Citric Acid Cycle

step	reactant	product	enzyme	details
1	acetyl CoA + oxaloacetate	citrate	citrate synthase	
2	citrate	isocitrate	aconitase	one water lost one water gained
3	isocitrate	α-ketoglutarate	isocitrate dehydrogenase	uses one NAD+ produces NADH + CO_2
4	α-ketoglutarate	succinyl CoA	ketoglutarate dehydrogenase	uses one NAD+ produces NADH + CO_2
5	succinyl CoA	succinate	succinyl CoA synthetase	uses one phosphate produces GTP
6	succinate	fumarate	succinate dehydrogenase	uses one FAD produces $FADH_2$
7	fumarate	malate	fumarase	one water gained
8	malate	oxaloacetate	malate dehydrogenase	uses one NAD+ produces NADH

• Citric acid, Tricarboxylic acid cycle (TCA) or Krebs cycle is a central pathway used by all aerobic organisms to generate energy through the oxidation of acetate (in the form of acetyl CoA) into CO2 and ATP. Also it releases the energy-rich molecules: NADH and FADH2

• It occurs in mitochondrial matrix except reaction 6 in which succinate dehydrogenase enzyme is found in inner mitochondrial membrane (it is the only transmembrane protein in Krebs cycle)

• To proceed in Krebs cycle we need the presence of:
1. Mitochondria
2. O2

• Two phases in Krebs cycle:
1. Phase I : the release of 2 CO2 molecules
2. Phase II : regeneration of oxaloacetate

• ***Step 1***: The irreversible condensation of acetyl CoA (2C) and oxaloacetate (4C) via citrate synthase to form citrate (6C)

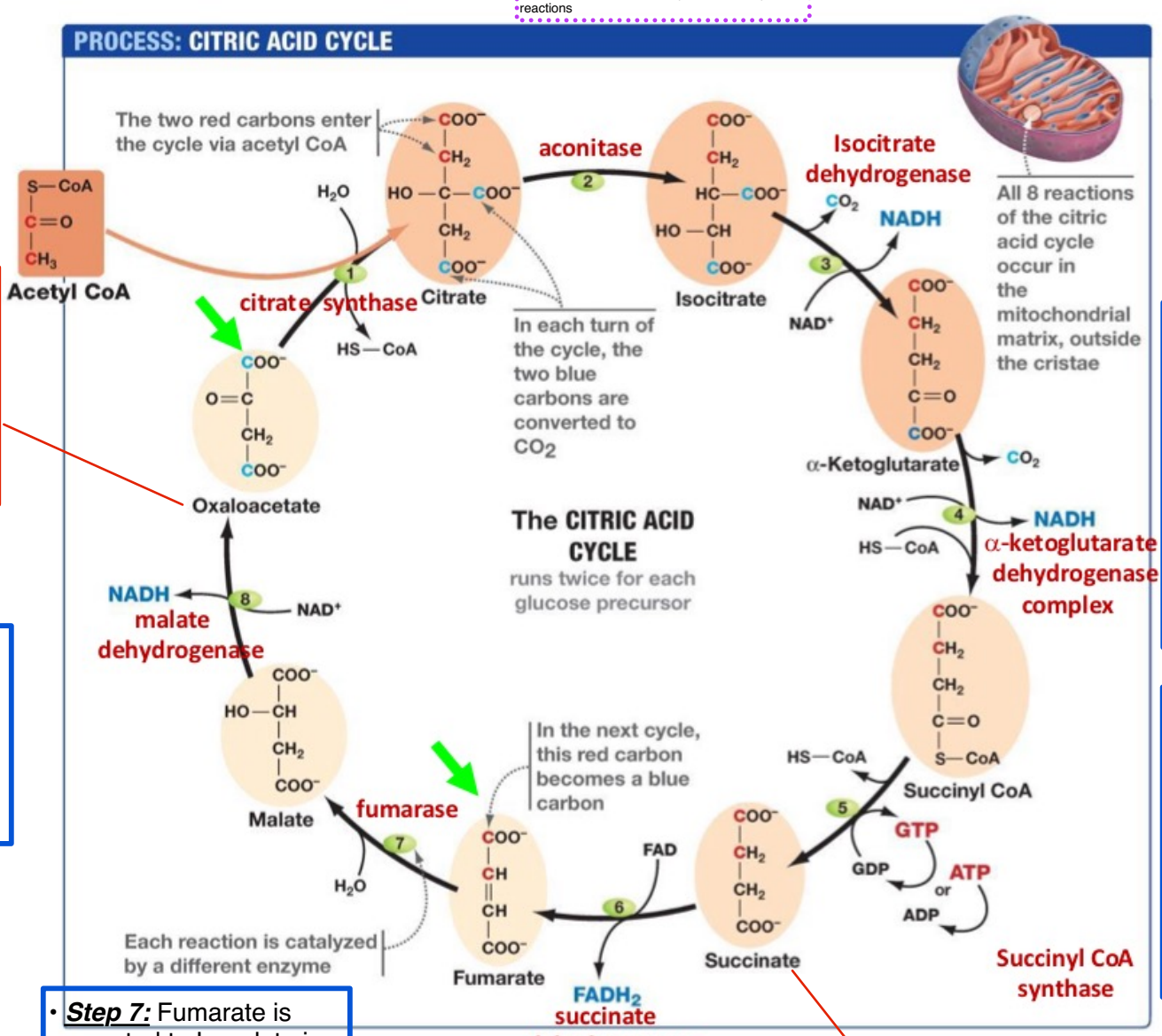
• ***Step 2***: Aconitase enzyme catalyzes the reversible isomerization of citrate to isocitrate (isomers differ in the position of OH group from C3 to C2)

