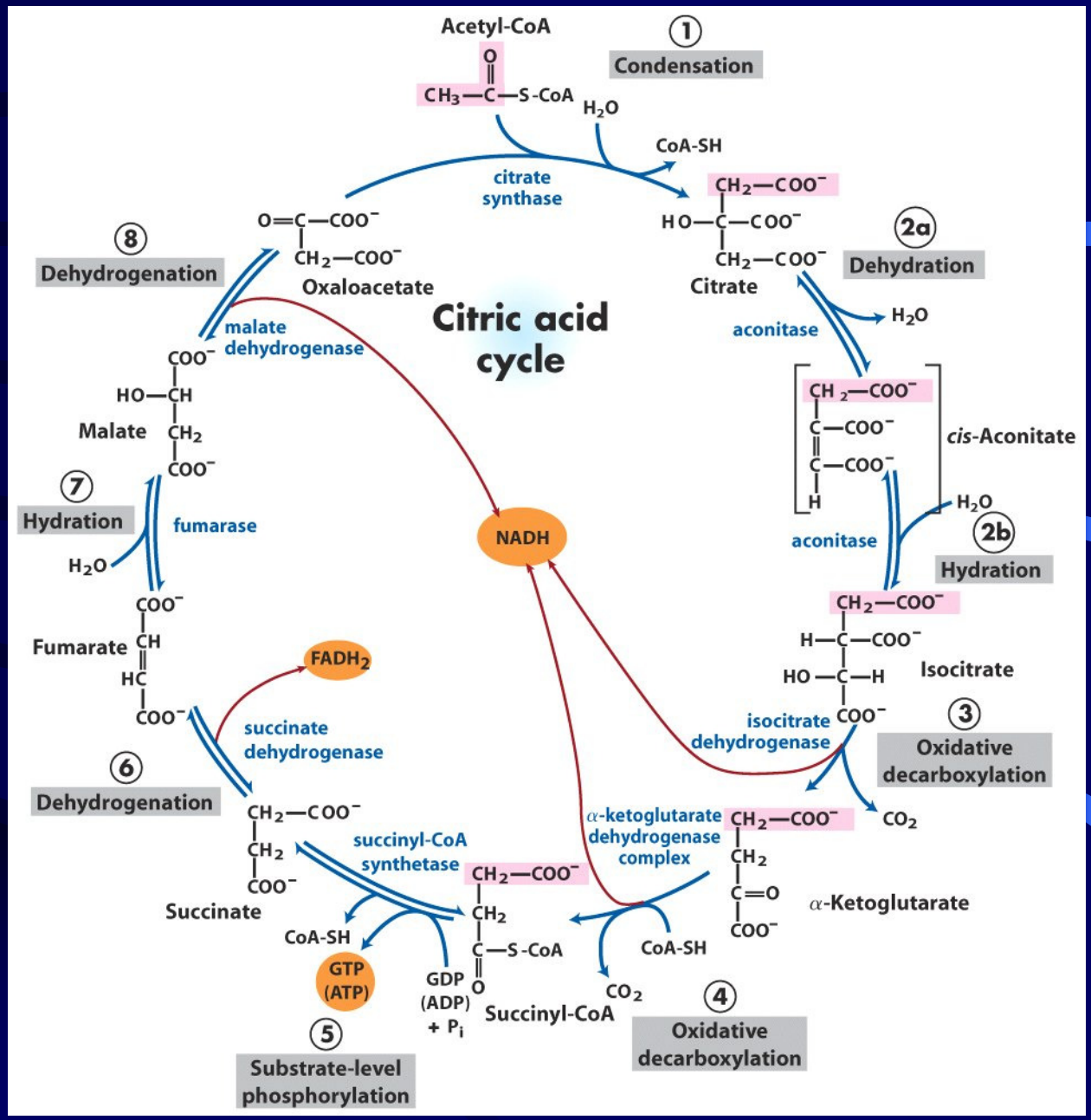
↓ descarboxilação oxidante

$H_3C-\overset{\overset{O}{\|}}{C}-S-CoA$

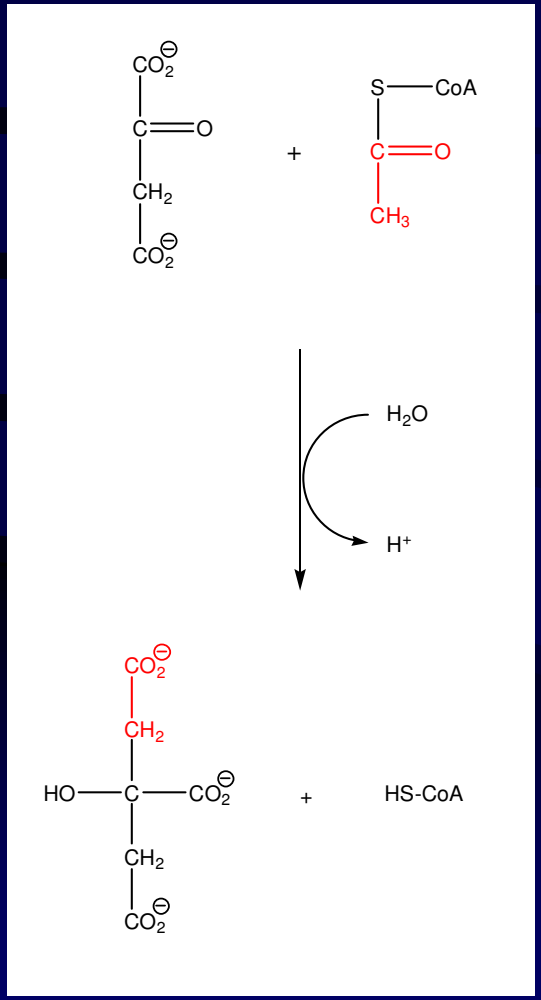
acetilcoenzima A

↓

Ciclo de Krebs:



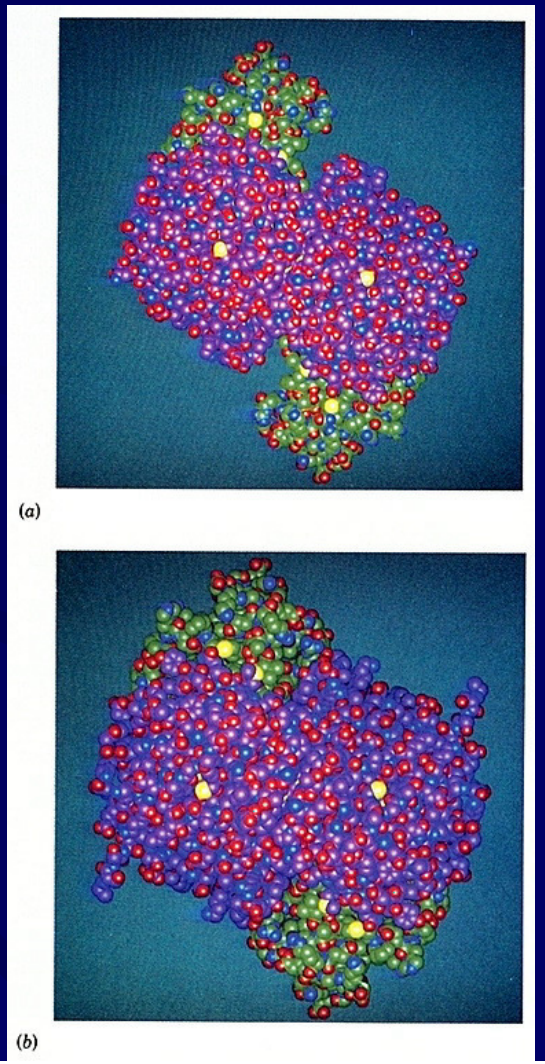
1. Formation of citrate



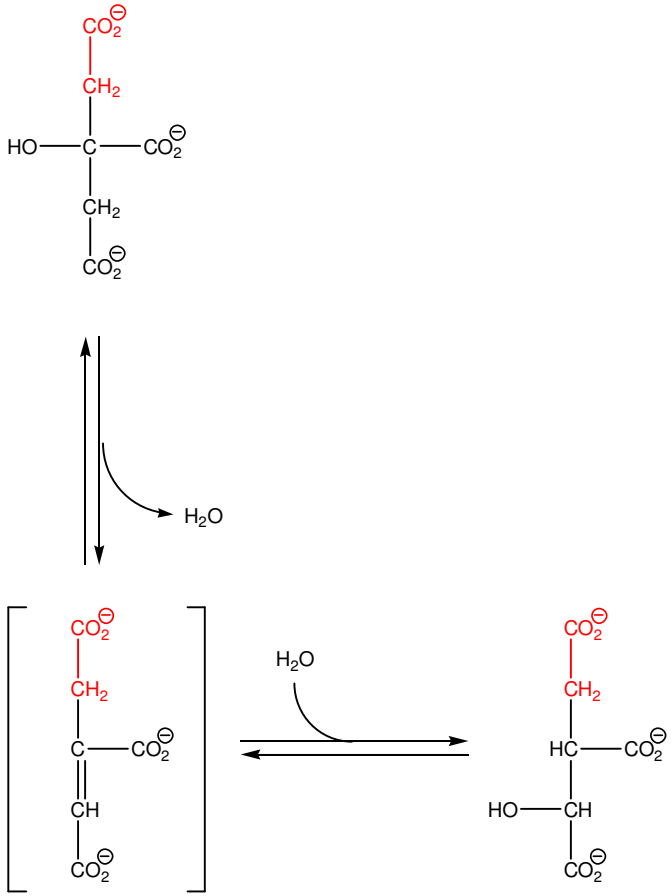
- Oxaloacetate reacts with acetyl-CoA to form citrate and coenzyme A
- Aldol condensation
  - Only C-C bond-forming reaction in cycle
- Irreversible
- Enzyme = citrate synthase

