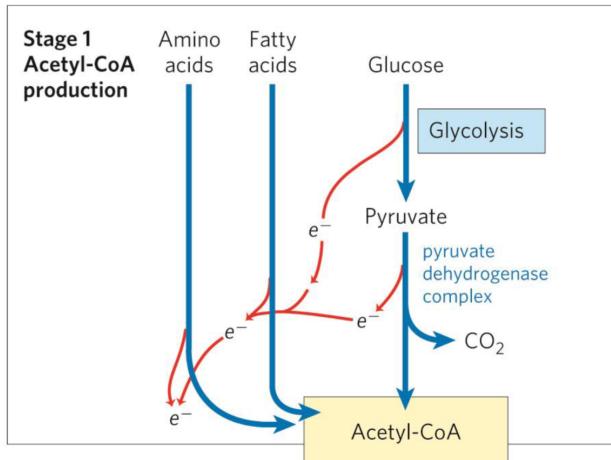


Cellular Respiration

cellular respiration = process by which the pyruvate produced by glycolysis is further oxidized to H_2O and CO_2

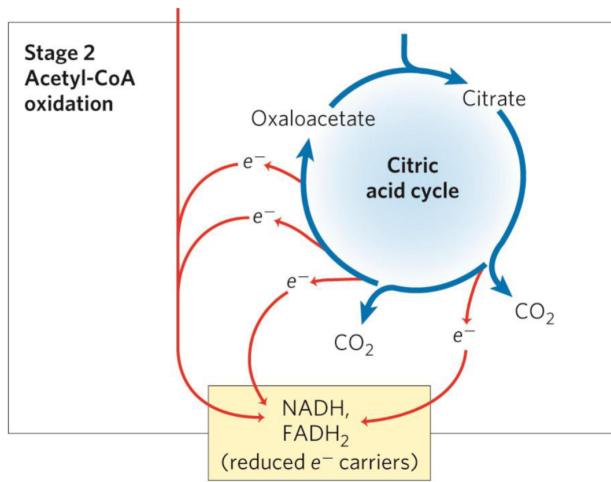
Stage 1 of Cellular Respiration

- Stage 1: Oxidation of fuels to acetyl-CoA
 - generates ATP, NADH, FADH₂



Stage 2 of Cellular Respiration

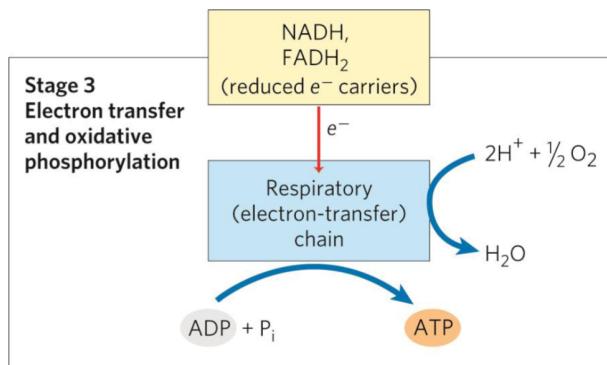
- Stage 2: oxidation of acetyl groups to CO_2 in the **citric acid cycle (tricarboxylic acid (TCA) cycle, Krebs cycle)**
 - generates NADH, FADH₂, and one GTP



Stage 3 of Cellular Respiration

- Stage 3: electron transfer chain and oxidative phosphorylation

- generates the vast majority of ATP from catabolism



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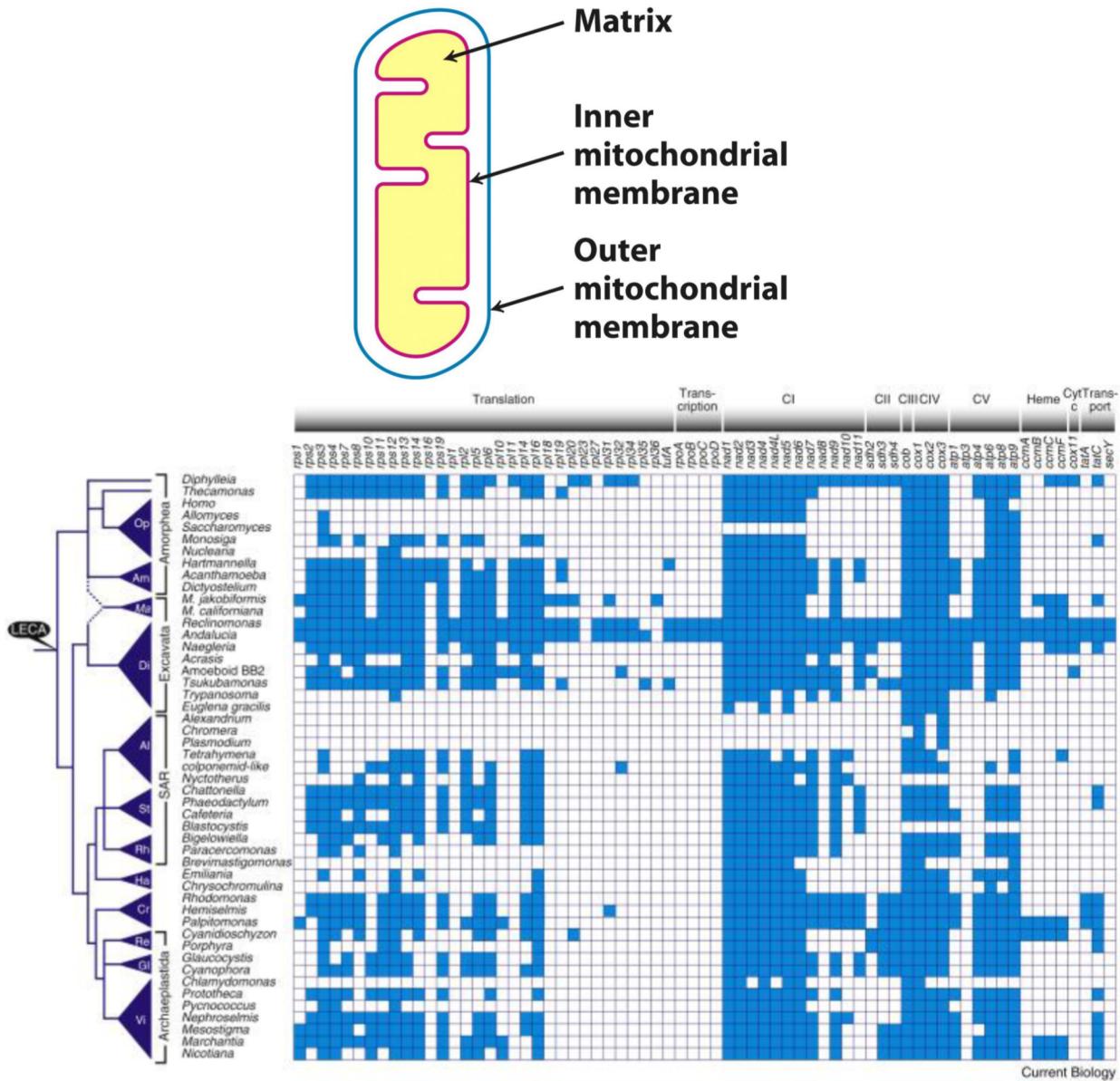
Pyruvate is the metabolite that links two central catabolic pathways, glycolysis, and the citric acid cycle. It is therefore a logical point for regulation that determines the rate of catabolic activity and the partitioning of pyruvate among its possible uses.

