Ciclo del Acido Cítrico o Ciclo de Krebs

Ideas previas

Planteo de preguntas

- ¿En qué consiste?
- ¿Dónde ocurre?
- ¿Cómo se regula?
- Interacción con otras vías metabólicas

Repaso de clases anteriores

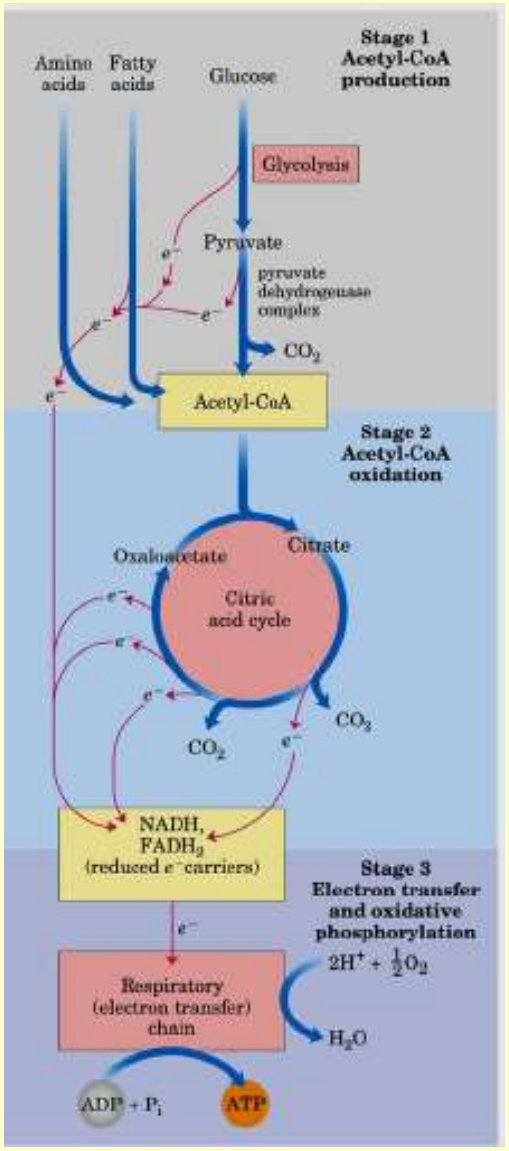
- ¿Destino de la glucosa?

  Glucólisis → Piruvato

- ¿Destino del piruvato?

  Anaerobios → vías fermentativas

  Aerobios → CO_2 + H_20 + energía



Respiración celular

Liberación de la energía de CHOs, AA y Ac grasos mediante reacciones bioquímicas

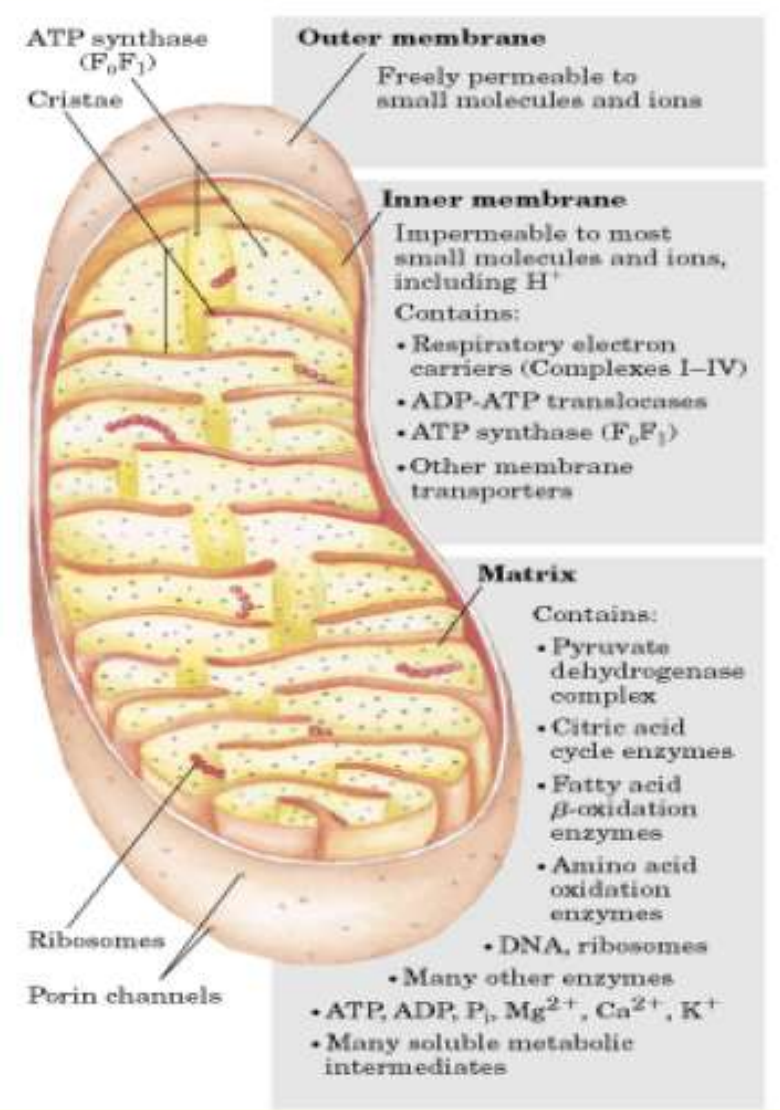
Fases

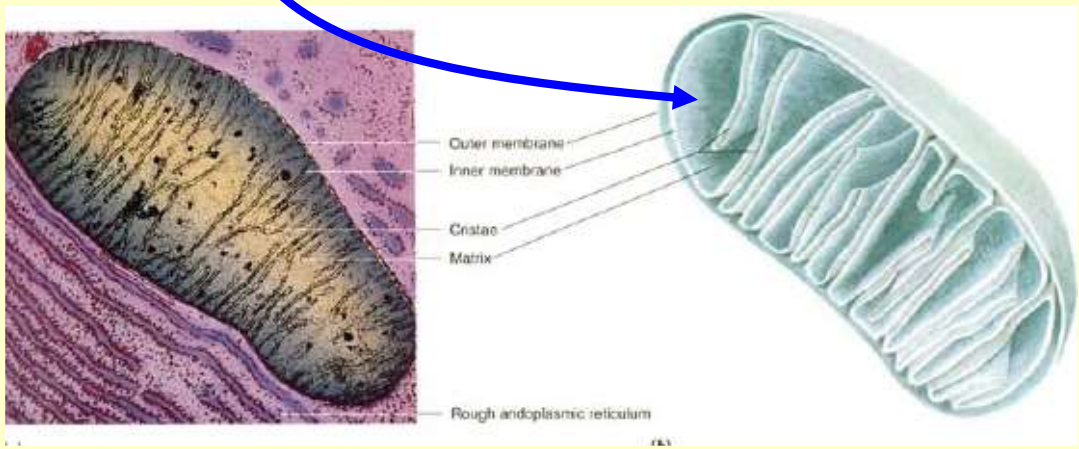
A- La oxidación de ácidos grasos, glucosa y algunos aminoácidos produce Acetil-CoA

B- El Acetil-CoA se oxida a CO_2 en una vía cíclica con producción de coenzimas reducidas y energía

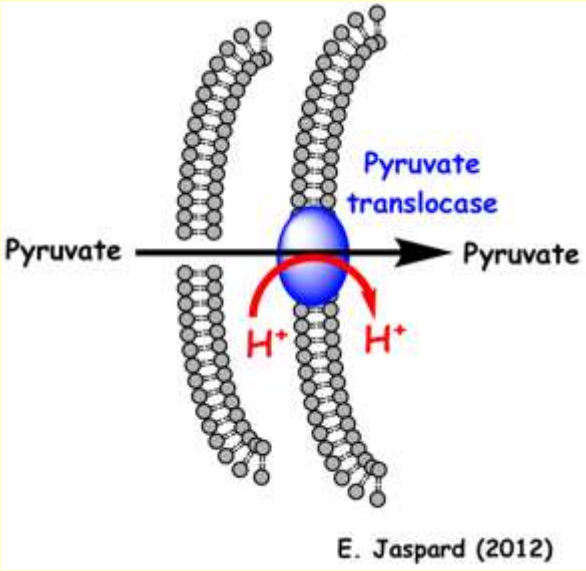
C- Oxidación de las coenzimas en la cadena de transferencia de electrones y generación de ATP