Citrate synthase

- Dimer of two identical subunits
- Changes in conformation
  - Binding of oxaloacetate
  - Domains move closer to form binding site for acetyl-CoA
  - Formation of intermediate
  - Enzyme closes around intermediate
    - Prevent side reactions by shielding thiol ester linkage of acetyl-CoA from hydrolysis by solvent
    - Intermediate hydrolyzed by bound water molecule
  - Enzyme opens and products leave active site

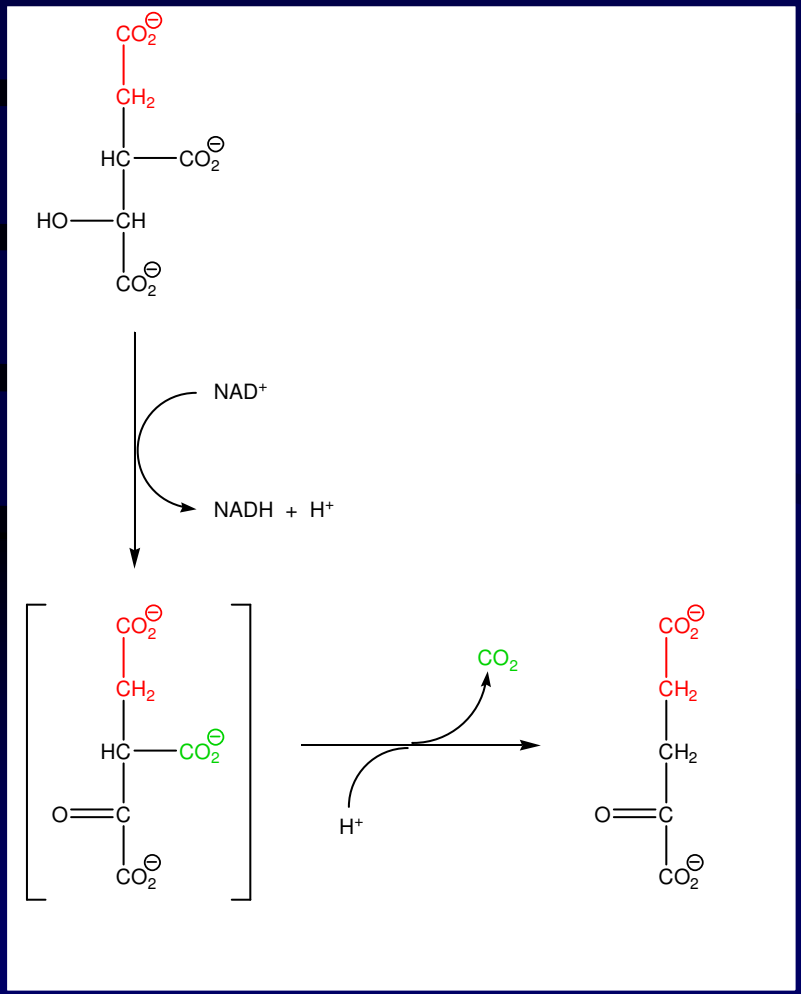


2. Isomerization of citrate to isocitrate



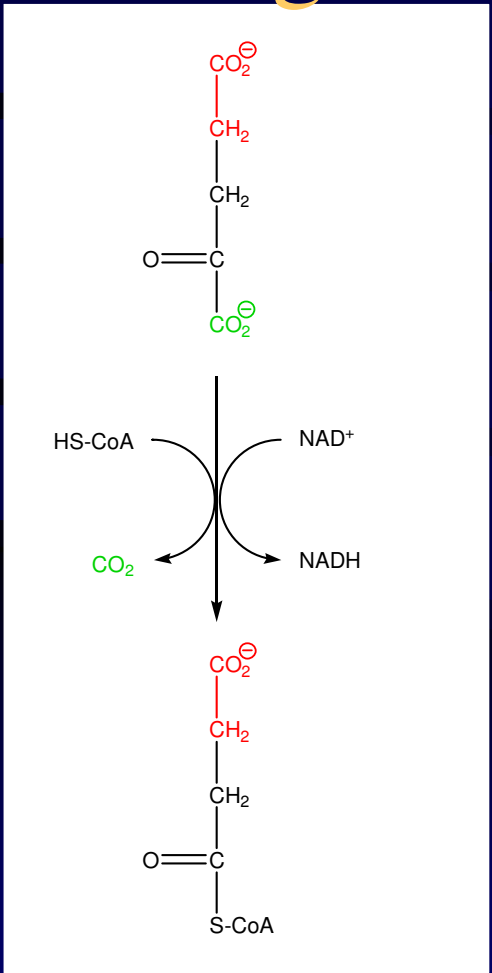
- Citrate is a 3° alcohol
  - Cannot be oxidized to keto acid
- Isocitrate is a 2° alcohol
  - Easily oxidized
- Mechanism:
  - First step: elimination of H_2O to from alkene intermediate (*cis*-aconitate)
  - Second step: stereospecific addition of water to form (2*R*, 3*S*)-isocitrate
  - Reaction near equilibrium
- Enzyme = aconitase
  - aka aconitate hydratase
  - Named for intermediate
  - Binds C3 carboxylate and hydroxyl groups
    - Substrate positioning essential for stereospecificity

3. Oxidative decarboxylation of isocitrate to form α-ketoglutarate



- First of four oxidation-reduction reactions
  - NAD^+ is oxidizing agent
- Mechanism:
  - First step: alcohol oxidized by transfer of $H:^-$ from C2 to NAD^+
    - Intermediate = oxalosuccinate, an unstable β-keto acid
    - First molecule of NADH formed
  - Second step: intermediate undergoes β-decarboxylation to form an α-keto acid, which is released from enzyme
  - First molecule of CO_2 produced
  - Irreversible
  - One of rate-limiting steps in cycle
- Enzyme = isocitrate dehydrogenase

4. Oxidative decarboxylation of α-ketoglutarate to form succinyl-CoA

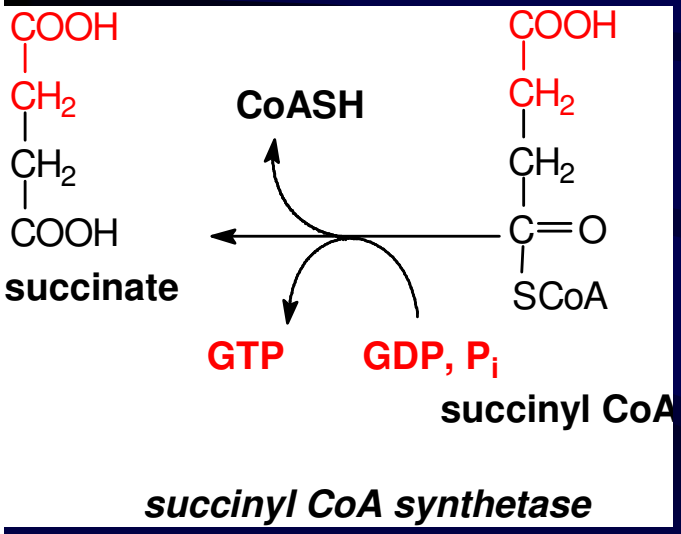


- Catalyzed by multi-enzyme α-ketoglutarate dehydrogenase complex
  - α-ketoglutarate dehydrogenase (E_1)
  - Dihydrolipoamide succinyltransferase (E_2)
  - Dihyrdolipoamide dehydrogenase (E_3)
  - Analogous to pyruvate-to-acetyl-CoA reaction catalyzed by pyruvate dehydrogenase complex
    - Same coenzymes
    - Similar complicated mechanism
- Product is high-energy thioester
- Key regulatory step of citric acid cycle
- Second molecule of NADH produced
- Second molecule of CO_2 produced