Pyruvate is Oxidized to Acetyl-CoA and CO₂

- Mitochondrial **pyruvate carrier (MPC)** = an H⁺-coupled pyruvate specific symporter in the inner mitochondrial membrane
- **pyruvate dehydrogenase (PDH) complex** = highly ordered cluster of enzymes and cofactors that oxidizes pyruvate in the mitochondrial matrix to acetyl-CoA and CO₂
 - the series of chemical intermediates remain bound to the enzyme subunits
 - regulation results in precisely regulated flux

The Mitochondrion

- Energy production: Site of **aerobic respiration**, oxidizing pyruvate to CO₂ and generating ATP
- Diverse biochemical processes:
 - Protein synthesis
 - Amino acid and nucleotide metabolism
 - Fatty-acid catabolism
 - Lipid, quinone, and steroid biosynthesis
 - Iron-sulfur (Fe/S) cluster biogenesis
 - Apoptosis (programmed cell death)
- The mitochondrial proteome contains over 1000 proteins, all (ETC subunits) contributing to many cellular pathways beyond ATP synthesis



The Endosymbiotic Origin of Mitochondria

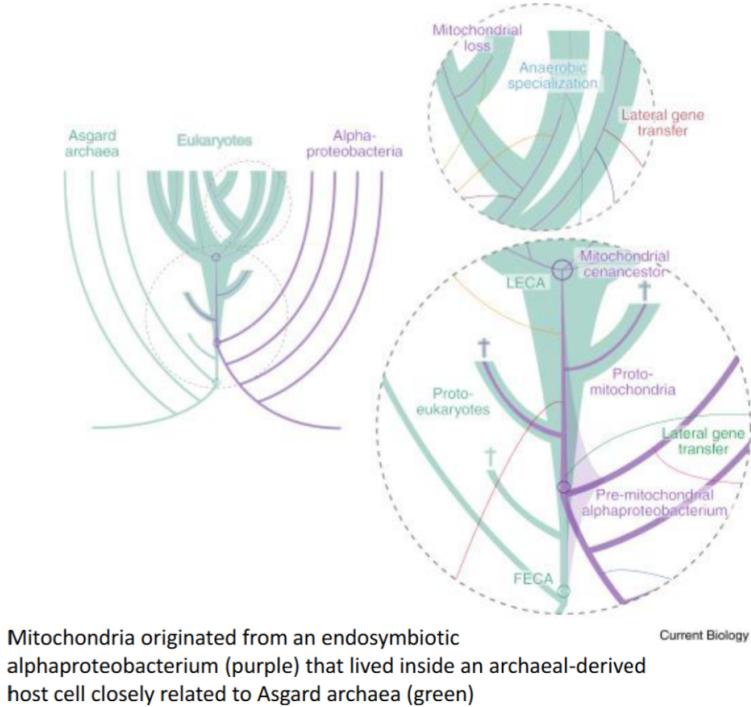
Lynn Margulis and the Endosymbiotic Theory (1967)

- Mitochondria evolved from an **endosymbiotic relationship** with an ancestral organism
 - Phylogenetic analyses confirmed:
 - Mitochondria originated from a lineage related to **alphaproteobacteria**
 - The host lineage is closely related to **Asgard Archaea**
 - Early controversy turned into widespread acceptance with advances in sequencing and proteomics

Prokaryotic Feature of Mitochondria:

- Double membrane
 - Circular DNA similar to bacteria

- Prokaryote-like ribosomes



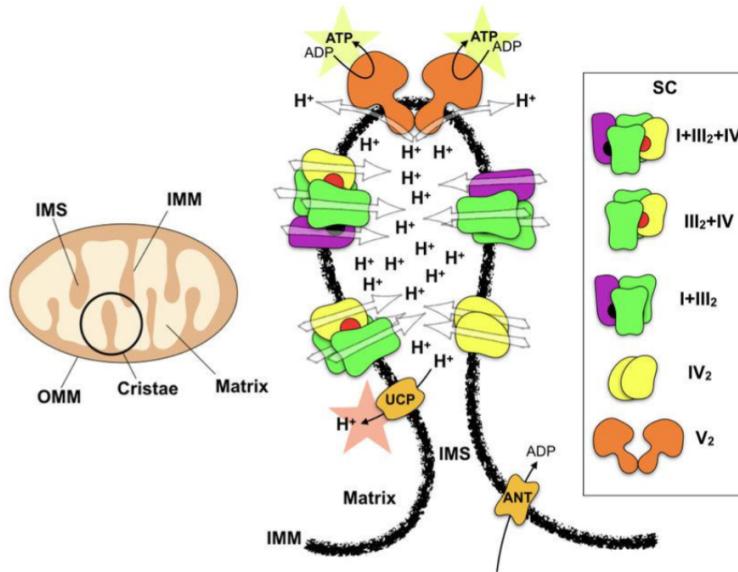
Mitochondria and the Evolution of Eukaryotes

- The Last Eukaryotic Common Ancestor (LECA)
 - All modern eukaryotes are descended from a mitochondrion-containing ancestor
 - LECA had many features of modern eukaryotes, including a fully functional mitochondrion
- Mitochondria's Evolutionary Role:
 - Enabled eukaryotes to thrive in oxygen-rich environments through efficient ATP production
 - Supported the evolution of multicellularity and cellular complexity
- Ongoing Research:
 - Genomic and cell biology studies reveal diversity in mitochondrial structure and function across
 - Controversy remains regarding the exact bacterial lineage that gave rise to mitochondria

The Mitochondrion

- Double Membrane Structure
 - The mitochondrion is enclosed by an **outer membrane** and an **inner membrane (IMM)**
 - Both membranes are **semi-permeable**, with the IMM being **impermeable to charged molecules** like protons, ensuring the separation of compartments necessary for energy production
- Cristae - Maximizing Efficiency:
 - Cristae are the **folded structures of the IMM**, significantly increasing its surface area
 - This expanded surface area accommodates more **Electron Transport Chain (ETC) complexes** and **ATP synthase**, enhancing the mitochondrion's capacity for ATP production