• This isomerization reaction is pre-required step to prepare substrates for decarboxylation reaction

• It involves successive dehydration and hydration reactions

• ***Step 3:*** Isocitrate dehydrogenase catalyzes the first oxidative decarboxylation of isocitrate (6C) to a-ketoglutarate (5C) resulting in the release of first CO2 and the formation of first NADH molecule

• It involves successive oxidation and decarboxylation reactions



• Oxaloacetate is already found in matrix. It can be produced in several ways in nature. For example, it is generated from an ATP-dependent carboxylation of pyruvate catalyzed by pyruvate carboxylase. This reaction occurs in the matrix

• ***Step 4:***a-ketoglutarate dehydrogenase complex catalyzes the oxidative decarboxylation of a-ketoglutarate (5C) to succinyl CoA (4C) releasing the second CO2 and producing the second NADH molecule

• ***Step 5***: Succinyl CoA synthetase (also known as succinate thiokinase) generates the first ATP (e.g. brain & heart tissues) or GTP (e.g. liver tissues).
The thioester bond of succinyl-CoA is energy-rich and can drive the phosphorylation of ADP or GDP
(substrate-level phosphorylation)

• ***Step 8:*** L-malate is oxidized to regenerate oxaloacetate via malate dehydrogenase enzyme thus generating the third NADH (reversible)

• ***Step 7:*** Fumarate is converted to L-malate in a hydration reaction catalyzed by fumarase (reversible reaction)

• ***Step 6:*** Succinate dehydrogenase catalyzes the oxidation of succinate to fumarate and consequently, the reduction of prosthetic group FAD into FADH2

• Succinate dehydrogenase is
the only enzyme found in the inner membrane of mitochondria

• FAD is more powerful oxidizing agent than NAD+

• It is stereoselective enzyme and only the trans isomer “fumarate” is formed but not the cis isomer H “maleate”

• At the end of krebs cycle, the products of oxidation one glucose via glycolysis and TCA are:
4 ATP + 6 CO2 + 10 NADH + 2 FADH2

Stage	ATP produced by substrate-level phosphorylation
Glycolysis	2 ATP
Acetyl CoA production	none
Krebs Cycle	2 ATP
Total/glucose 4 ATP molecules	

Stage	Electron-carrier molecule	Total H^+ pumped	ATP synthase $4H^+ \longrightarrow$ 1 ATP
Glycolysis	2 NADH	12-20	3-5 ATP
Acetyl CoA production	2 NADH	20	5 ATP
Krebs Cycle	6 NADH 2 $FADH_2$	60 12	15 ATP 3 ATP
Total/glucose 26-28 ATP produced by oxidative phosphorylation			

• TCA is considered as a part of aerobic metabolism although it does not use O2 in any of its reaction ??