El cliclo de Krebs transcurre en la matriz mitocondrial

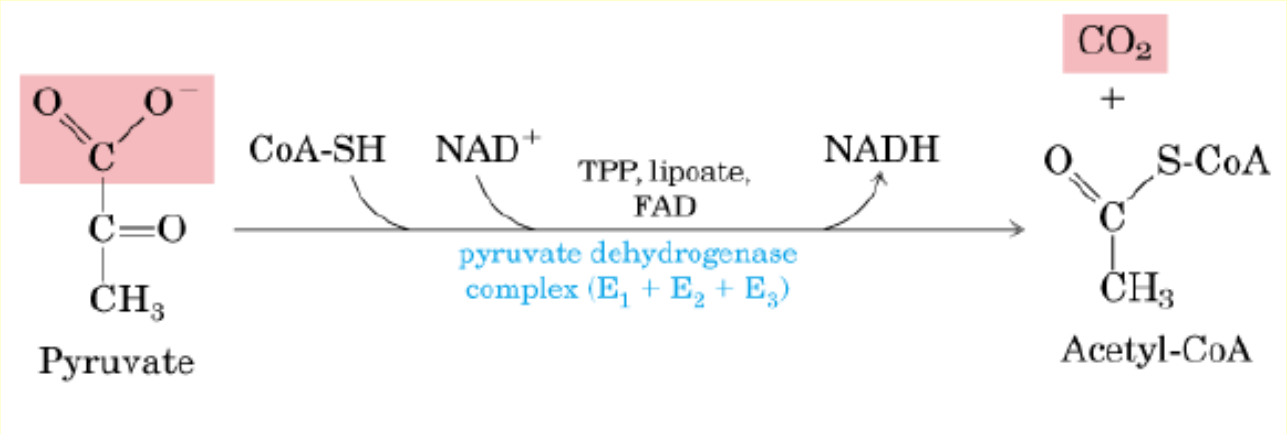




Entrada de Piruvato a la Mitocondria



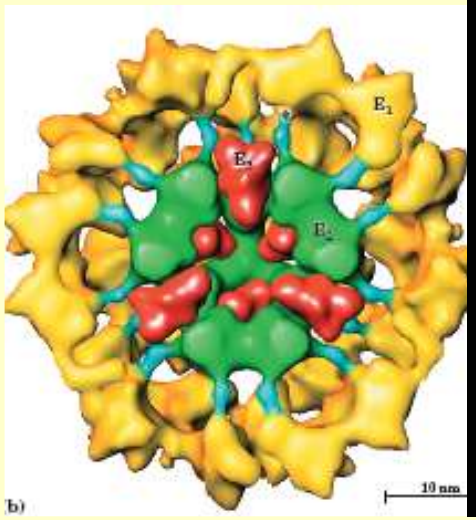
Conversion de Piruvato en Acetil-CoA

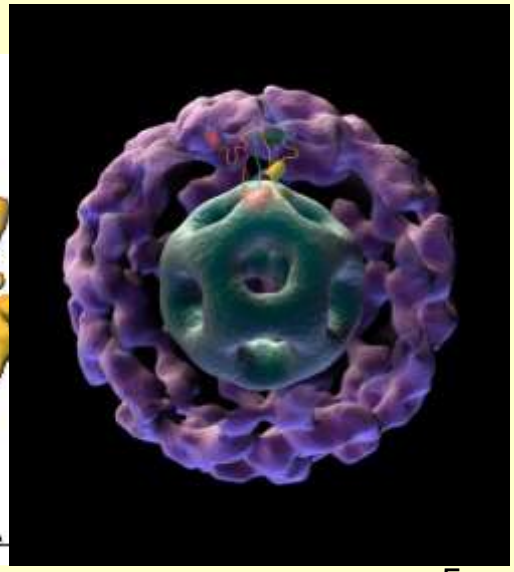


Complejo Piruvato Deshidrogenasa

- E1: piruvato deshidrogenasa (60)
- E2: dihidrolipoil transacetilasa (60)
- E3: dihidrolipoil deshidrogenasa (12)
- Quinasa
- Fosfatasa

50 nm de diámetro y 4.600.000 Da

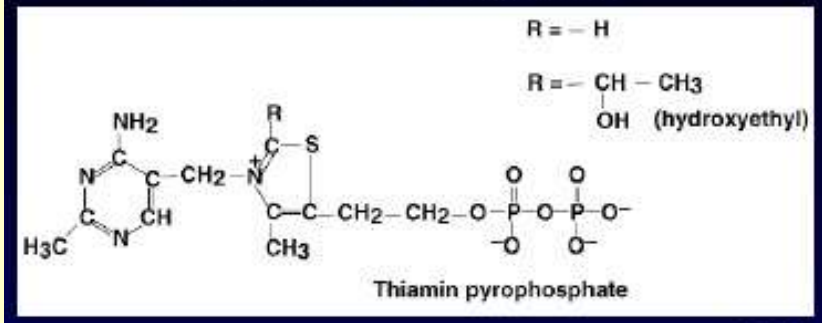




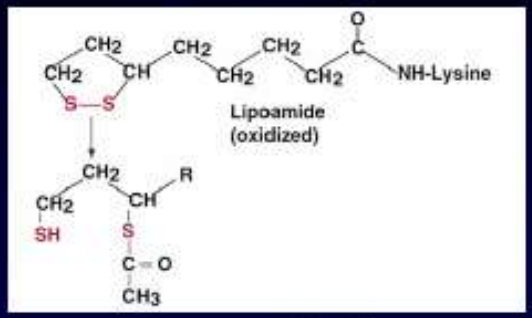
Fuente Imagen del complejo Pirivato DH: https://imagebank.nih.gov/details.cfm?imageid=1279