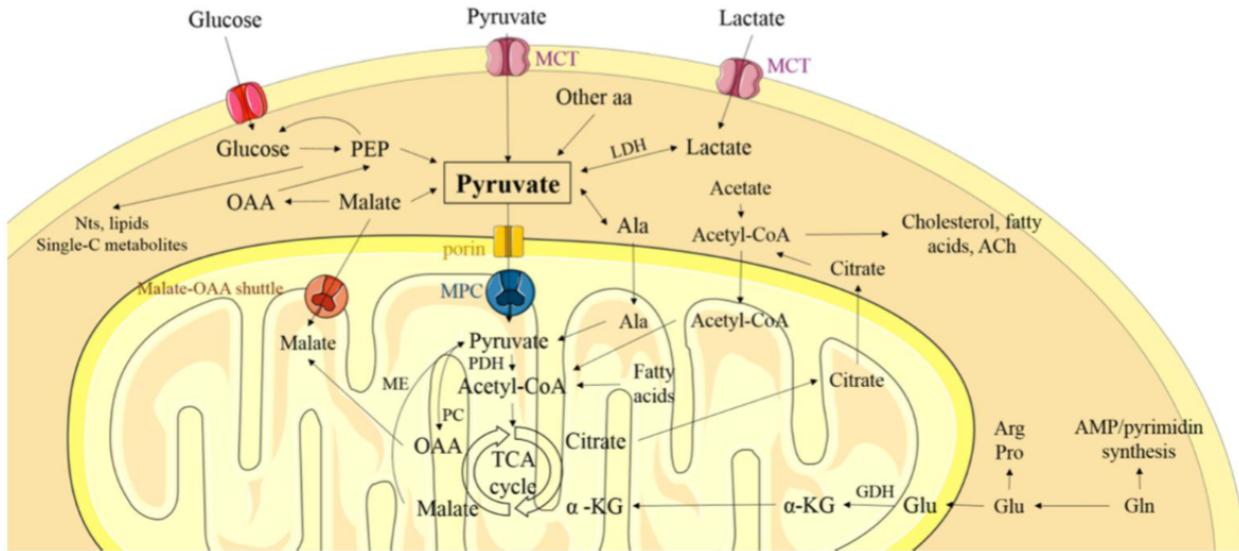
- Cristae also facilitate the **compartmentalization and concentration of protons**, creating a stronger electrochemical gradient for ATP synthesis
- **Mitochondrial Matrix:** The **matrix** is the internal space of the mitochondrion. It houses the enzymes of the **TCA cycle (Krebs cycle)**, which generate NADH and FADH₂, essential electron carriers for the electron transport chain.



The ETC complexes and accessory proteins are organized into individual **complexes** and **supercomplexes** embedded in the IMM. These complexes pump **protons (H⁺)** from the **matrix** into the **intermembrane space (IMS)**, creating an **electrochemical gradient**. The electrochemical gradient drives **Complex V (ATP synthase)** to produce ATP (lime-colored stars) from ADP

Pyruvate Transport

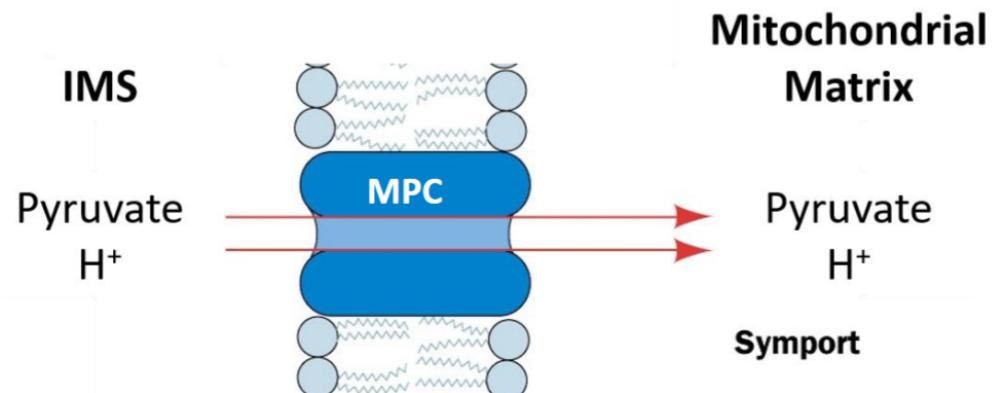
- The transport of pyruvate into the mitochondria involves crossing **two membranes**: the **outer mitochondrial membrane (OMM)** and the **inner mitochondrial membrane (IMM)**
 - The OMM contains **porins**, which are large, non-selective protein channels. These porins allow **small molecules like pyruvate** (and other metabolites up to ~5 kDa) to diffuse freely between the cytosol and the **intermembrane space (IMS)**
 - **Mechanism:** Pyruvate diffuses through the porins in a passive manner, driven by its concentration gradient



- The IMM is impermeable to charged or polar molecules, including pyruvate, so it requires a **specific transporter** for pyruvate to enter the matrix. Transport is mediated by the **Mitochondrial Pyruvate Carrier (MPC)**, a protein complex embedded in the IMM:
- Pyruvate is transported into the mitochondrial matrix together with a proton (H^+) via the MPC
- This symport is powered by the **proton gradient** across the IMM:
 - The **intermembrane space (IMS)** has a lower pH (7.0-7.4), while the **matrix** has a higher pH (7.8)
 - Protons moving down their gradient into the matrix drive the transport of pyruvate into the matrix

Energy Source:

- The transport is **secondary active transport**, as it indirectly uses the energy from the proton gradient created by the electron transport chain (ETC)



Pyruvate is imported into the mitochondrial matrix for oxidation by the TCA cycle

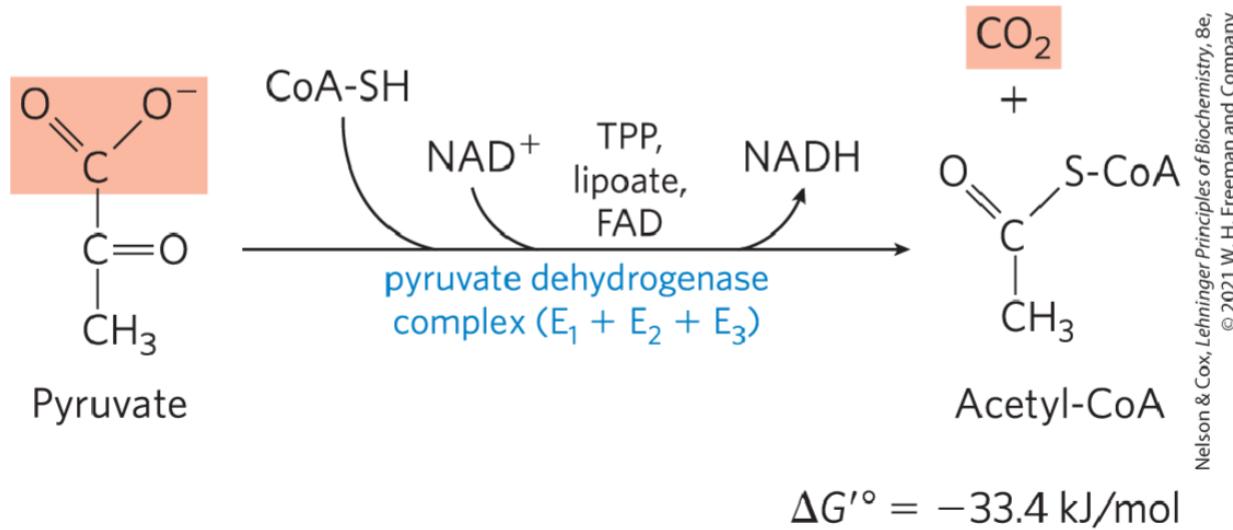
- What happens?** Pyruvate and H^+ symport into the matrix via mitochondrial pyruvate carrier (MPC)
- What powers the transport?** Driven by the pH gradient: matrix (pH 7.8) vs. IMS (pH 7.0-7.4)
- Why is it important?** Essential for TCA cycle and ATP production

Symport mechanism:

- Pyruvate is transported into the matrix together with a proton (H^+) via the MPC in a process called **symport**
- The inward flow of protons (driven by the gradient) provides the energy to "pull" pyruvate into the matrix, even if the pyruvate concentration is higher inside the matrix than in the IMS. This pH gradient (a component of the proton-motive force) is the **driving force** for this transport, leveraging the natural movement of protons down their gradient to "power" the symport of pyruvate

The PDH Complex Catalyzes an Oxidative Decarboxylation

- **oxidative decarboxylation:** an irreversible oxidation process in which the carboxyl group is removed, forming CO_2 .