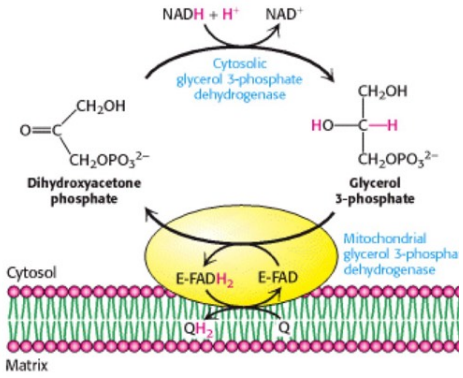
the TCA cycle (Krebs cycle) is part of aerobic metabolism because its products (NADH and $FADH_2$) are used in the electron transport chain, which requires oxygen to produce ATP. So, even though O_2 is not directly used in the TCA cycle itself, the cycle cannot function without oxygen.

Cytosolic NADH Shuttling

• The electrons carried by cytosolic NADH (i.e. NADH generated by glycolysis) will be shuttled to the matrix by one of two mechanisms:

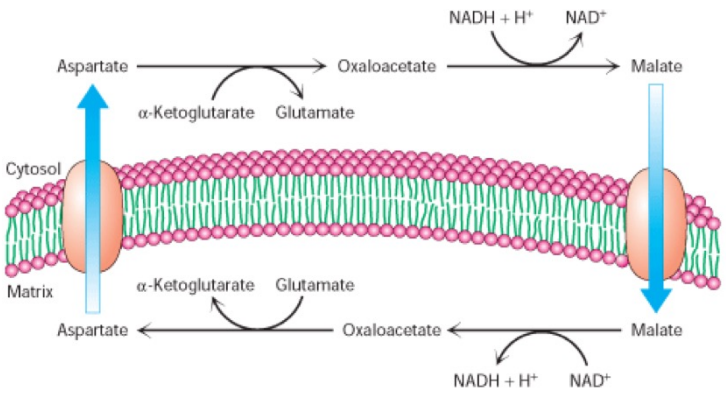
1. DHAP/G3P shuttle:

it is active in brain and skeletal muscle. This pathway delivers the 2e from cytosolic NADH to mitochondrial FAD



2. Aspartate/malate shuttle:

it is active in liver and heart. This pathway delivers the 2e from cytosolic NADH to mitochondrial NAD+ (found in the matrix)



extra:

Cytosolic NADH Shuttling

NADH produced in the cytosol (e.g. during glycolysis) cannot enter the mitochondria directly, so the electrons ($2e^-$) from NADH must be transferred into the mitochondria using shuttle systems. There are two main shuttles:

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1. DHAP/G3P Shuttle
   - Active in: Brain and skeletal muscle.
   - How it works:
   - Cytosolic NADH transfers its electrons to DHAP, converting it to G3P.
   - G3P enters the mitochondria and gives the electrons to FAD, forming $FADH_2$.
   - Result: Electrons from NADH reach the mitochondrial electron transport chain via FAD, which produces less ATP than NADH.

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2. Aspartate/Malate Shuttle
   - Active in: Liver and heart.
   - How it works:
   - Cytosolic NADH transfers its electrons to oxaloacetate, forming malate.
   - Malate enters the mitochondria and transfers the electrons to NAD^+, forming NADH inside the mitochondria.
   - Result: The electrons are transferred to mitochondrial NAD^+, so the cell gets more ATP compared to the G3P shuttle.

Summary:
Both shuttles help move electrons from cytosolic NADH into the mitochondria, but they use different molecules and result in different energy yields.

Anaplerotic Pathway

- Anaplerotic pathways (from Greek word meaning filling up) are the processes that replenish

TCA intermediates so that the flow of carbon out of the cycle is balanced by these reactions

extra:

* Anaplerotic pathways (from Greek “anaplerosis” meaning “filling up”) are reactions that refill or replenish the intermediates of the TCA cycle.

Why are they important?
Because TCA cycle intermediates are sometimes taken out of the cycle to be used for other purposes (like making amino acids, glucose, etc.). If too many are removed, the cycle can’t work properly.

So, anaplerotic reactions add new carbon to the cycle to keep it running smoothly.