Coenzimas del complejo PDH

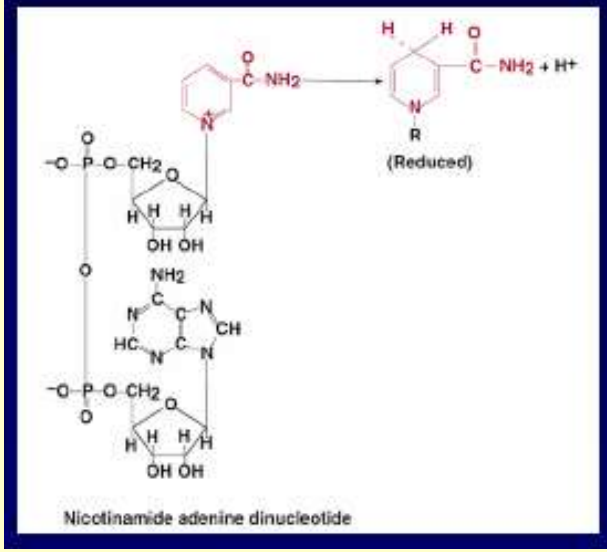
Pirofosfato de tiamina



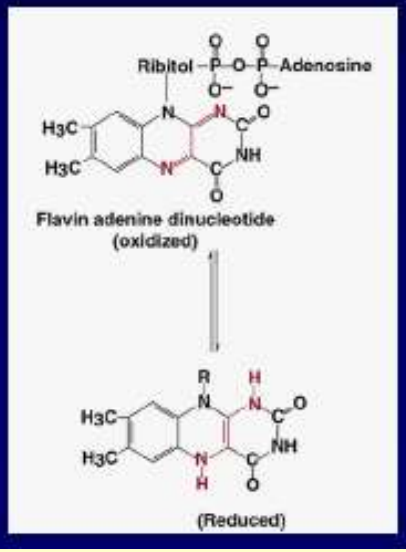
Lipoamida



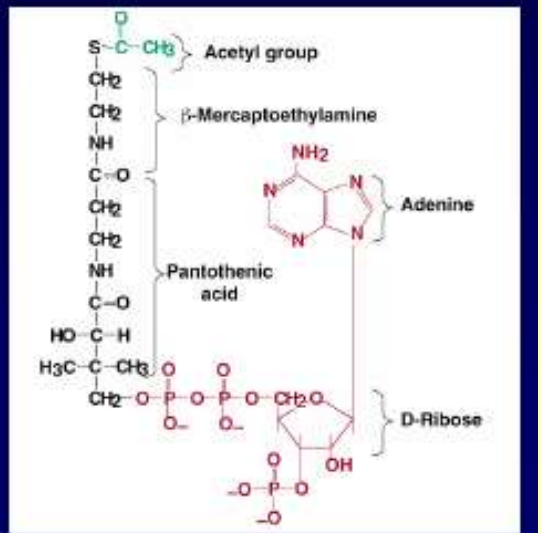
NAD



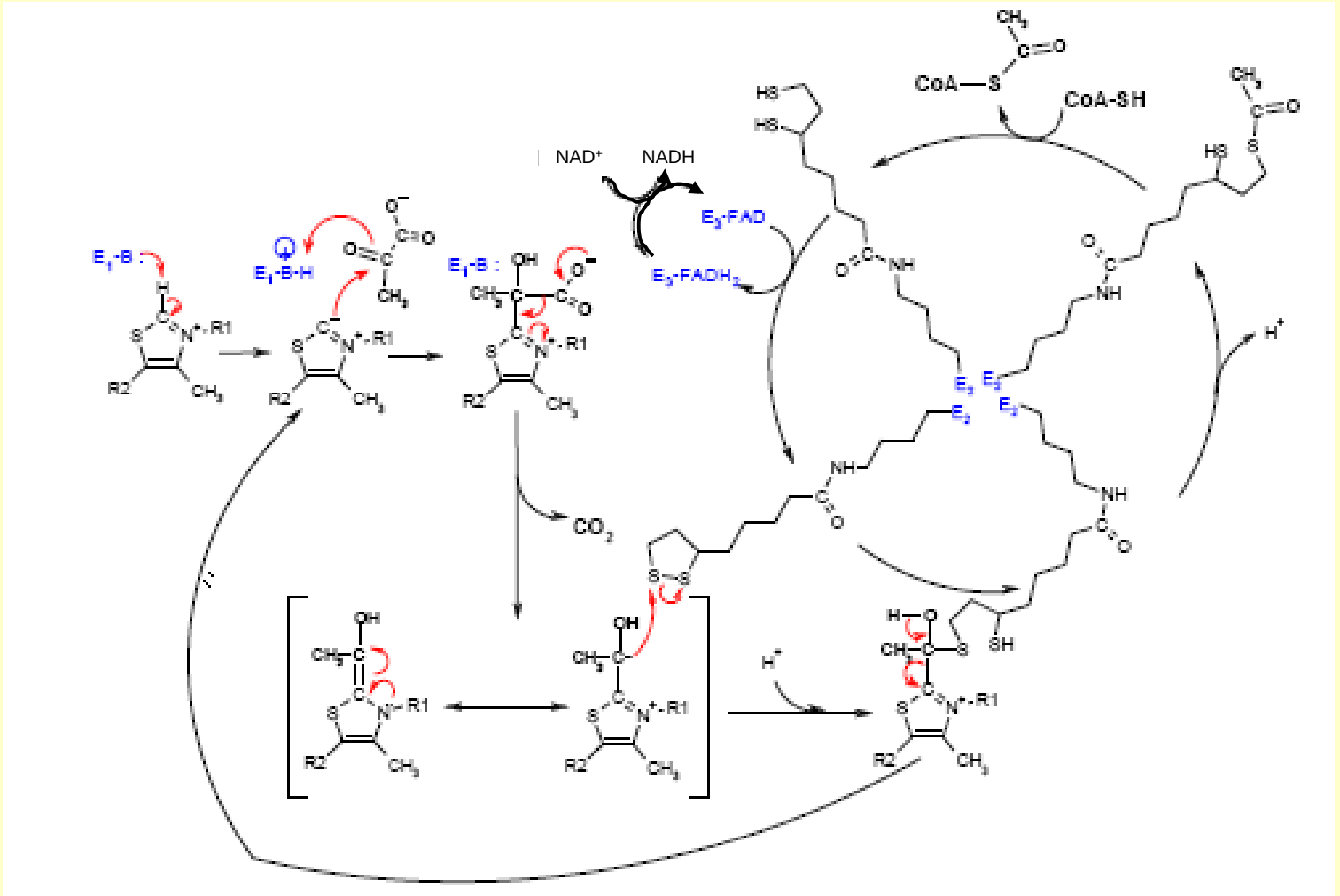
FAD



CoA



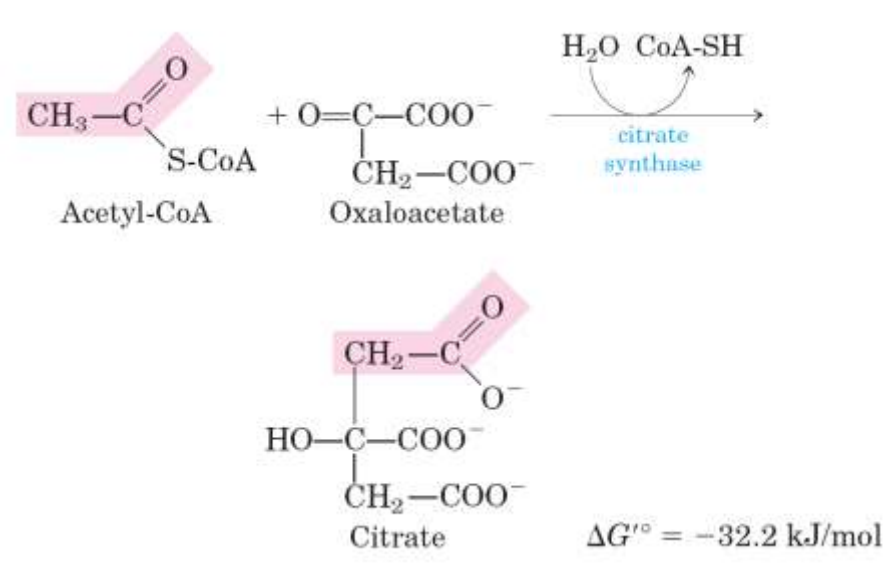
Mecanismo de reacción

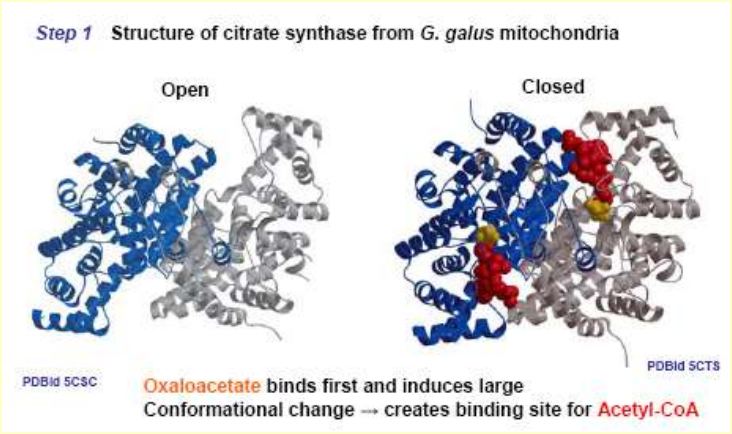


Reacciones del Ciclo de Krebs

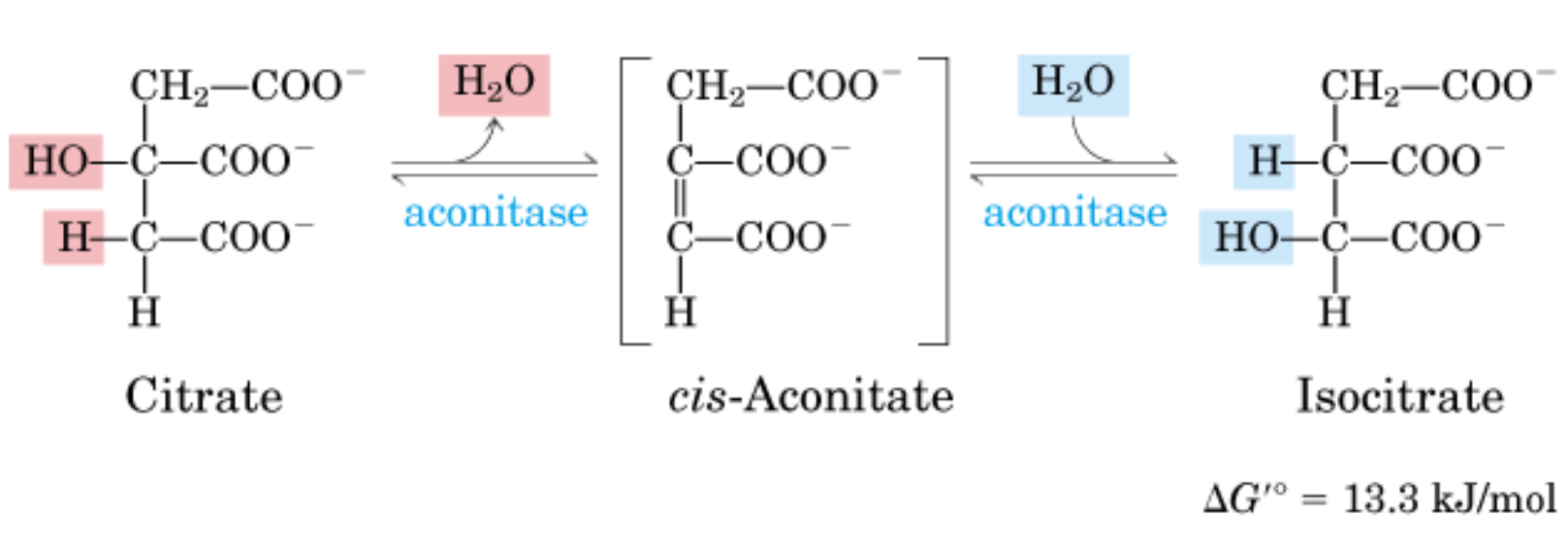
Reacciones del Ciclo de Krebs

1 - Formación de Citrato

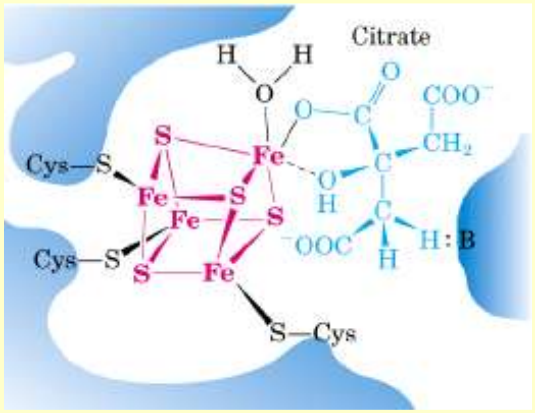




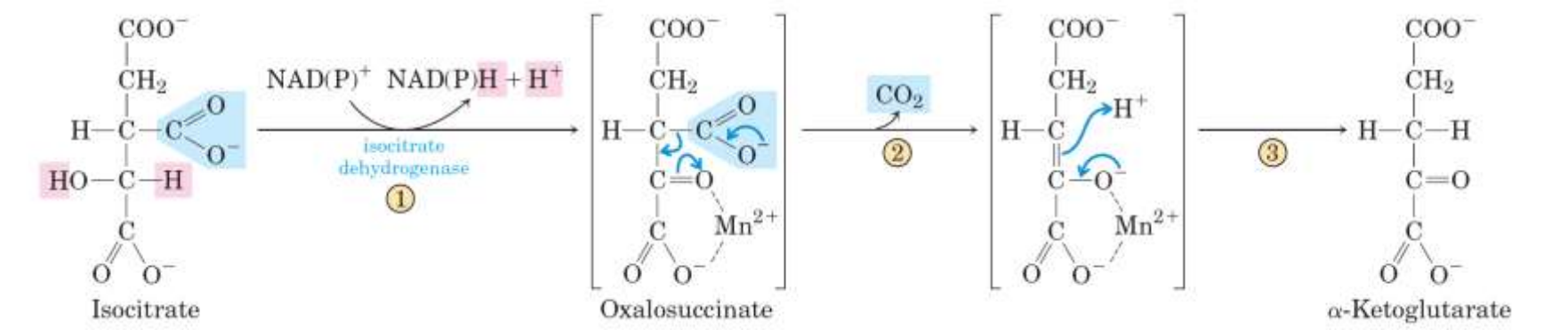
2 - Formación de Isocitrato vía *cis*-Aconitato