The PDH Complex Employs Three Enzymes and Five Coenzymes to Oxidize Pyruvate

Three enzymes:

- pyruvate dehydrogenase, E_1
- dihydrolipoyl transacetylase, E_2
- dihydrolipoyl dehydrogenase, E_3

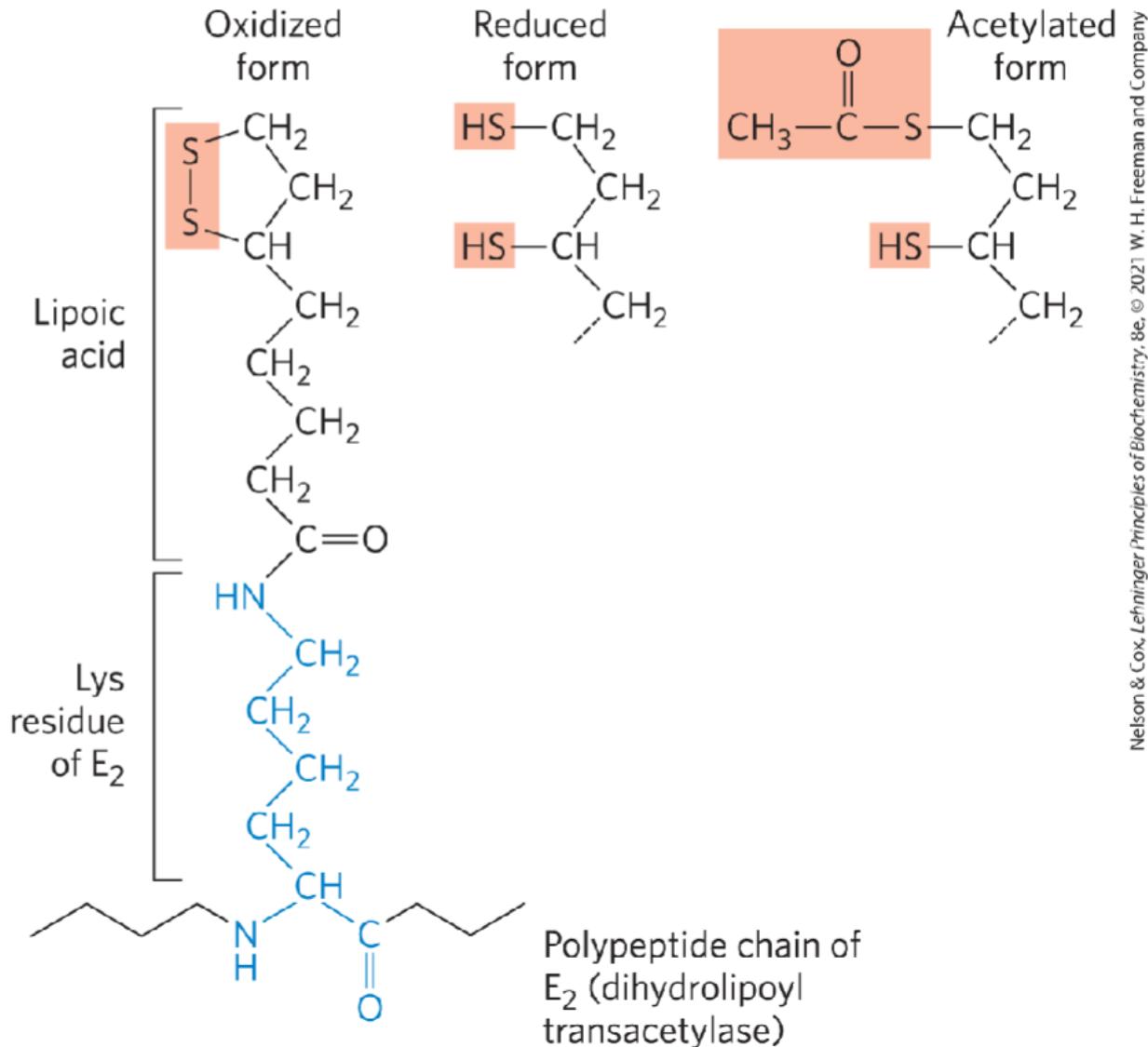
Five coenzymes:

- thiamine pyrophosphate (TPP)
- lipoate
- coenzyme A (CoA, CoA-SH)
- flavin adenine dinucleotide (FAD)
- nicotinamide adenine dinucleotide (NAD)