Halfway through the cycle…

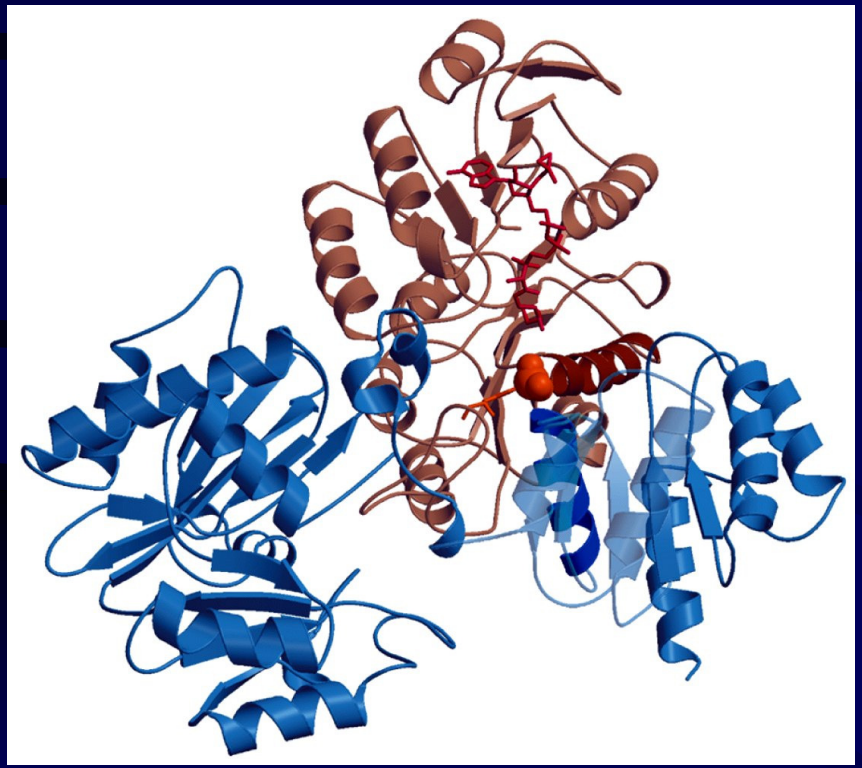
- So far…
  - Net oxidation of two carbon atoms to produce two molecules CO_2
- In the next four reactions…
  - Four-carbon succinyl group of succinyl CoA converted back to oxaloacetate
  - As oxaloacetate is regenerated, additional acetyl- CoA enters the citric acid cycle to be oxidized

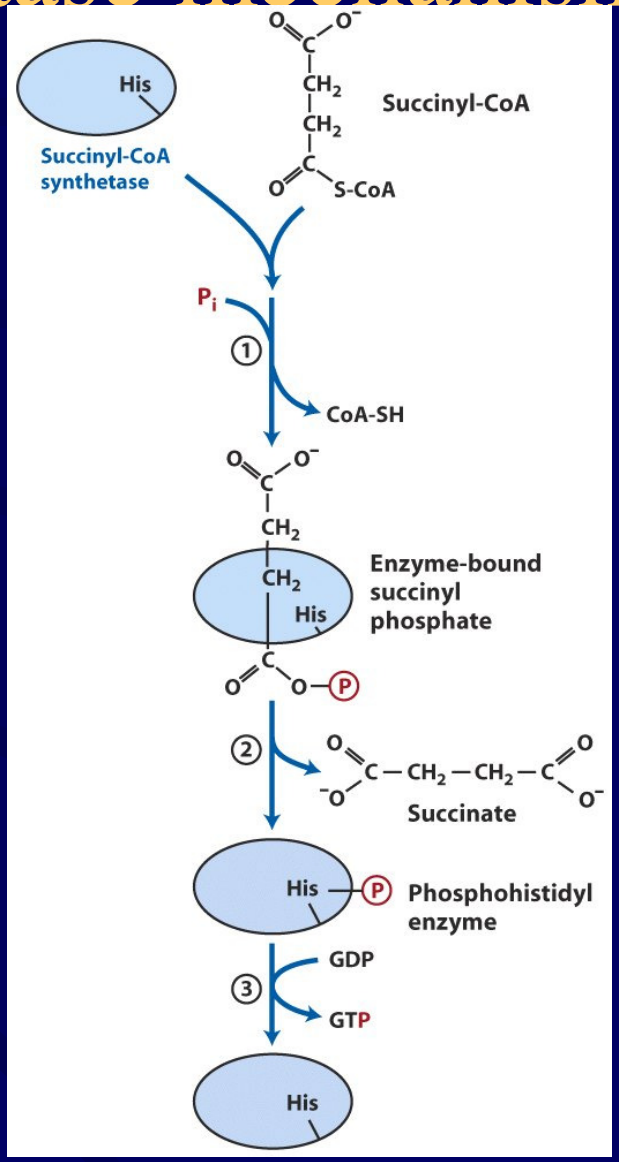
5. Conversion of succinyl-CoA to succinate



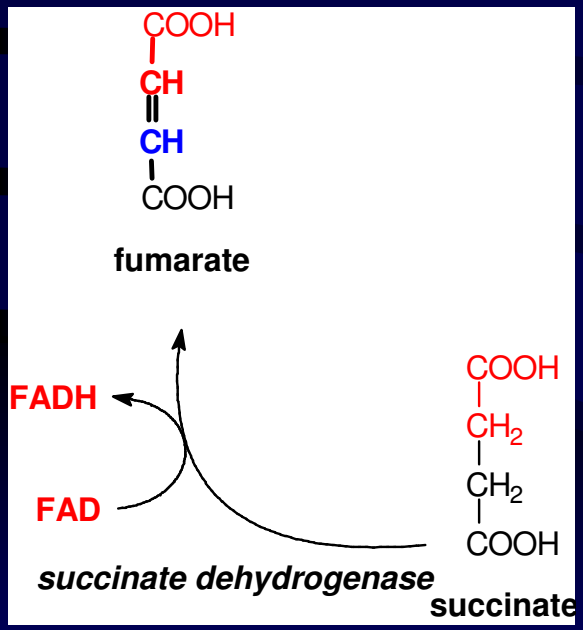
- Substrate-level phosphorylation
  - Cleavage of high-energy thioester bond
  - Free energy conserved through the synthesis of nucleoside triphosphate
    - GTP in mammals
    - ATP in plants and bacteria
  - GDP regenerated and ATP produced from the reaction of GTP with ADP
    - GTP + ADP GDP + ATP
    - Nucleoside diphosphate kinase
- Enzyme = succinyl-CoA synthetase
  - aka succinate thiokinase
- Mechanism:
  - Phosphate displace CoA from bound succinyl-CoA molecule
  - Phosphoryl group transfers to His residue of enzyme
  - Succinate released
  - Phosphoryl group transferred to GDP (or ADP)