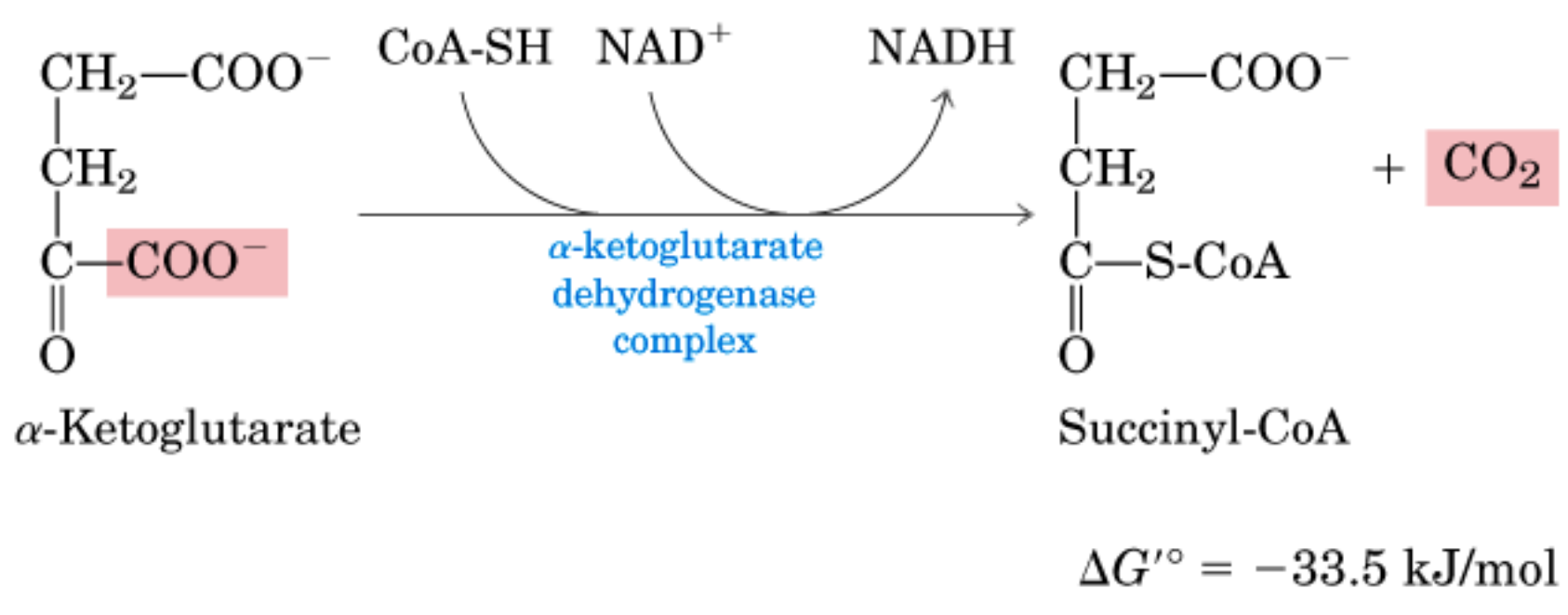
La aconitasa contiene un centro hierro azufre que actúa como centro de fijación de sustratos y centro catalítico.



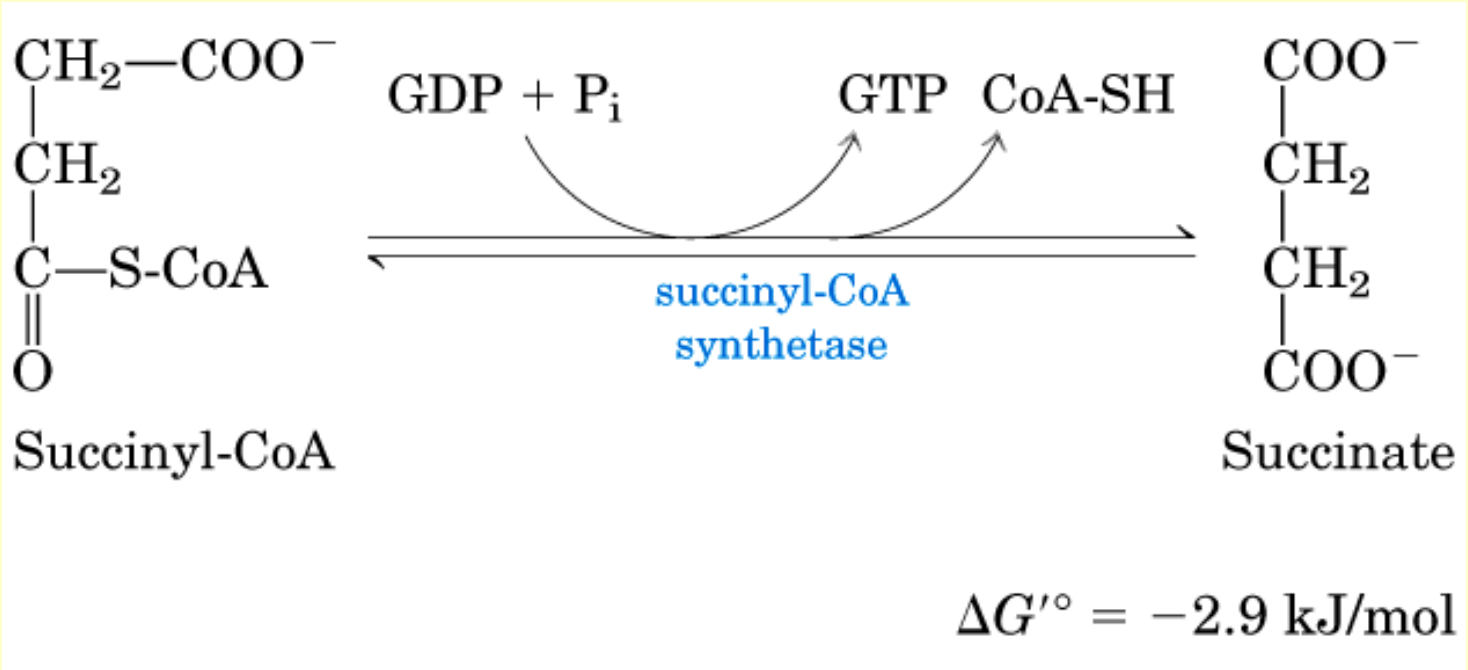
3 - Oxidación del Isocitrato a Alfacetoglutarato y CO_2



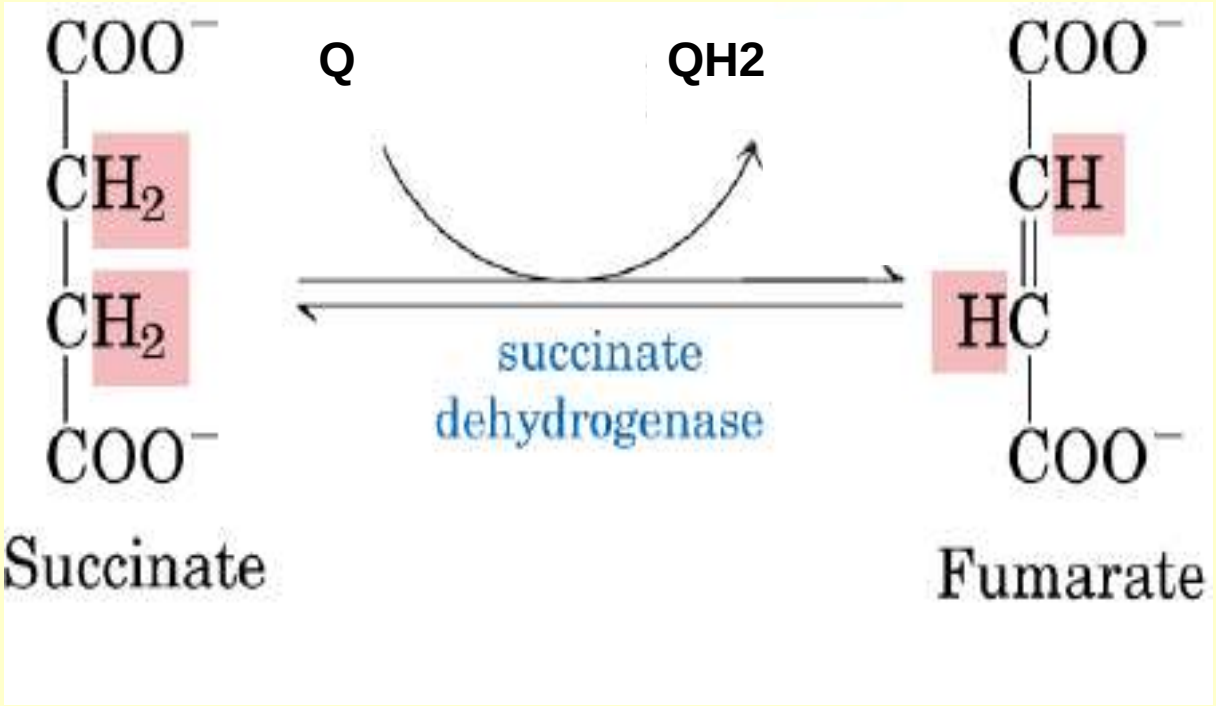
4 – Oxidación del Alfacetoglutarato a Succinil CoA y CO_2