Lipoate

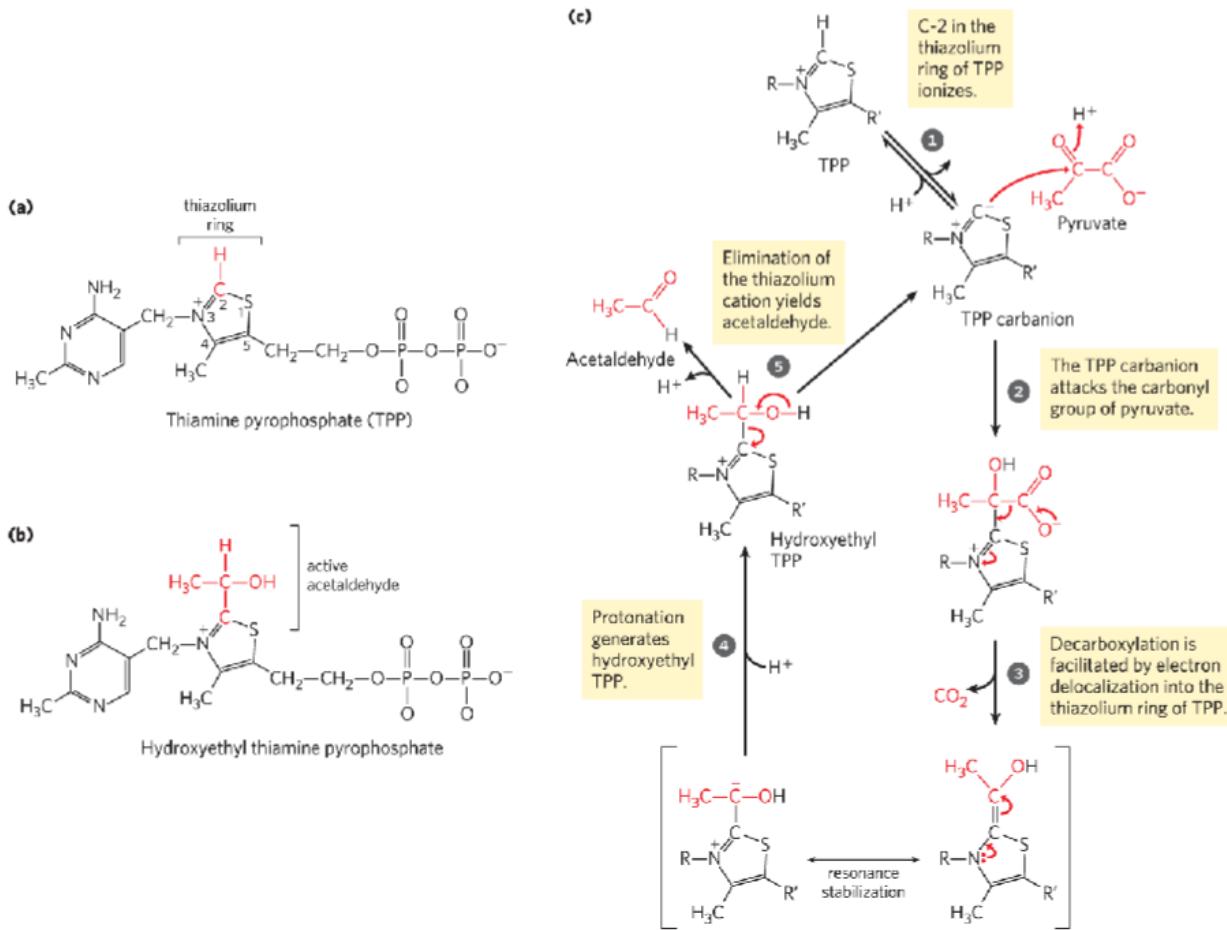
Lipoate is a coenzyme with two thiol groups that can undergo reversible oxidation to a disulfide bond (-S-S-)

- serves as an electron (hydrogen) carrier and an acyl carrier
- covalently linked to E₂ via a lysine residue



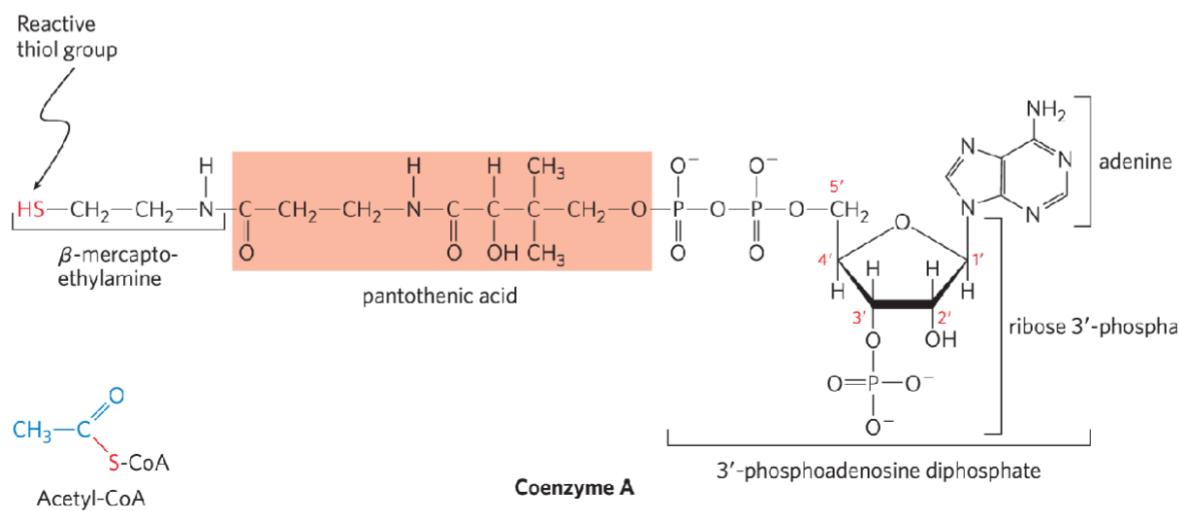
Thiamine Pyrophosphate (TPP)

- **thiamine pyrophosphate:** coenzyme derived from vitamin B₁
 - the thiazolium ring plays an important role in the cleavage of bonds adjacent to a carbonyl group

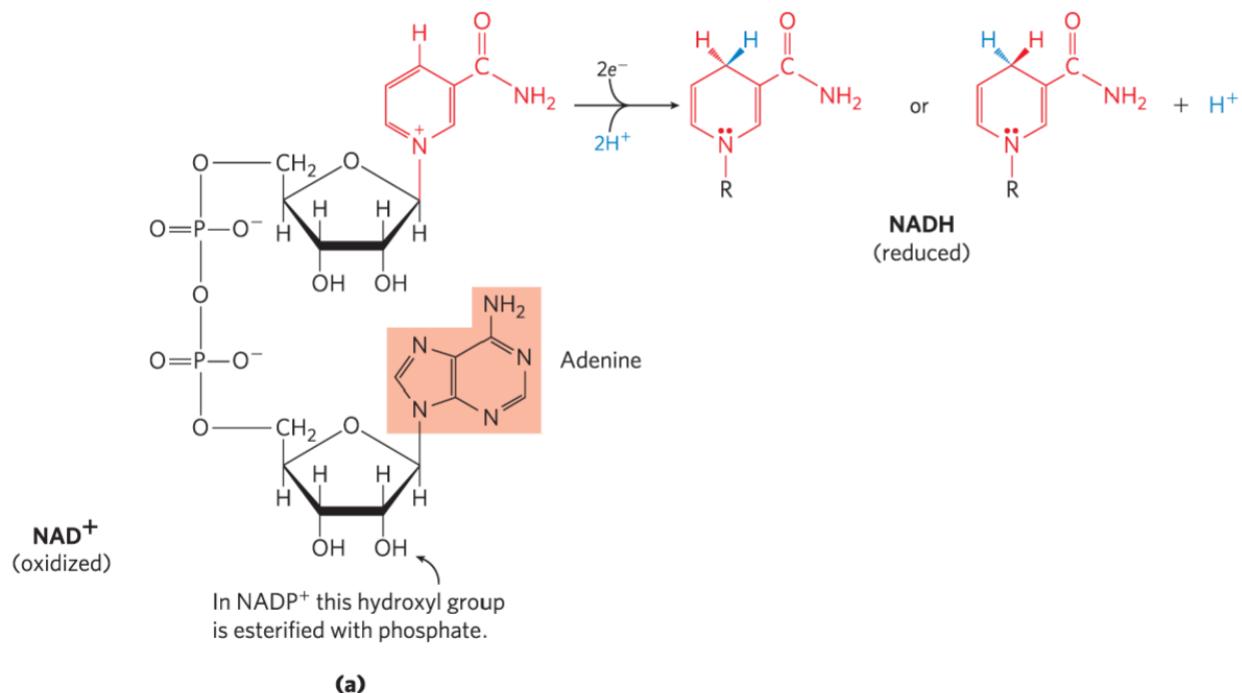


Coenzyme A (CoA-SH)