Succinyl-CoA synthetase mechanism



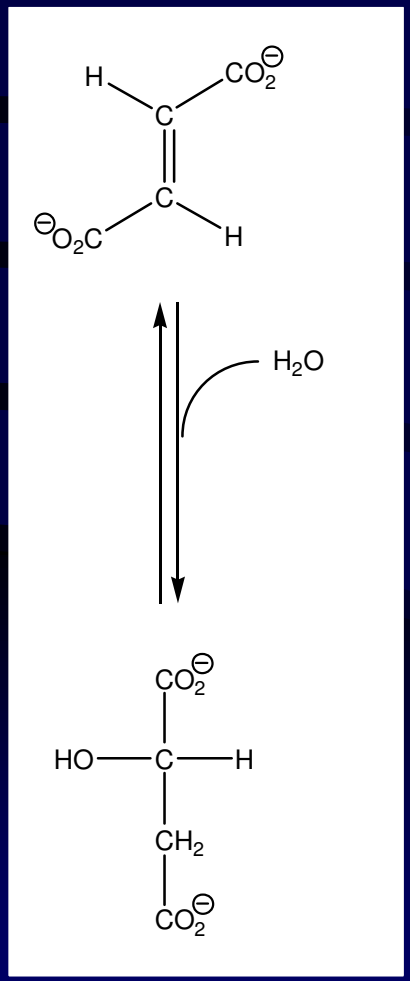


6. Oxidation of succinate to fumarate



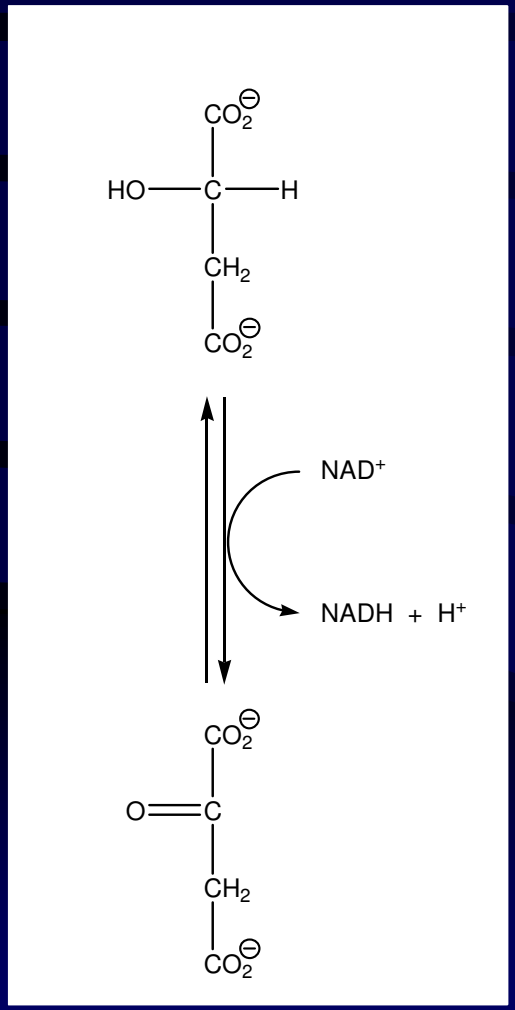
- Dehydrogenation (loss of H_2; oxidation)
  - Stereospecific to form *trans* double bond only
- Catalyzed by succinate dehydrogenase complex
  - aka succinate dehydrogenase
  - aka Complex II
  - Embedded in inner mitochondrial membrane, rather than in mitochondrial matrix
- Oxidation of alkane requires stronger oxidizing agent than NAD^+ (hence FAD)
  - $FADH_2$ produced is re-oxidized by coenzyme ubiquinone (Q) to reform FAD and ubiquinol (QH_2)
- Competitive inhibitor = malonate
  - $^-O_2C\text{-}CH_2\text{-}CO_2^-$
  - Binds to active site through carboxylate groups
  - Cannot undergo dehydrogenation
  - Inhibition reactions used by Krebs to determine citric acid cycle reaction sequence
- Symmetrical molecule evenly distributes carbons in remainder of products throughout the cycle

7. Hydration of fumarate to form L-malate



- Reversible reaction, near equilibrium
- Stereospecificity
  1. *trans* addition of water to double bond of fumarate
  2. Only *trans* double bond will react
- Enzyme = fumarase
  - aka fumarate hydratase

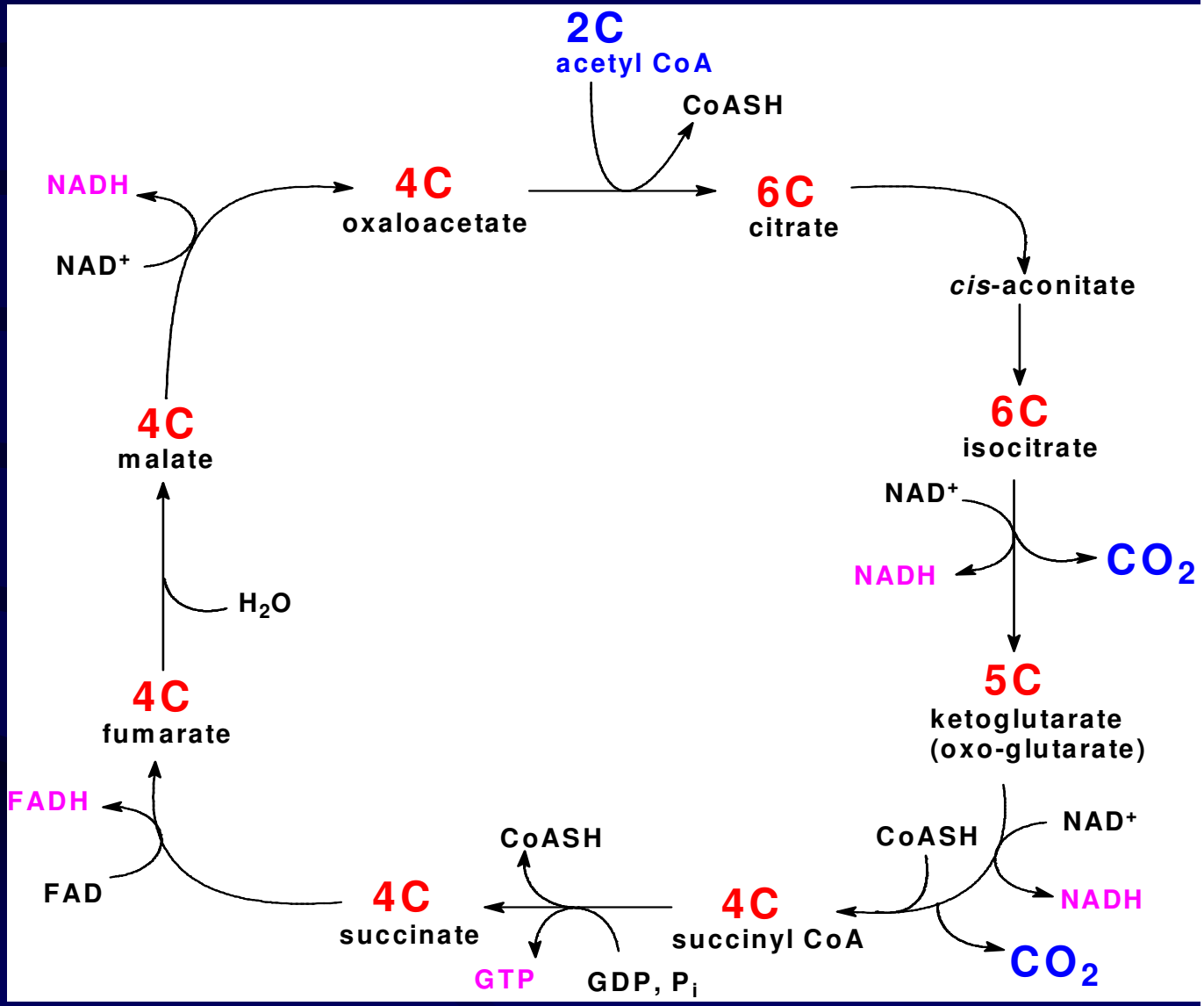
8. Oxidation of L-malate to regenerate oxaloacetate



- Formation of third molecule of NADH
- Reaction is endergonic, and concentration of product is low at equilibrium
  - Next reaction in cycle (1) is highly exergonic
  - Product used immediately
- Enzyme = malate dehydrogenase