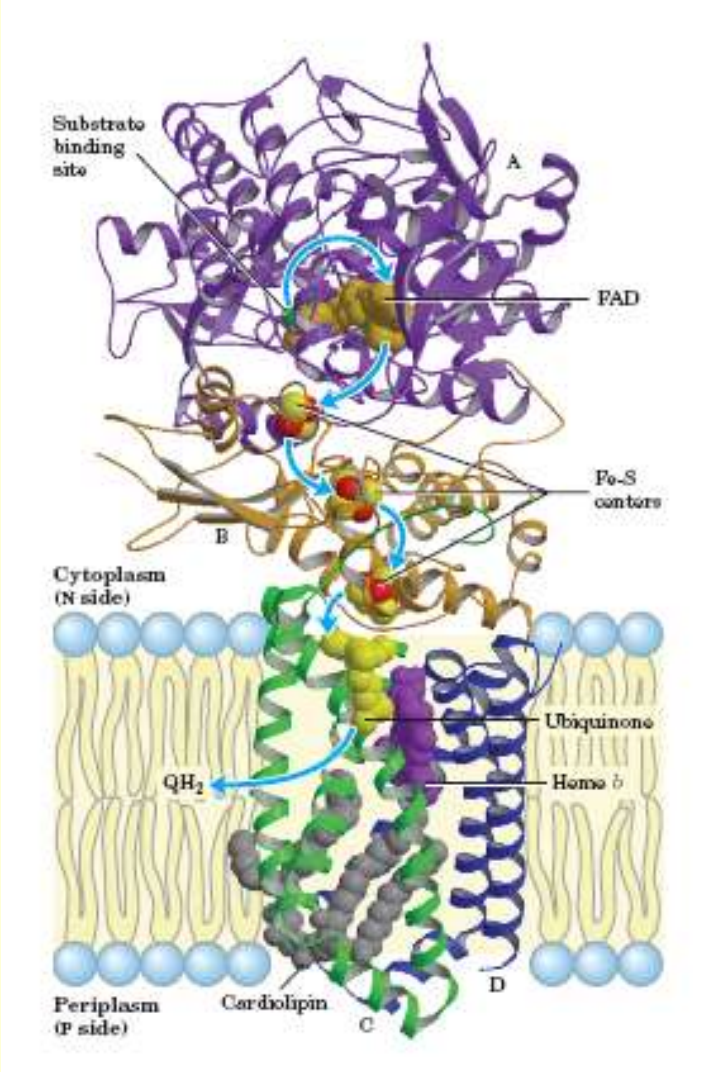
5 - Conversión de Succinil-CoA en Succinato