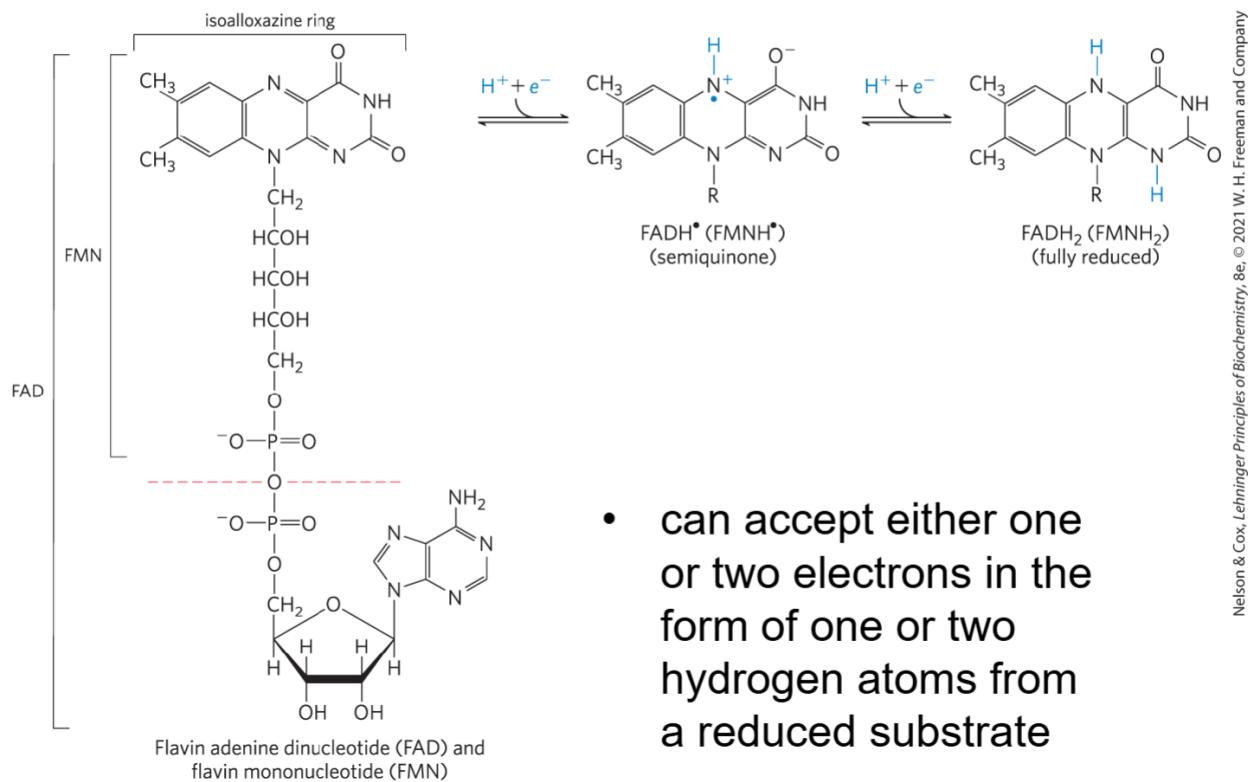
- coenzyme A has a reactive thiol (-SH) group that is critical to its role as an acyl carrier
 - the -SH group forms a **thioester** with acetate in acetyl-CoA



NAD and NADP Undergo Reversible Reduction of the Nicotinamide Ring



Oxidized and Reduced FAD and FMN



Coenzyme and Prosthetic Group Roundup

Table 17-1

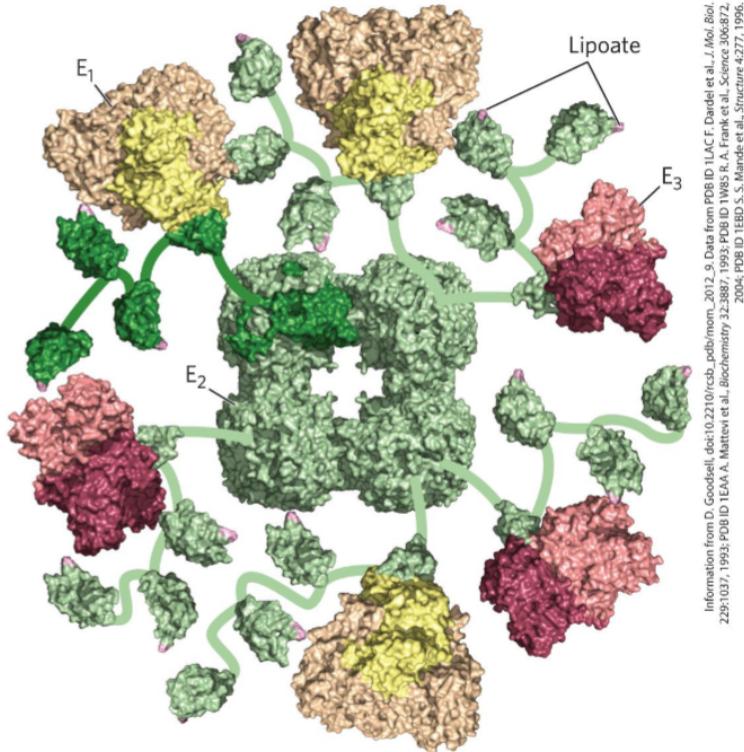
The Coenzymes and Prosthetic Groups of Pyruvate Dehydrogenase

| Cofactor | Location | Function |
|---|--|--|
| Thiamine pyrophosphate (TPP) | Bound to E ₁ | Decarboxylates pyruvate yielding a hydroxyethyl-TPP carbanion |
| Lipoic acid | Covalently linked to a Lys on E ₂ (lipoamide) | Accepts the hydroxyethyl carbanion from TPP as an acetyl group |
| Coenzyme A (CoA) | Substrate for E ₂ | Accepts the acetyl group from lipoamide |
| Flavin adenine dinucleotide (FAD) | Bound to E ₃ | Reduced by lipoamide |
| Nicotinamide adenine dinucleotide (NAD ⁺) | Substrate for E ₃ | Reduced by FADH ₂ |

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The PDH Complex Enzymes

- the PDH complex contains multiple copies of:
 - pyruvate dehydrogenase (E₁)
 - dihydrolipoyl transacetylase (E₂)
 - dihydrolipoyl dehydrogenase (E₃)
- an E₂ core (of 24-60 copies) is surrounded by multiple and variable numbers of E₁ and E₃ copies



Enzymes have evolved to form complexes to efficiently achieve a series of chemical transformations without releasing the intermediates into the bulk solvent. This strategy, seen in the pyruvate dehydrogenase complex of the metabolons of the citric acid cycle, is ubiquitous in other pathways of metabolism, in respiration, and in the many complexes.