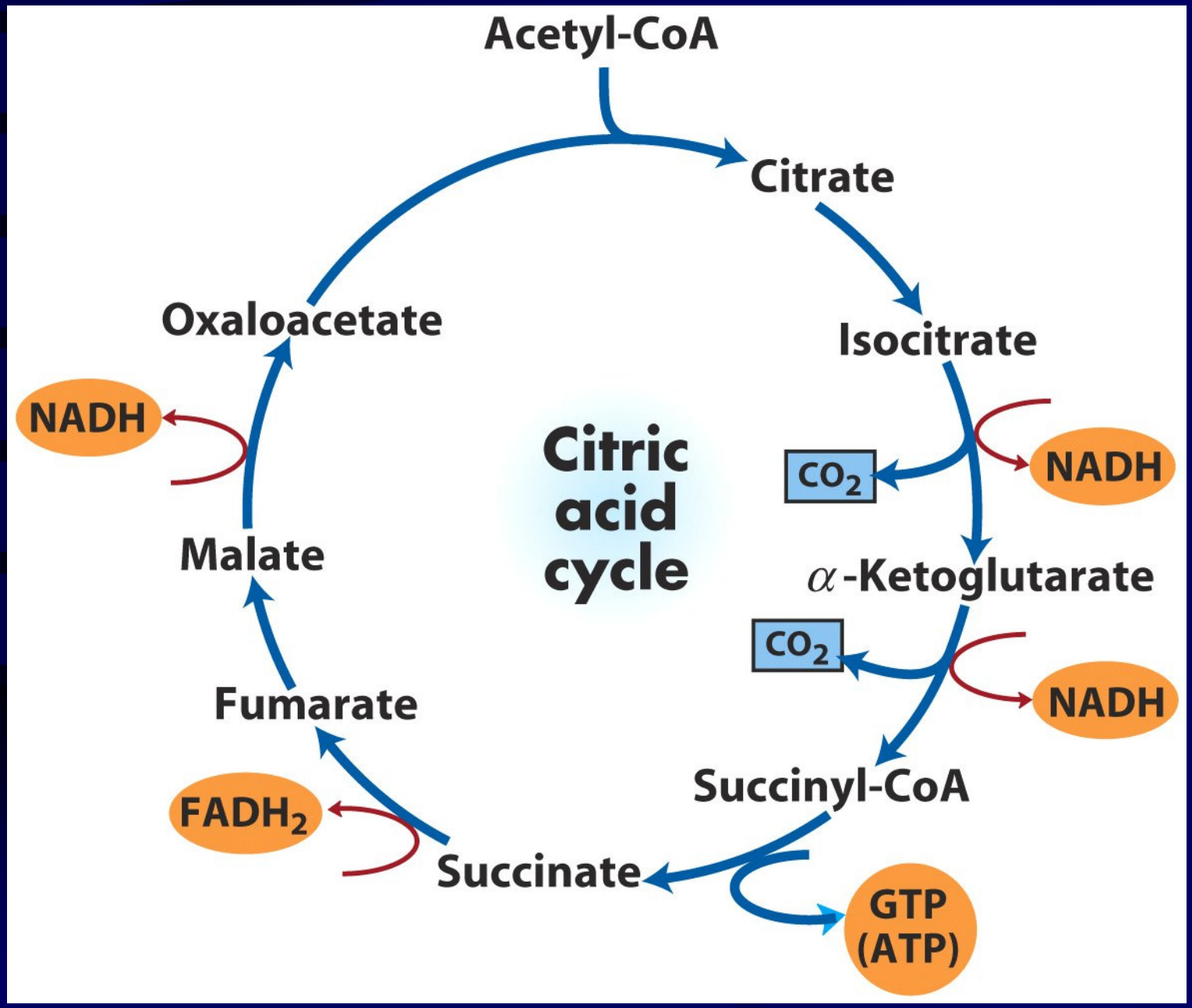
TCA Cycle Summary

1 acetate through the cycle produces 2 CO_2, 1 GTP, 3NADH, 1$FADH_2$



Net profit of aerobic metabolism

- For each acetyl group that enters the TCA cycle as acetyl-CoA, 2 CO_2 are produced along with:
  - 3 NADH
  - 1 $FADH_2$ (succinate DH)
  - 1 GTP
- Oxidation of NADH yields 2.5 ATP
- Oxidation of $FADH_2$ yields 1.5 ATP
- Thus, oxidation of the 2 carbons of acetyl-CoA will produce 10 ATP.


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

Aerobic Nature of the Cycle

- NADH and $FADH_2$ must be reoxidized by the electron transport chain.
- Succinate Dehydrogenase is part of electron transport chain in the inner membrane of mitochondria.

Net profit of aerobic metabolism

If we start from glucose:

- Glycolysis to 2 pyruvate yields:
  - 2 ATP
  - 2 NADH (= 5 ATP)
- Pyruvate DH converts 2 pyruvate to 2 ac-CoA + 2 CO_2
  - 2 NADH (= 5 ATP)
- Oxidation of the 2 ac-CoA to 2 CO_2 by TCA yields
  - 20 ATP

The net yield is **32 ATP** per glucose oxidized to CO_2.
(Compare this to fermentation's yield of 2 ATP.)

TABLE 16–1 Stoichiometry of Coenzyme Reduction and ATP Formation in the Aerobic Oxidation of Glucose via Glycolysis, the Pyruvate Dehydrogenase Complex Reaction, the Citric Acid Cycle, and Oxidative Phosphorylation

Reaction	*Number of ATP or reduced coenzyme directly formed*	*Number of ATP ultimately formed**
Glucose ⟶ glucose 6-phosphate	−1 ATP	−1
Fructose 6-phosphate ⟶ fructose 1,6-bisphosphate	−1 ATP	−1
2 Glyceraldehyde 3-phosphate ⟶ 2 1,3-bisphosphoglycerate	2 NADH	3 or 5†
2 1,3-Bisphosphoglycerate ⟶ 2 3-phosphoglycerate	2 ATP	2
2 Phosphoenolpyruvate ⟶ 2 pyruvate	2 ATP	2
2 Pyruvate ⟶ 2 acetyl-CoA	2 NADH	5
2 Isocitrate ⟶ 2 α-ketoglutarate	2 NADH	5
2 α-Ketoglutarate ⟶ 2 succinyl-CoA	2 NADH	5
2 Succinyl-CoA ⟶ 2 succinate	2 ATP (or 2 GTP)	2
2 Succinate ⟶ 2 fumarate	2 $FADH_2$	3
2 Malate ⟶ 2 oxaloacetate	2 NADH	5
Total		30–32

* This is calculated as 2.5 ATP per NADH and 1.5 ATP per $FADH_2$. A negative value indicates consumption.

† This number is either 3 or 5, depending on the mechanism used to shuttle NADH equivalents from the cytosol to the mitochondrial matrix; see Figures 19–27 and 19–28.

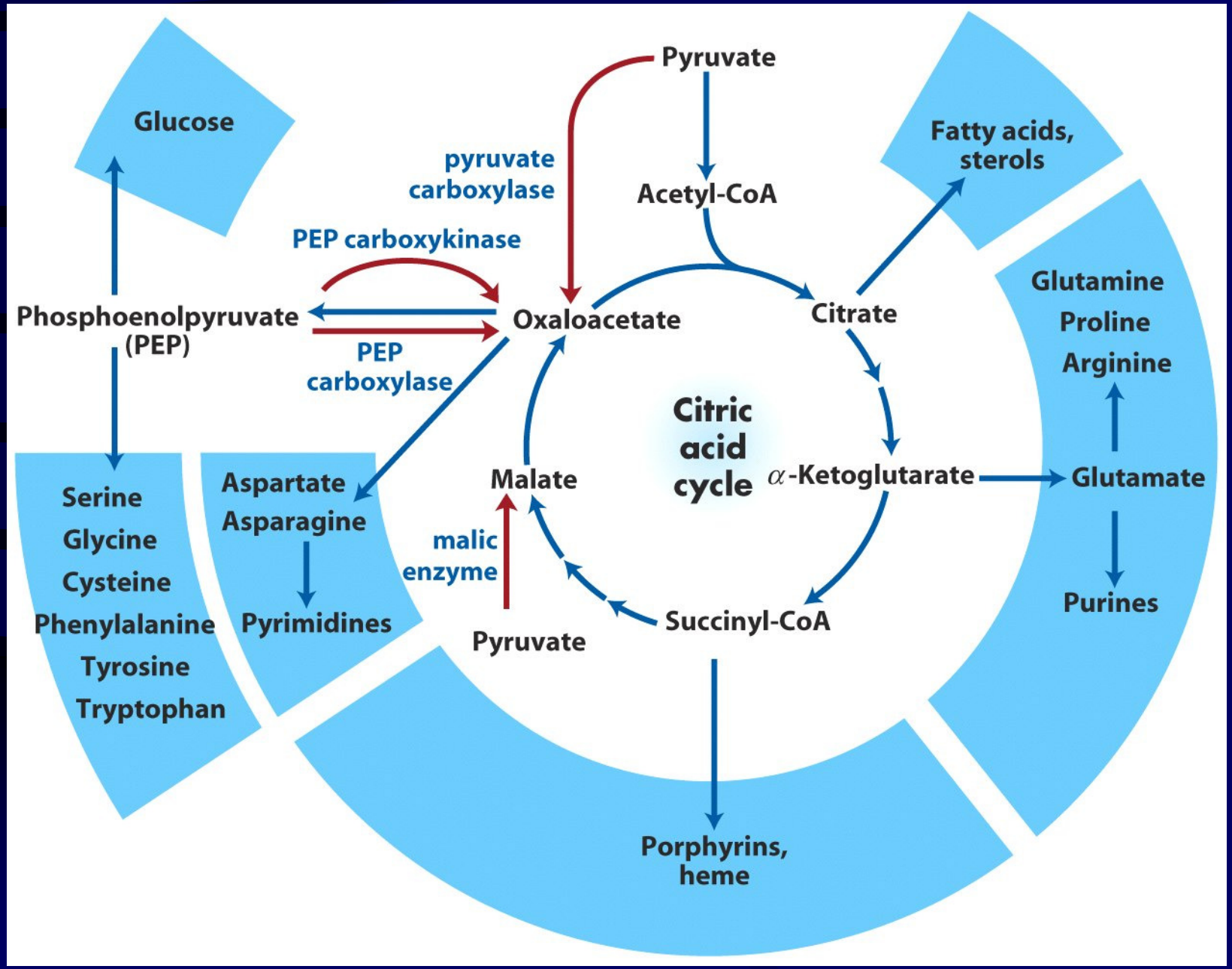
Amphibolic nature of the TCA cycle

- The TCA cycle is used for:

  catabolism – generation of most cellular ATP

  anabolism – provides precursors for amino acids, nucleic acids, *etc.*

- Adequate levels of the TCA cycle intermediates must be maintained to keep the cycle going.
- The beauty of a cycle is that you can take any of the 7 intermediates from the cycle that you want and replenish it with any of the other 6.