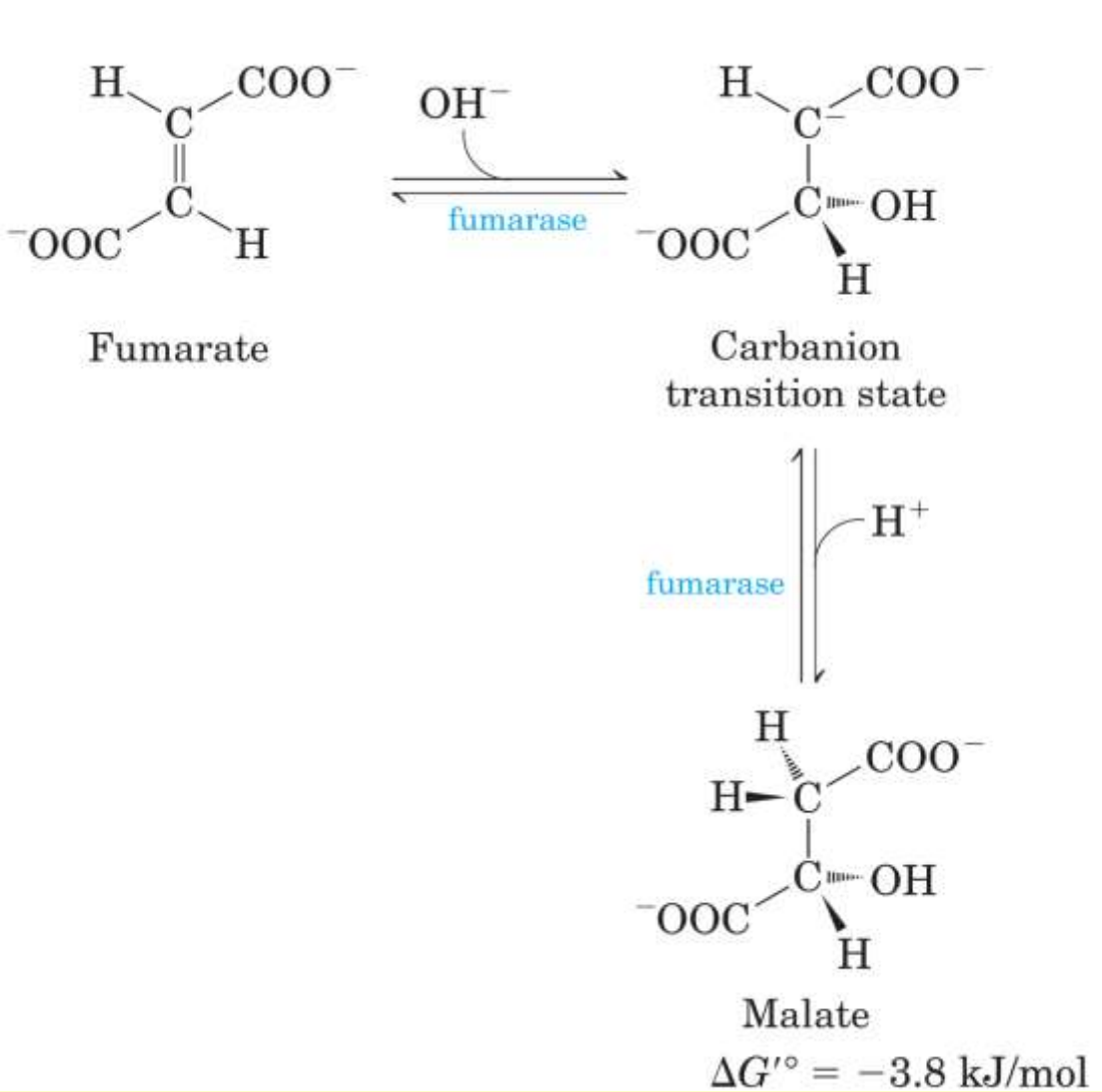
6 - Oxidación de Succinato a Fumarato



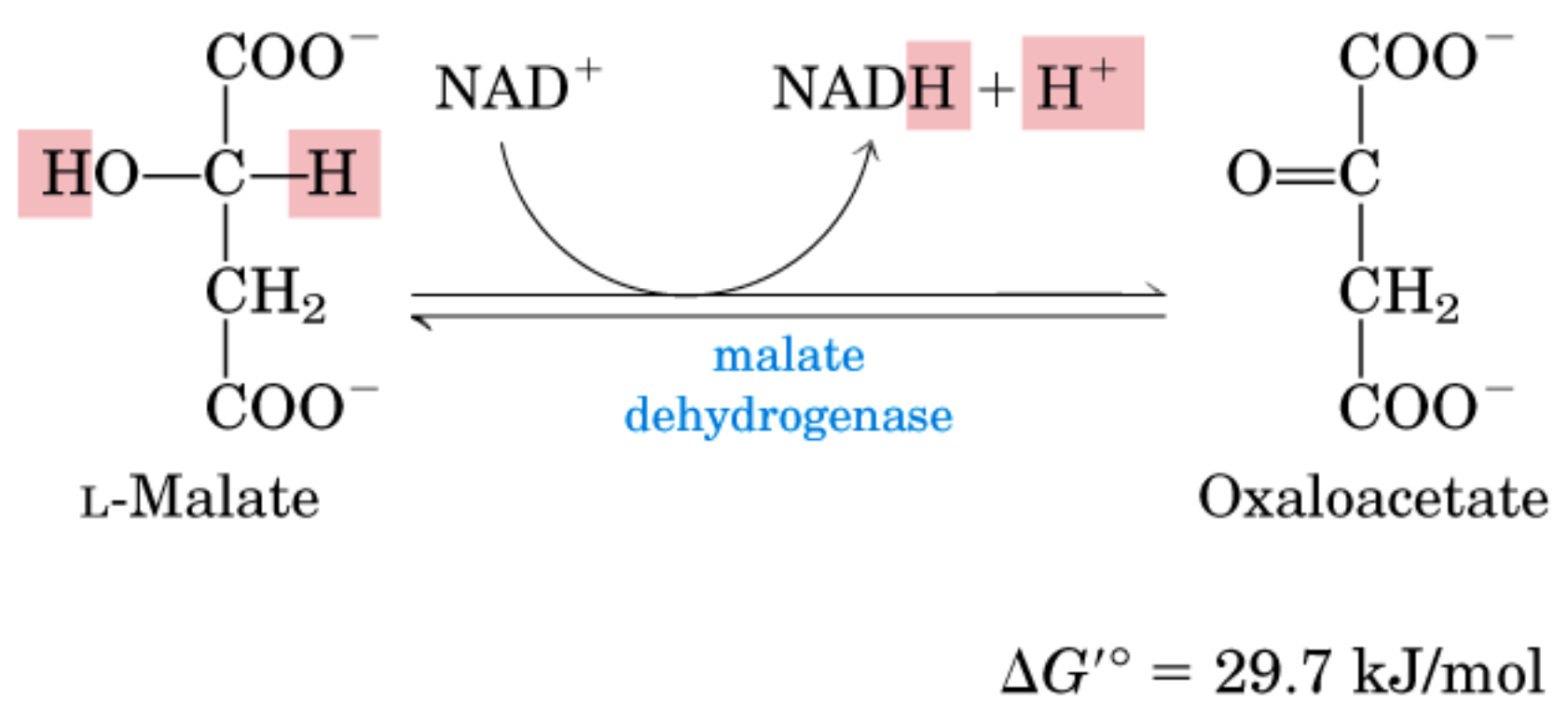
Succinato deshidrogenasa



7 - Hidratación del Fumarato y producción de Malato

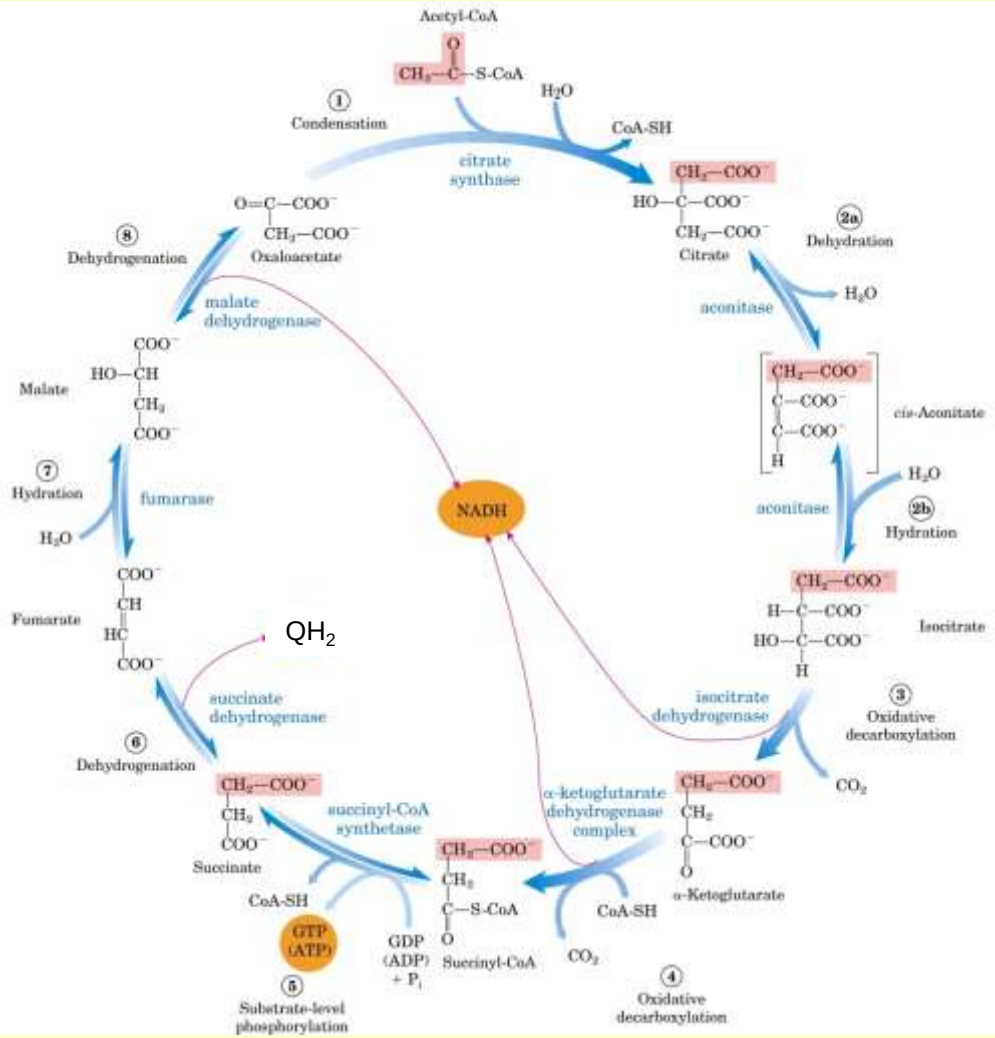


8 - Oxidación de Malato y regeneración del Oxalacetato



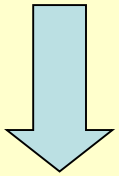
Ciclo de Krebs, visión global

http://www.sigmaaldrich.com/life-science/metabolomics/learning-center/metabolic-pathways/tca-cycle/tca-cycle-animation.html



Balance del Ciclo de Krebs

- Acetil-CoA + 3 NAD^+ + Q + GDP(o ADP) + Pi + 2 H_2O



- 2 CO_2 + CoASH + 3 NADH + QH_2 + GTP (o ATP) + 2 H^+

Ganancia energética a partir de una molécula de Glucosa

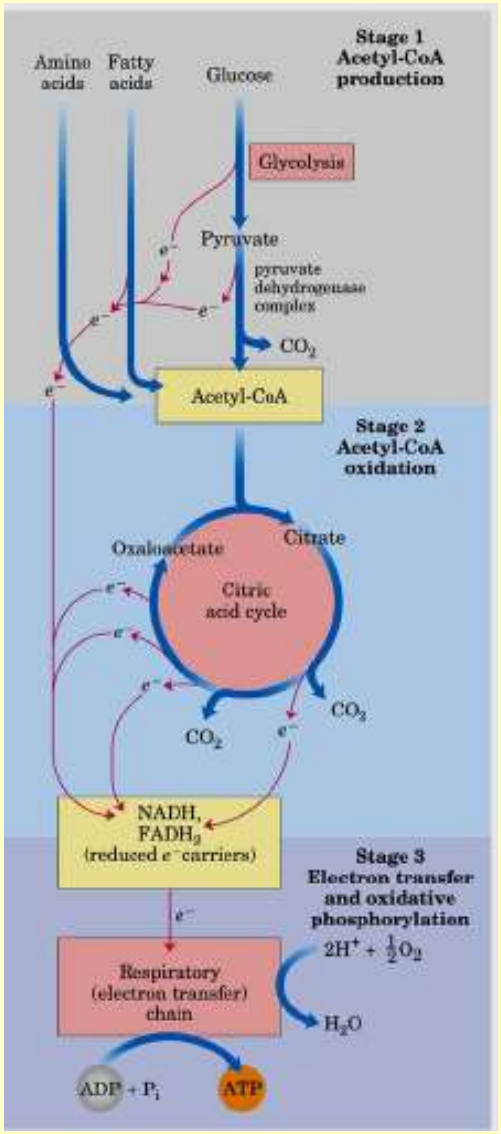


Table 16-1

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

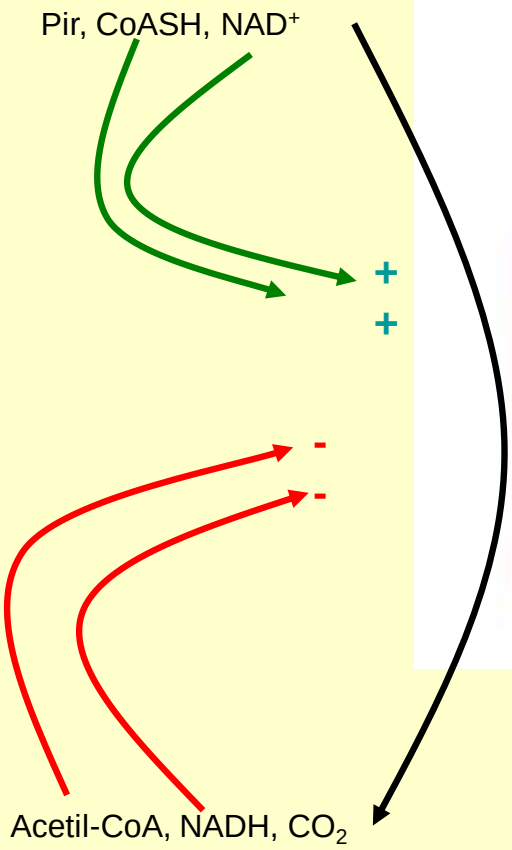
Reaction	Number of ATP or reduced coenzymes directly formed	Number of ATP ultimately formed*
Glucose ⟶ glucose 6-phosphate	−1 ATP	−1
Fructose 6-phosphate ⟶ fructose 1,6-bisphosphate	−1 ATP	−1
2 Glyceraldehyde 3-phosphate ⟶ 2 1,3-bisphosphoglycerate	2 NADH	3–5
2 1,3-Bisphosphoglycerate ⟶ 2 3-phosphoglycerate	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.