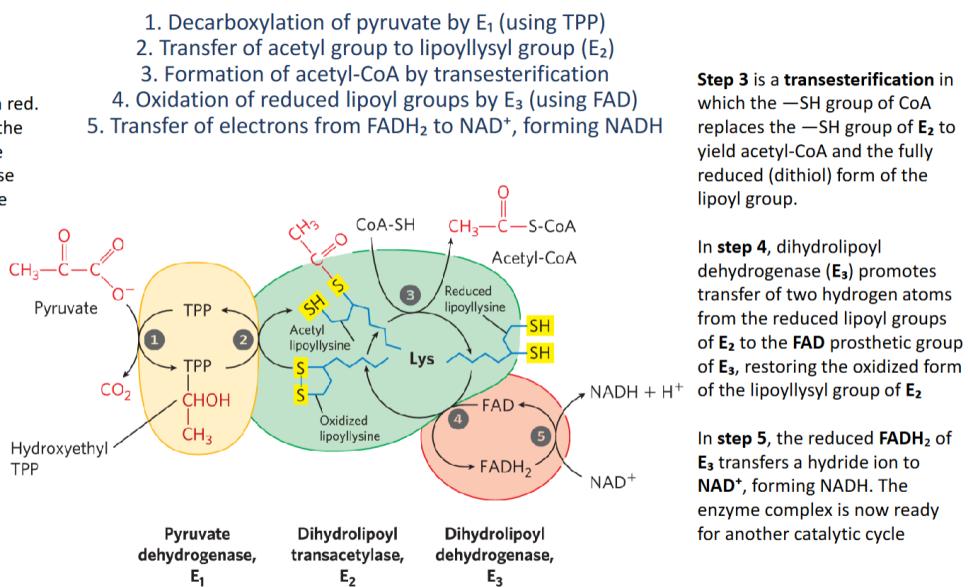
The PDH Complex Enzymes

- **E1: Pyruvate Dehydrogenase (Decarboxylase)**
 - Catalyzes the decarboxylation of pyruvate, releasing CO₂ and forming hydroxyethyl-TPP (a covalent intermediate with thiamine pyrophosphate, TPP)
 - Association: E1 binds to E2 non-covalently, allowing close interaction with E2's lipoyl domain
- **Dihydrolipoyl Transacetylase**
 - Transfers the acetyl group from hydroxyethyl-TPP to coenzyme A (CoA) forming acetyl-CoA
 - Structure: E2 forms the **core structure** of the complex, providing a scaffold for E1 and E3 to associate
 - The flexible **lipoyl arms** of E2 (with covalently attached lipoic acid cofactor) shuttle intermediates between the active sites of E1, E2, and E3
- **Dihydroilipoyl Dehydrogenase**
 - Reoxidizes the reduced lipoyl group of E2 and transfers electrons to NAD⁺, forming NADH
 - Association: E3 is non-covalently attached to the E2 core and interacts with the lipoyl domain during electron transfer

The PDH Complex Integrates Five Reactions to Convert Pyruvate into Acetyl-CoA

The fate of pyruvate is traced in red. In step 1, pyruvate reacts with the bound thiamine pyrophosphate (TPP) of pyruvate dehydrogenase (E_1) and is decarboxylated to the hydroxyethyl derivative

Pyruvate dehydrogenase also carries out step 2, the transfer of two electrons and the acetyl group from TPP to the oxidized form of the lipoyllysyl group of the core enzyme, dihydrolipoyl transacetylase (E_2), to form the acetyl thioester of the reduced lipoyl group



Oxidative Decarboxylation of Pyruvate

Pyruvate dehydrogenase, E_1 , with bound TPP catalyzes:

- Step 1: decarboxylation of pyruvate to the hydroethyl derivative
 - Rate-limiting step
- Step 2: Oxidation of the hydroethyl derivative to an acetyl group
 - Electrons and the acetyl group are transferred from TPP to the lipoyllysyl group of E_2

Dihydrolipoyl Transacetylase, E_2 , catalyzes:

- Step 3: esterification of the acetyl moiety to one of the lipoyl-SH groups, followed by transesterification to CoA to form acetyl-CoA

Dihydrolipoyl dehydrogenase, E_3 , catalyzes:

- Step 4: Electron transfer to regenerate the oxidized form of the lipoyllysyl group
- Step 5: Electron transfer to regenerate the oxidized FAD cofactor, forming NADH

The Five-Reaction Sequence of the PDH Complex is an Example of Substrate Channeling

- **Substrate Channeling** = the passage of intermediates from one enzyme directly to another enzyme without release
- the long lipoyllysyl arm of E_2 channels the substrate from the active site of E_1 to E_2 to E_3
 - tethers intermediates to the enzyme complex
 - increases the efficiency of the overall reaction
 - minimizes side reactions

Regulation of the PDH Complex Ensures Cellular Energy Balance

- Activation by Dephosphorylation (via PDP)
- Inactivation by Phosphorylation (via PDK)

Activators of PDC (Promote Dephosphorylation):

- Ca^{2+} : Directly activates PDP; important in muscle contraction and energy demand
- Insulin: Stimulates PDP; promotes glucose utilization in the fed state
- Pyruvate: Inhibits PDK, allowing PDC to stay active
- ADP: Inhibits PDK, signals low energy, promoting PDC activation
- NAD^+ : Competes with NADH to inhibit PDK, favoring PDC activation

Inhibitors of PDC (Promote Phosphorylation):

- ATP: Activates PDK; signals high energy, reducing pyruvate usage
- NADH: Activates PDK; indicates reduced state, suppressing PDC
- Acetyl-CoA: Activates PDK; signals sufficient TCA cycle substrate

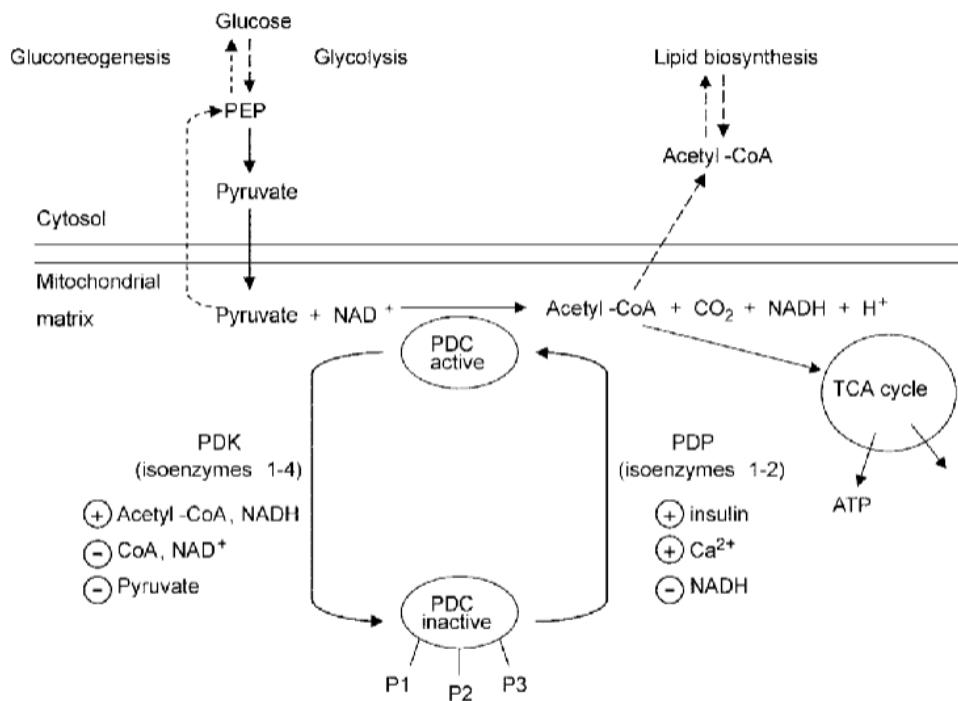


Figure 1. Regulation of PDC activity by interconversion between active (unphosphorylated) and inactive (phosphorylated) forms catalyzed by PDPs and PDKs.