Glucose
Pyruvate
pyruvate carboxylase
Acetyl-CoA
Fatty acids, sterols
PEP carboxykinase
Phosphoenolpyruvate (PEP)
Oxaloacetate
Citrate
PEP carboxylase
Glutamine
Proline
Arginine
Citric acid cycle
Serine
Glycine
Cysteine
Phenylalanine
Tyrosine
Tryptophan
Aspartate
Asparagine
Malate
α-Ketoglutarate
Glutamate
malic enzyme
Pyrimidines
Purines
Pyruvate
Succinyl-CoA
Porphyrins, heme

The Pyruvate Partition

- The main TCA cycle intermediate used to replenish the cycle is **oxaloacetate** (OAA).
- The main source of OAA in eukaryotic cells that are metabolizing carbohydrates is the **pyruvate carboxylase** reaction:

  pyruvate + HCO_3^- + ATP $\rightarrow$ OAA + ADP + P_i

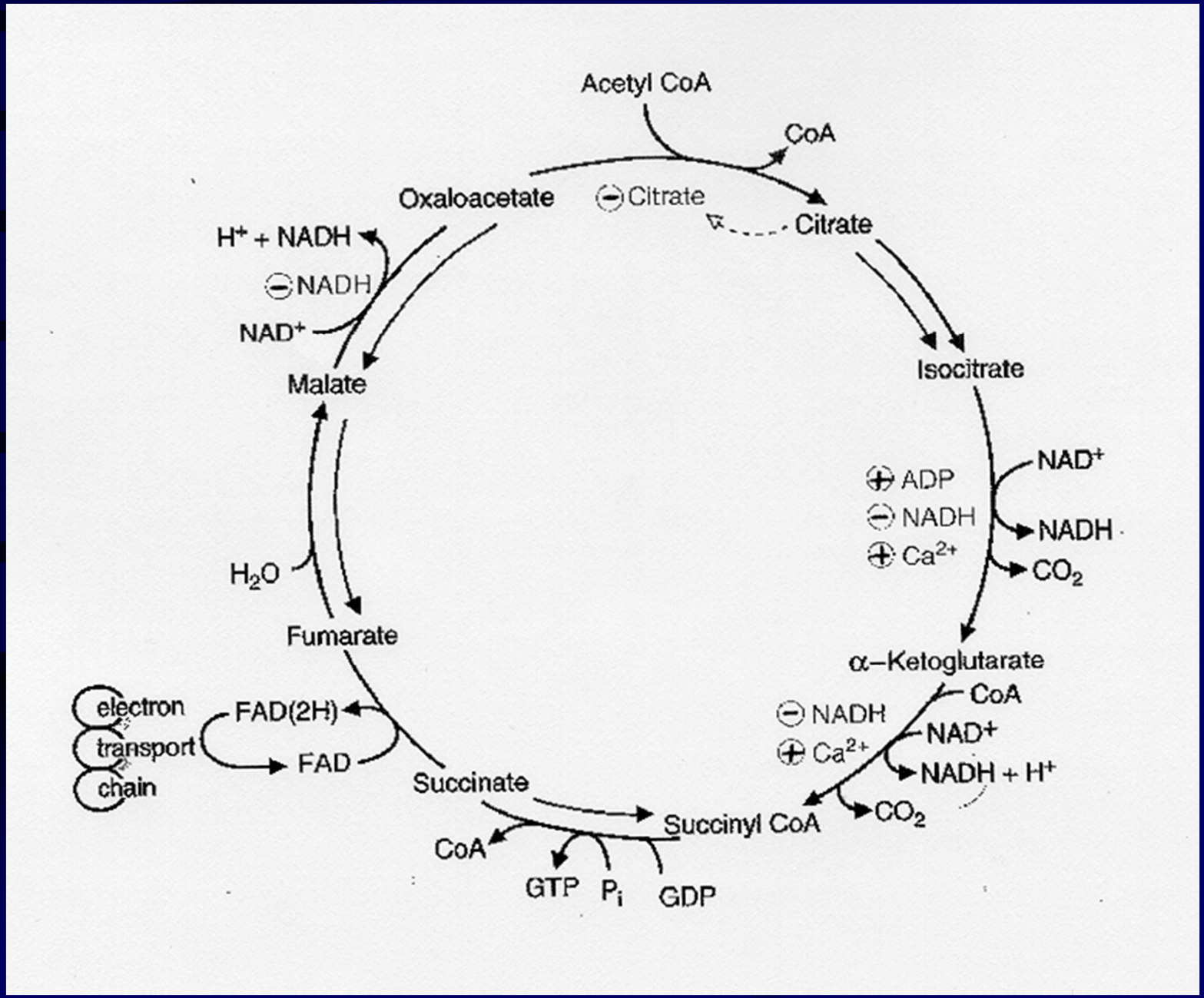
  (You saw this reaction used in gluconeogenesis.)

The Pyruvate Partition

Thus, pyruvate entering the mitochondria can be partitioned into one of 2 paths:

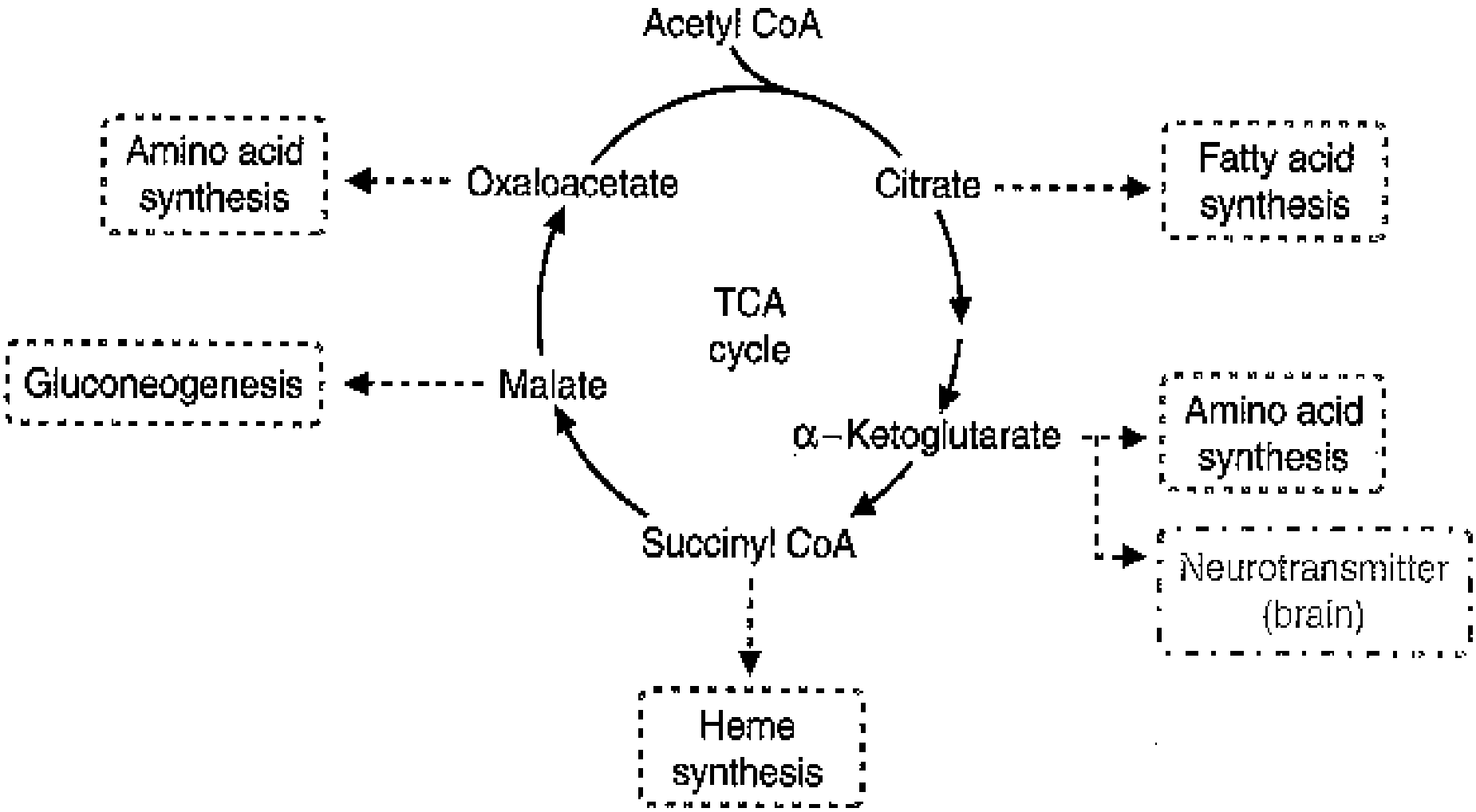
1. **acetyl-CoA**: 2-carbon, destined to be completely oxidized to CO_2
2. **OAA**: 4-carbon, TCA cycle intermediate, precursor to carbohydrates (PEP), amino acids, etc.