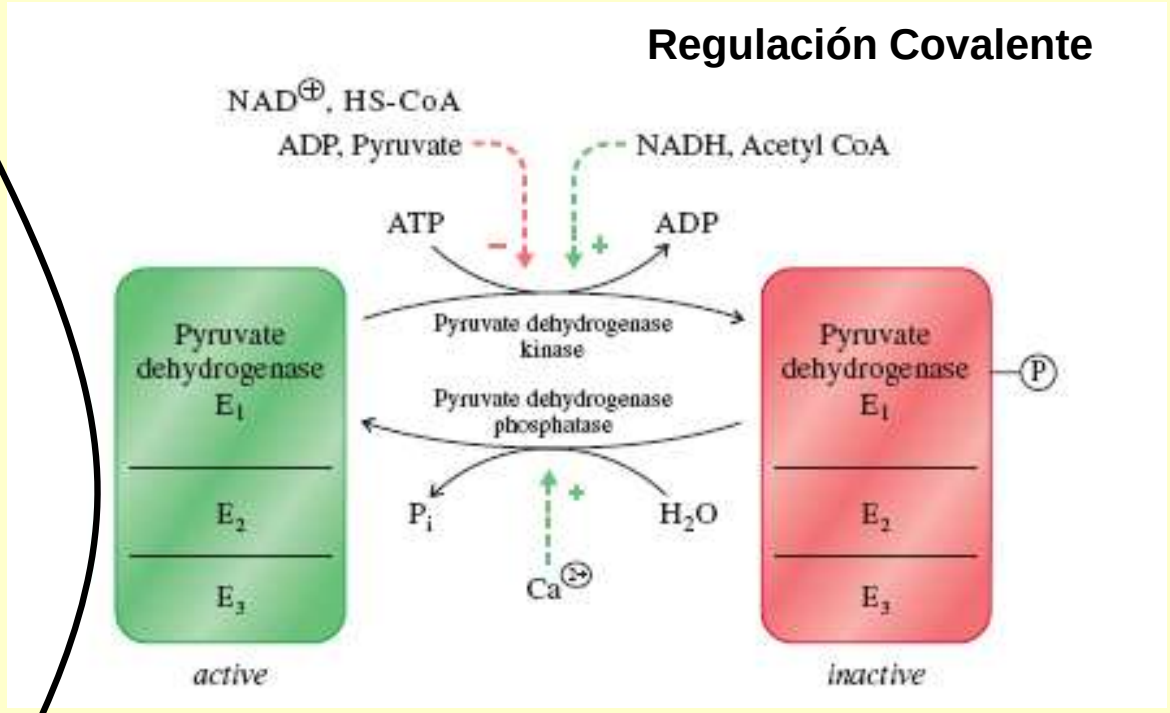
Regulación del Ciclo de Krebs

Complejo Piruvato deshidrogenasa

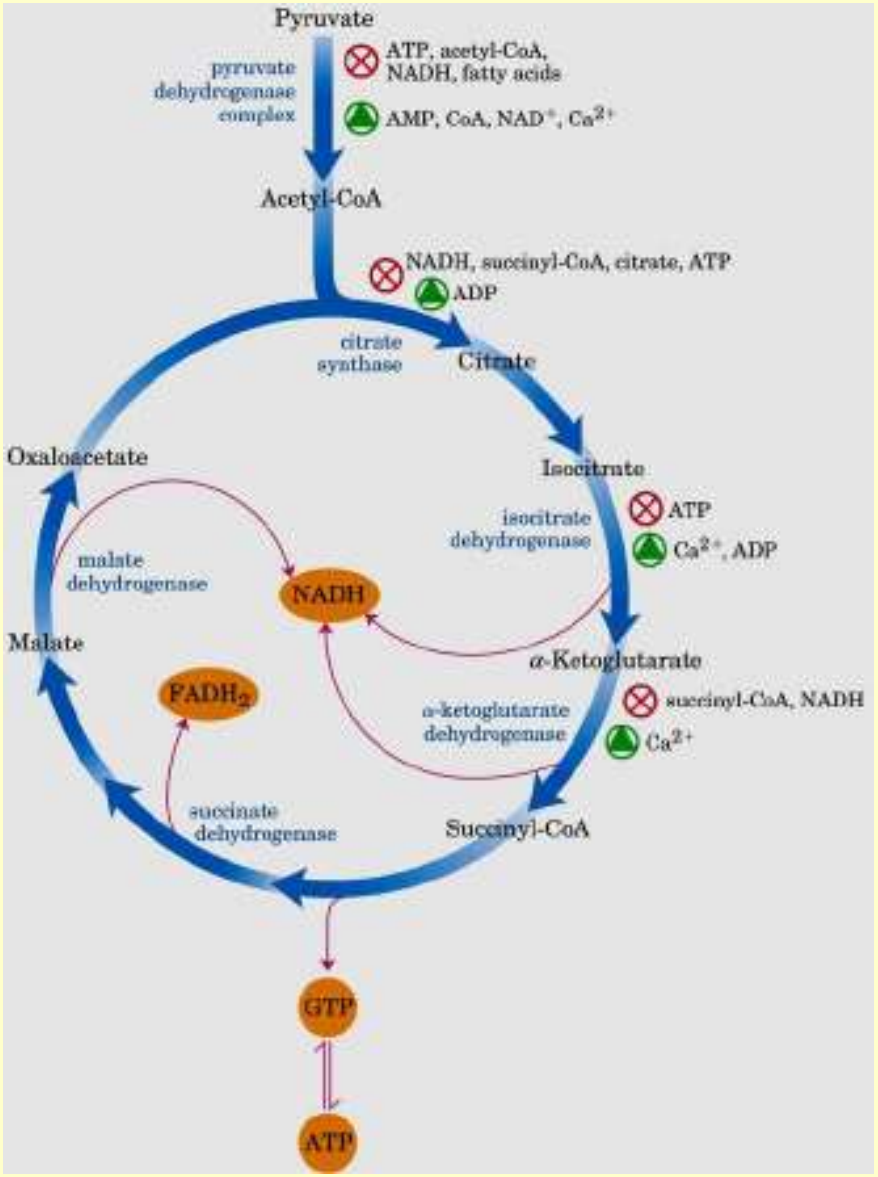
Regulación Alostérica



Regulación Covalente



Regulación del Ciclo de Krebs



Regulación alostérica de enzimas claves

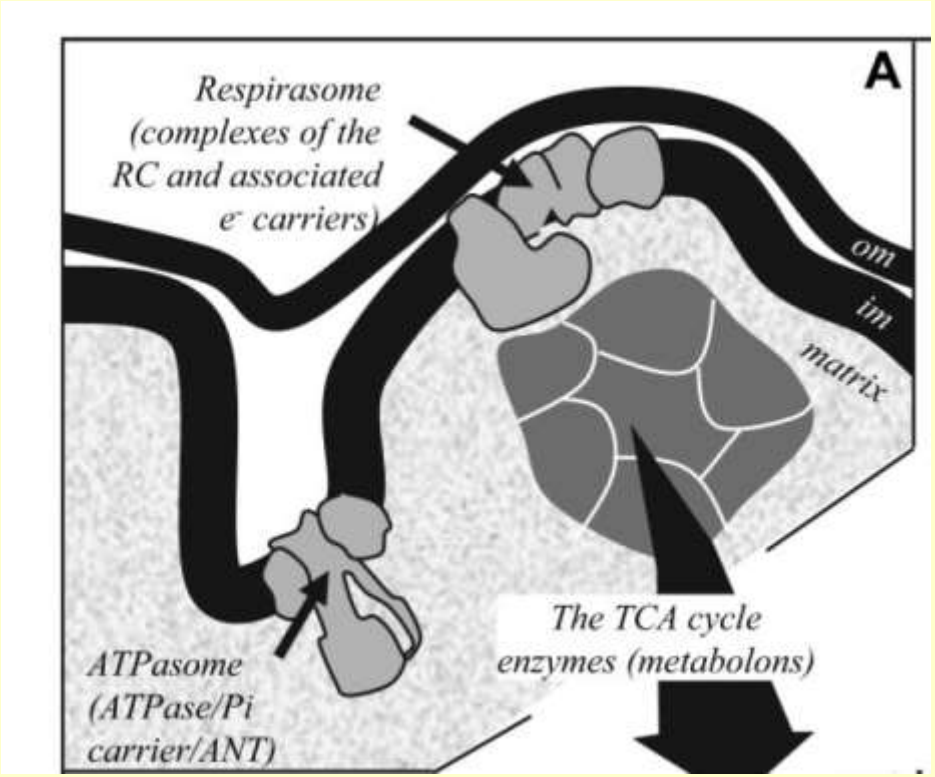
Citrato sintasa
Inhibidores: NADH, Succinil-CoA, Citrato, ATP
Activadores: ADP

Isocitrato deshidrogenasa
Inhibidores: ATP
Activadores: Ca^{+2} (músculo), ADP

Alfacetoglutarato deshidrogenasa
Inhibidores: Succinil-CoA, NADH
Activadores: Ca^{+2} (músculo)

Regulador clave: relación mitocondrial de [NAD+] / [NADH]

Asociación funcional de las enzimas del ciclo de Krebs



Briere et al., 2006.

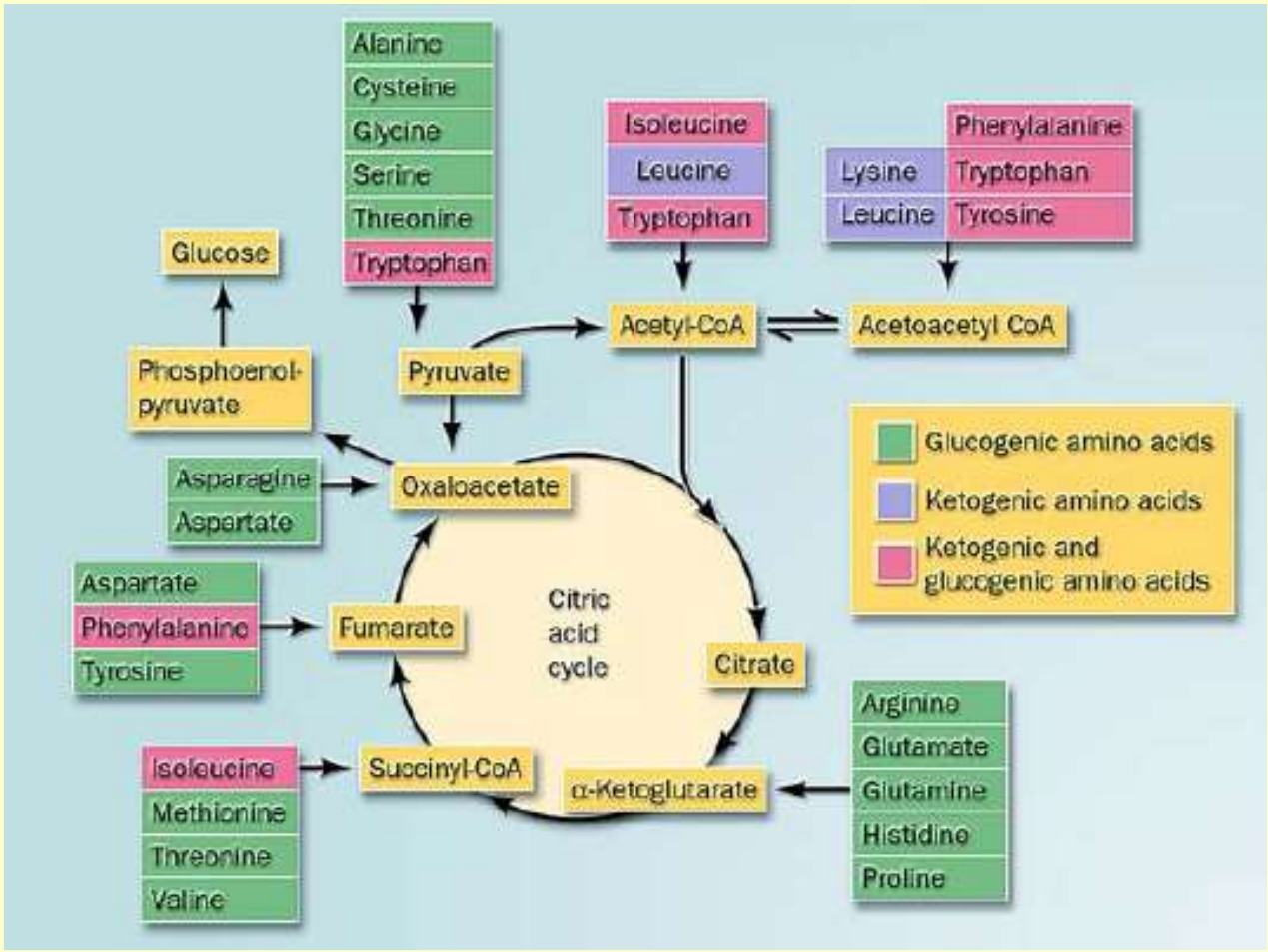
Interrelaciones del ciclo de Krebs con otras vías metabólicas