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Example:

- Pyruvate → Oxaloacetate (by the enzyme pyruvate carboxylase) is a key anaplerotic reaction.

It helps replenish oxaloacetate, an important TCA intermediate.

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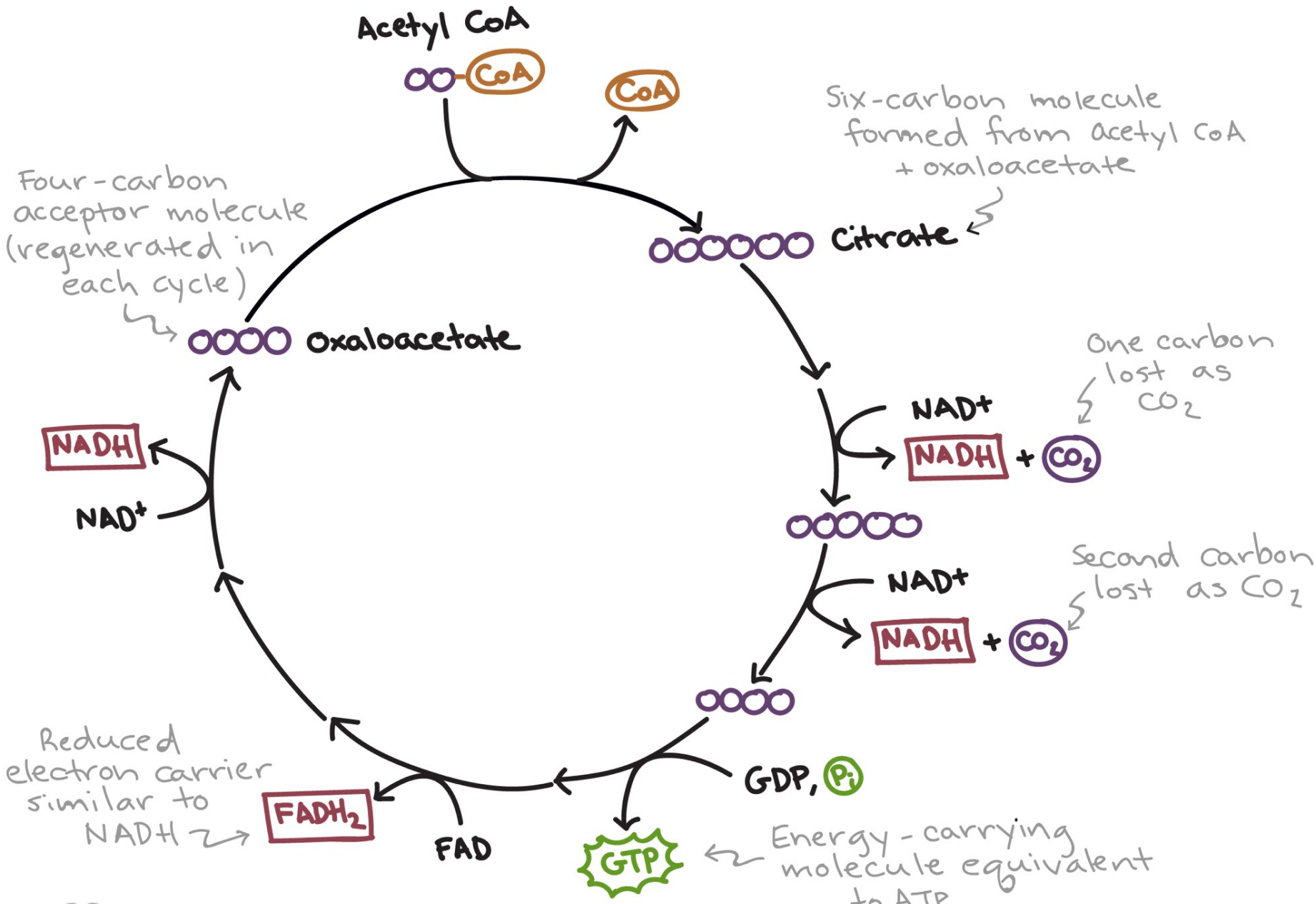
In short:
Anaplerotic pathways refill the TCA cycle when intermediates are lost.



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