- The relative levels of these 2 pathways depend upon the needs of the cell and they are tightly regulated.


Acetyl CoA
CoA
Oxaloacetate
⊖ Citrate
Citrate
H+ + NADH
⊖ NADH
NAD+
Malate
Isocitrate
⊕ ADP
⊖ NADH
⊕ Ca2+
NAD+
NADH
CO2
H2O
Fumarate
α–Ketoglutarate
CoA
⊖ NADH
⊕ Ca2+
NAD+
NADH + H+
CO2
electron
transport
chain
FAD(2H)
FAD
Succinate
Succinyl CoA
CoA
GTP
Pi
GDP

Anaplerotic reactions

- **Anaplerotic** (filling up) reactions replenish citric acid cycle intermediates
- Amphibolic Nature of TCA Cycle means it both Anabolic and Catabolic. TCA cycle provides several of Intermediates for Biosynthesis


Acetyl CoA
Amino acid synthesis
Oxaloacetate
Citrate
Fatty acid synthesis
TCA cycle
Gluconeogenesis
Malate
α–Ketoglutarate
Amino acid synthesis
Succinyl CoA
Neurotransmitter (brain)
Heme synthesis

Anaplerotic reactions

- PEP carboxylase - converts PEP to oxaloacetate , Anaplerotic reaction in plants and bacteria
- Pyruvate carboxylase - converts pyruvate to oxaloacetate, a major anaplerotic reaction in mammalian tissues
- Malic enzyme converts pyruvate into malate

The citric acid cycle is *amphibolic*

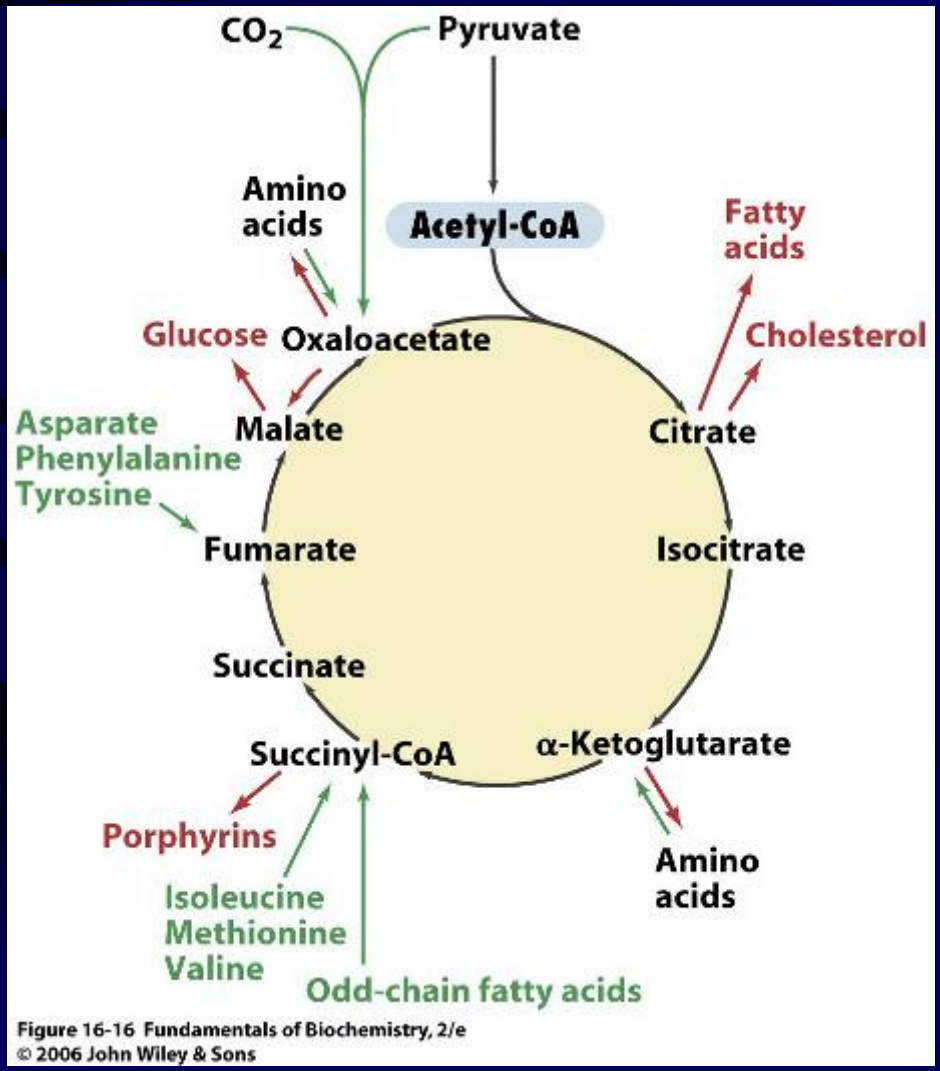


Figure 16-16 Fundamentals of Biochemistry, 2/e

Green:
Anaplerotic reactions
(replenish)

Red:
Cataplerotic reactions
(drain)

Regulation of the TCA Cycle

Again, 3 irreversible reactions are the key sites

- Citrate synthase - regulated by availability of substrates - acetyl-CoA and oxaloacetate, citrate is a competitive inhibitor;

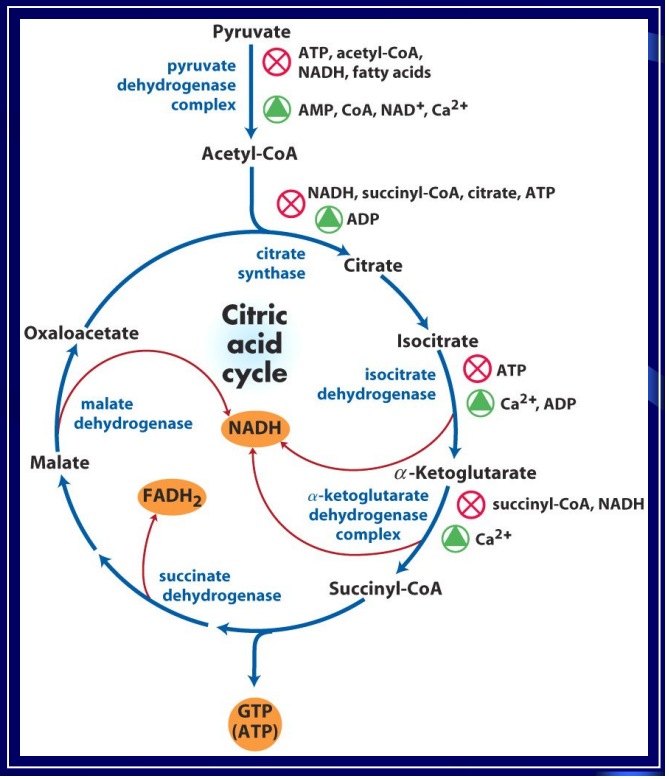
  Allosteric: - NADH , ATP,succinyl-CoA

- Isocitrate dehydrogenase – NADH,ATP inhibit, ADP and NAD^+ Ca^{++} activate
- α -Ketoglutarate dehydrogenase - NADH and succinyl-CoA inhibit, AMP Ca^{++}activate

Regulation of the Citric Acid Cycle

- Achieved by the modulation of key enzymes and the availability of certain substrates
- Recall that build-up of citrate slows glycolysis/production of pyruvate
- Regulation also depends on continuous supply of acetyl-CoA (from pyruvate), NAD^+, FAD, and ADP
- Regulated enzymes in the citric acid cycle:
  - Citrate synthase (reaction 1)
    - Allosterically inhibited by high concentrations of citrate, succinyl-CoA, NADH, ATP
  - Isocitrate dehydrogenase (reaction 3)
    - Activity stimulated by ADP, NAD^+, and Ca^{2+} (muscle)
    - Inhibited by ATP and NADH

    α-Ketoglutarate dehydrogenase (reaction 4)
    - Inhibited by ATP, GTP, NADH and succinyl-CoA
  - All three of these enzymes catalyze reactions that represent important metabolic branch points



Allosteric control (TCA cycle)

Enzyme	**Activated by**	**Inhibited by**
Pyruvate dehydrogenase	AMP, NAD^+, CoA, (Ca^{2+})	ATP, NADH, acetyl-CoA, FA's
Pyruvate carboxylase	acetyl-CoA	
Citrate synthase	ADP	ATP, NADH, succinyl-CoA, citrate
Isocitrate dehydrogenase	ADP, NAD^+, (Ca^{2+})	ATP, NADH
α-ketoglutarate dehydrogenase	(Ca^{2+})	NADH, succinyl-CoA

The Glyoxylate cycle

An Anabolic Variant of the Citric Acid Cycle for plants and bacteria

. The glyoxylate cycle is a metabolic pathway occurring in plants and several bacteria, but not animals.