Regulation ensures that the PDH Complex (PDC in this figure) integrates signals from the cell's energy status, substrate availability, and metabolic demands. It allows:

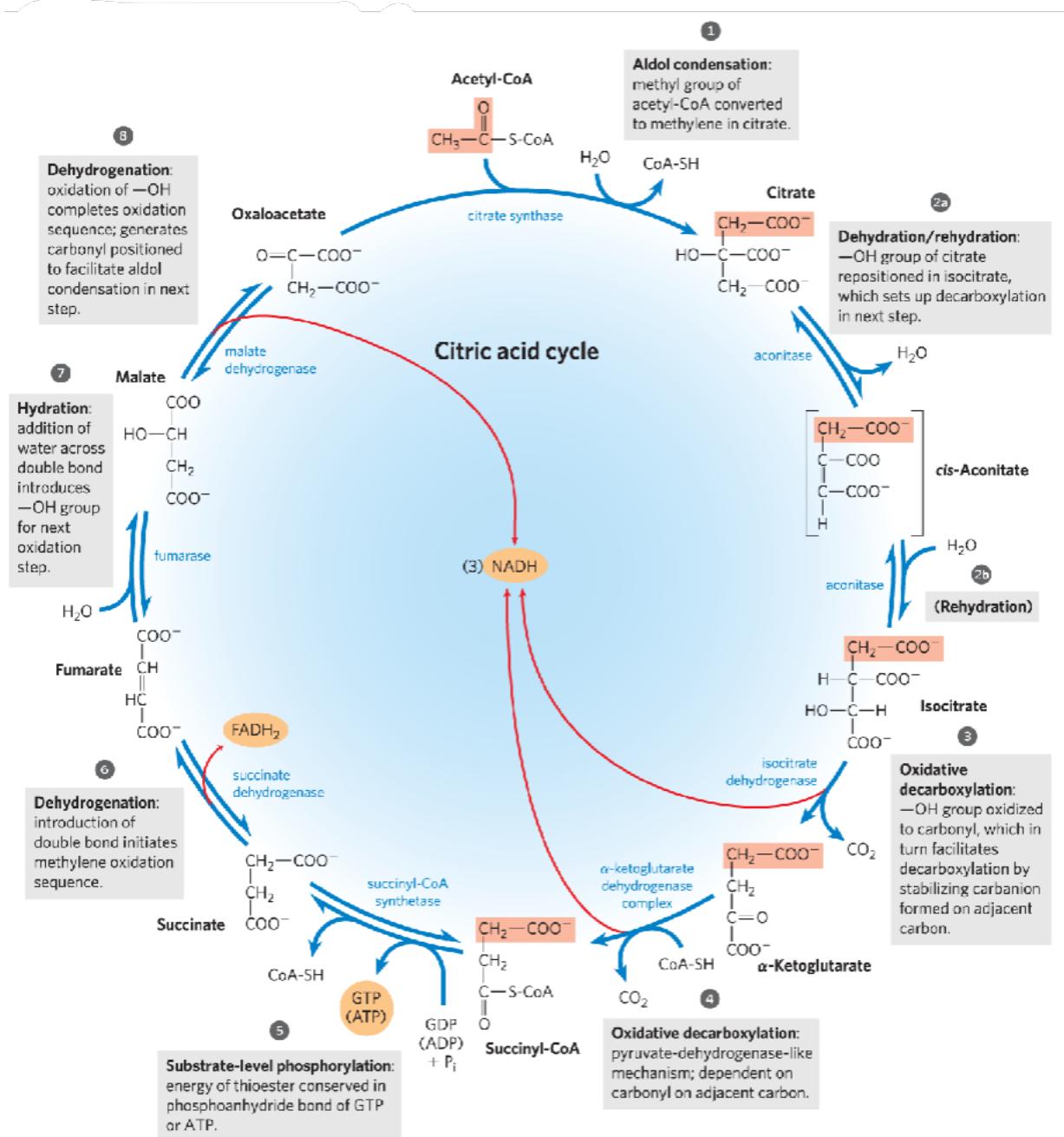
activation in conditions of high energy demand or substrate availability (e.g., pyruvate, ADP, NAD^+ , insulin), and **inhibition** in conditions of sufficient energy or biosynthetic precursor abundance (e.g., acetyl-CoA, NADH, ATP).

Reactions of the Citric Acid Cycle

Reactions of the citric acid cycle follow a chemical logic: In its catabolic role, the citric acid cycle oxidizes acetyl-CoA to CO₂ and H₂O. Energy from the oxidations in the cycle drives the synthesis of ATP. The chemical strategies for activating groups for oxidation and for conserving energy in the form of reducing power and high-energy compounds are used in many other biochemical pathways

The Citric Acid Cycle oxidizes acetyl-CoA to CO₂ and conserves energy:

- Produces 3 NADH, 1 FADH₂, and 1 GTP (or ATP) per cycle
- Regenerates oxaloacetate, allowing continuous substrate oxidation
- Feeds electrons into the electron transport chain for ATP production
- Citrate formed from acetyl-CoA and oxaloacetate is oxidized to yield:
 - CO₂
 - NADH
 - FADH₂
 - GTP or ATP
- energy from the **four** oxidations is conserved as NADH and FADH₂



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The cycle enables the sequential oxidation of acetyl-CoA carbons, capturing high-energy electrons as NADH and FADH₂. Regenerating oxaloacetate ensures that the process can continue indefinitely as long as acetyl-CoA is available, maximizing the energy yield from substrates like glucose and fatty acids.

In Eukaryotes, the Mitochondrion is the Site of Energy-Yielding Oxidative Reactions and ATP Synthesis

- Isolated mitochondria contain all enzyme, coenzymes, and proteins needed for:
 - the citric acid cycle
 - electron transfer and ATP synthesis by oxidative phosphorylation

- (and also:)
 - oxidation of fatty acids and amino acids to acetyl-CoA
 - oxidative degradation of amino acids to citric acid cycle intermediates

The Sequence of Reactions in the Citric Acid Cycle Makes Chemical Sense

- complete oxidation of acetyl-CoA and CO₂ extracts the maximum potential energy
- direct oxidation to yield CO₂ and CH₄ is not biochemically feasible because [most] organisms cannot oxidize CH₄
- carbonyl groups are more chemically reactive than a methylene group or methane
- **each step of the cycle involves either:**
 - an energy-conserving oxidation
 - placing functional groups in position to facilitate oxidation or oxidative decarboxylation