. The glyoxylate cycle allows these organisms to use fats for the synthesis of carbohydrates, a task which vertebrates, including humans, cannot perform.
Isocitrate --> succinate + glyoxylate ($O=CH\text{-}COO^-$)+acetyl-CoA--> malate-->> glucose

. When fatty acids are consumed by vertebrates they are degraded to many copies of small 2-carbon acetyl compounds.

. This acetyl group binds to the active thiol group of coenzyme A and enters the citric acid cycle, where it is fully oxidized to carbon dioxide, which is released into the environment.

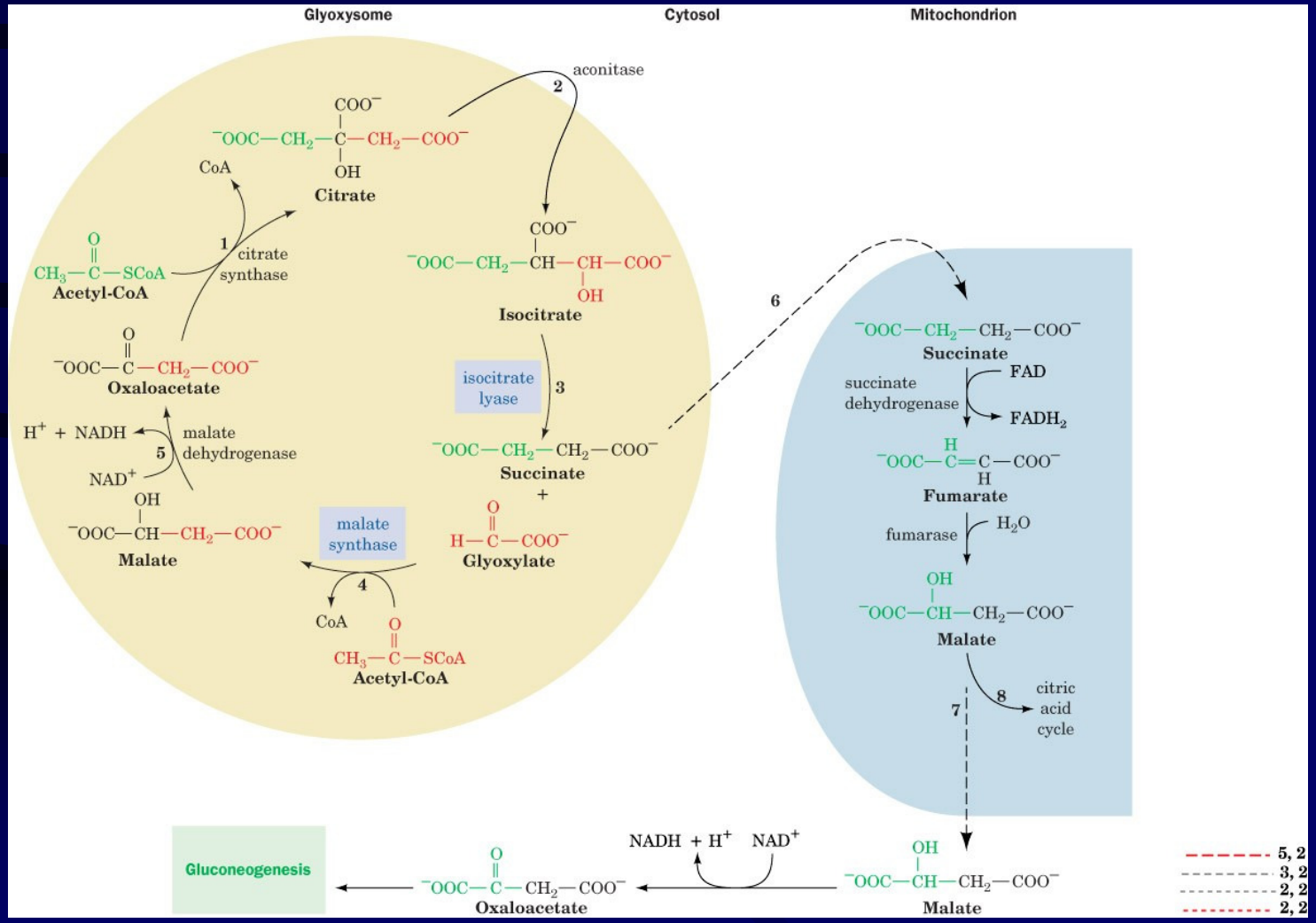
. This pathway allows the cell to obtain energy from fat.

In plants the glyoxylate cycle occurs in special peroxisomes which are called glyoxysomes.

. Glyoxysomes are membrane-bound organelles found in plants, particularly in the fat storage tissues of germinating seeds.

. The glyoxylate cycle involves 5 enzymes, 3 of which also participate in the citric acid cycle: citrate synthase, aconitase and malic dehydrogynase.

- The other 2 enzymes are unique to the glyoxylate cycle: isocitrate lyase and malate synthase.



- The glyoxylate cycle converts 2 Acetyl-CoA --> succinate instead of $2CO_2$ (as occurs in the Citric acid cycle).

- Succinate can be transported to mitochondria and enter the Citric acid cycle, or it can be transported to the cytosol where it is trans-formed to oxaloacetate and enters the gluconeogenesis pathway.

- Overall: $2\text{Acetyl-CoA}+2NAD^{+}+FAD \rightarrow \text{oxaloacetate}+ 2\text{CoA}+2NADH+FADH_2+2H^{+}$
- germinating seeds convert stored tryglycerides to glucose.

Why is this cycle important?

- **Especially important to seeds which can use their fatty acids (oxidation-> acetyl-CoA) for synthesizing ATP and glucose.**

- **Germination depends on carbohydrates which can not be formed from photosynthesis in the dark.**

- **The two initial stages of this cycle are identical to those of the citric acid cycle: acetate -> citrate -> isocitrate.**

- **The next step is different: isocitrate is cleaved into succinate and glyoxylate.**

- Succinate is channeled directly into the citric acid cycle and eventually forms oxaloacetate.
- Glyoxylate condenses with acetyl-CoA, yielding malate.
- Both malate and oxaloacetate can be converted into phosphoenolpyruvate and gluconeogenesis can be initiated.
- The net result of the glyoxylate cycle is the production of glucose from fatty acids.

The glyoxylate cycle.

The overall reaction of the glyoxylate cycle is the net formation of oxaloacetate from 2 molecules of acetyl-CoA:

2 AcetylCoA+2NAD$^+$+FAD --> oxaloacetate + 2CoA+ 2NADH+$FADH_2$+2H^+.

Summary

- Pyruvate is converted to acetyl-CoA by the action of pyruvate dehydrogenase complex, a huge enzyme complex.
- Acetyl-CoA is converted to 2 CO_2 via the eight-step citric acid cycle, generating three NADH, one $FADH_2$, and one ATP (by substrate-level phophorylation).
- Intermediates of citric acid cycle are also used as biosynthetic precursors for many other biomolecules, including fatty acids, steroids, amino acids, heme, pyrimidines, and glucose.
- Oxaloacetate can get replenished from pyruvate, via a carboxylation reaction catalyzed by the biotin-containing pyruvate carboxylase.

- The activity of pyruvate dehydrogenase complex is regulated by allosteric effectors and reversible phosphorylations.
- Net conversion of fatty acids to glucose can occur in germinating seeds, some invertebrates and some bacteria via the *glycoxylate cycle*, which shares three steps with the citric acid cycle but bypasses the two decarboxylation steps, converting two molecules of acetyl-CoA to one succinate.
- Acetyl-CoA (isocitrate) is partitioned into the glyoxylate cycle and citric acid cycle via a coordinately regulation of the isocitrate dehydrogenase and isocitrate lyase.