Vía anfibólica

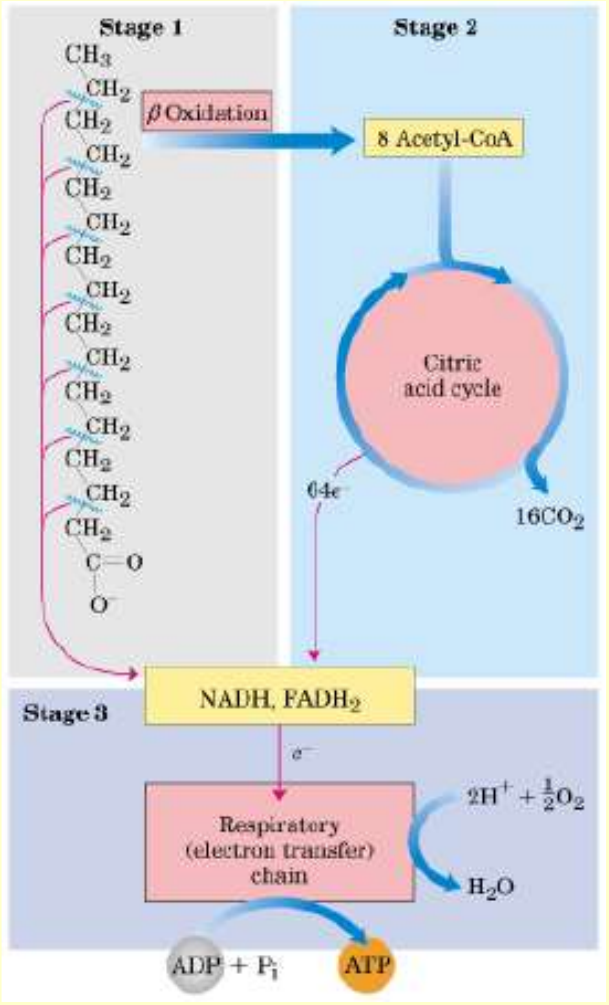
vías metabólicas con roles anabólicos y catabólicos a la vez

Degradación de aminoácidos

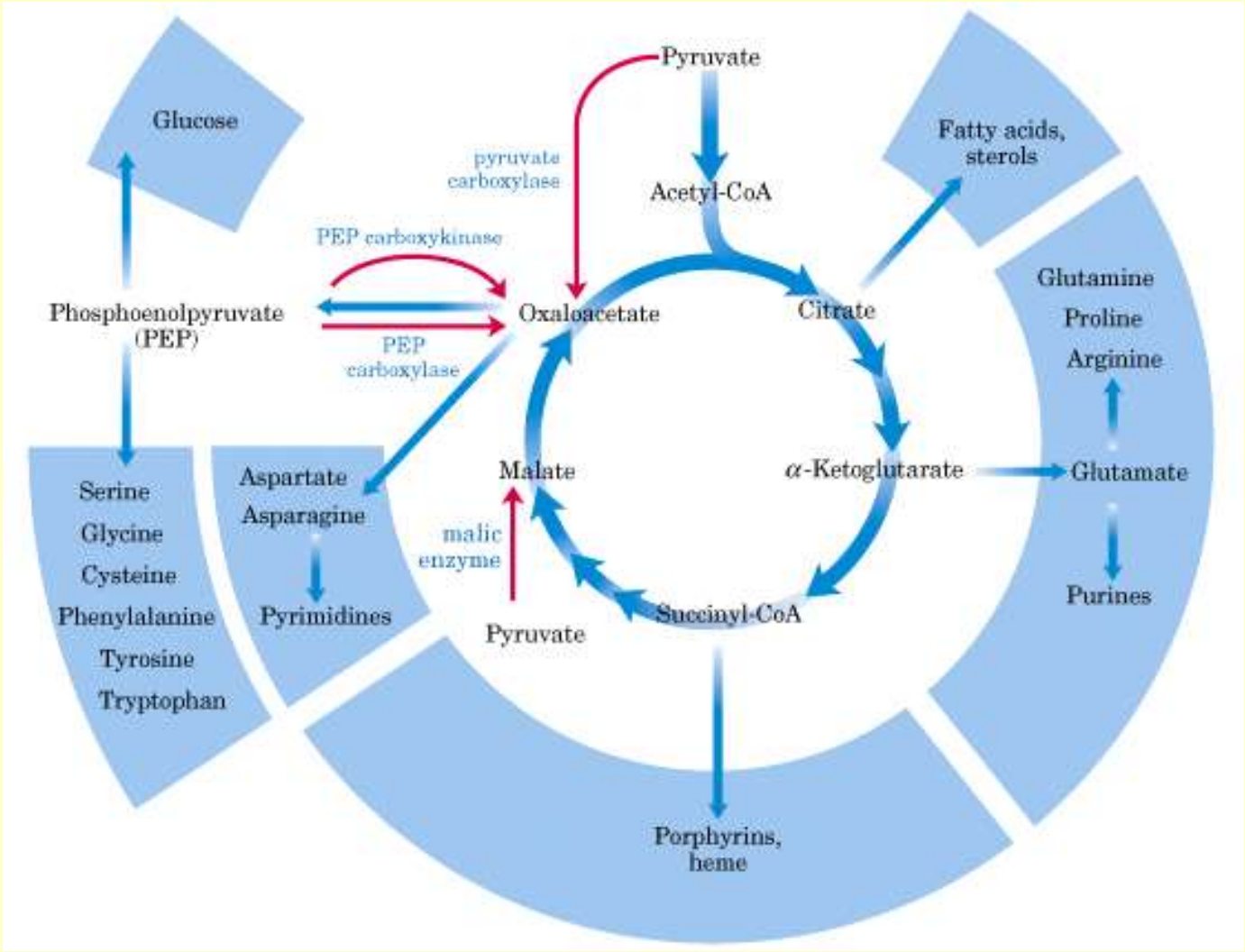
Los carbonos de los AA entran al ciclo en diferentes puntos



Degradación de ácidos grasos (Beta oxidación)



Rol del ciclo de Krebs en el anabolismo



Reacciones anapleróticas: en rojo

Reposición de intermediarios por reacciones anaplerótica

table 16–2

Anaplerotic Reactions

Reaction	Tissue(s)/organism(s)
Pyruvate + HCO_3^- + ATP $\xrightleftharpoons{\text{pyruvate carboxylase}}$ oxaloacetate + ADP + P_i	Liver, kidney
Phosphoenolpyruvate + CO_2 + GDP $\xrightleftharpoons{\text{PEP carboxykinase}}$ oxaloacetate + GTP	Heart, skeletal muscle
Phosphoenolpyruvate + HCO_3^- $\xrightleftharpoons{\text{PEP carboxylase}}$ oxaloacetate + P_i	Higher plants, yeast, bacteria
Pyruvate + HCO_3^- + NAD(P)H $\xrightleftharpoons{\text{malic enzyme}}$ malate + $NAD(P)^+$	Widely distributed in eukaryotes and prokaryotes

Mal funcionamiento del ciclo de Krebs como causa de enfermedades y tumores en humanos

Table 1. *TCA enzymopathies and diseases*

Enzyme	Gene	Disease
α-Ketoglutarate dehydrogenase	OGDH	Severe encephalopathy, hypotonia, psychotic behavior, pyramidal symptoms
Succinate dehydrogenase	SDHA	Leigh syndrome
	SDHB	Paraganglioma and pheochromocytoma
	SDHC	Paraganglioma
	SDHD	Paraganglioma and pheochromocytoma
Fumarase	FH	Early encephalopathy, seizures, and muscular hypotonia
		Leiomyomatosis and papilliary renal cell cancer
Succinyl-CoA synthetase (ADP forming)	SUCLA2	Encephalomyopathy and mtDNA depletion

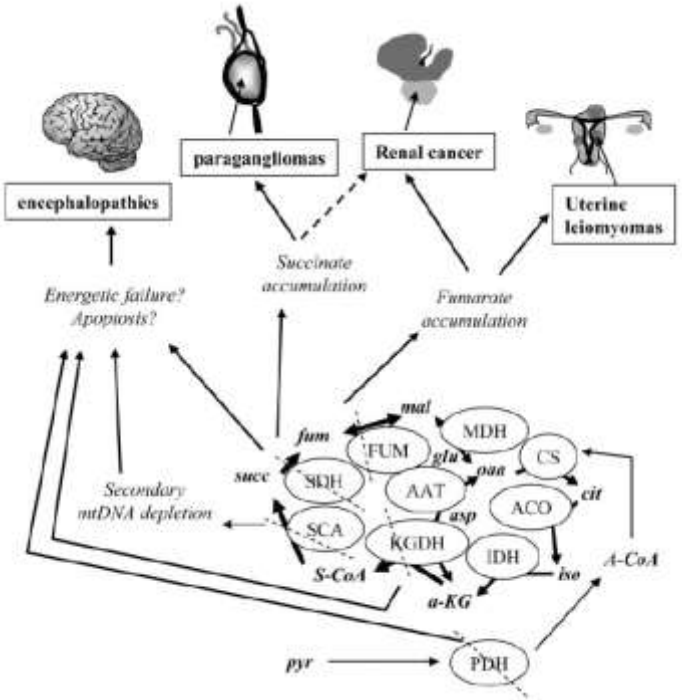


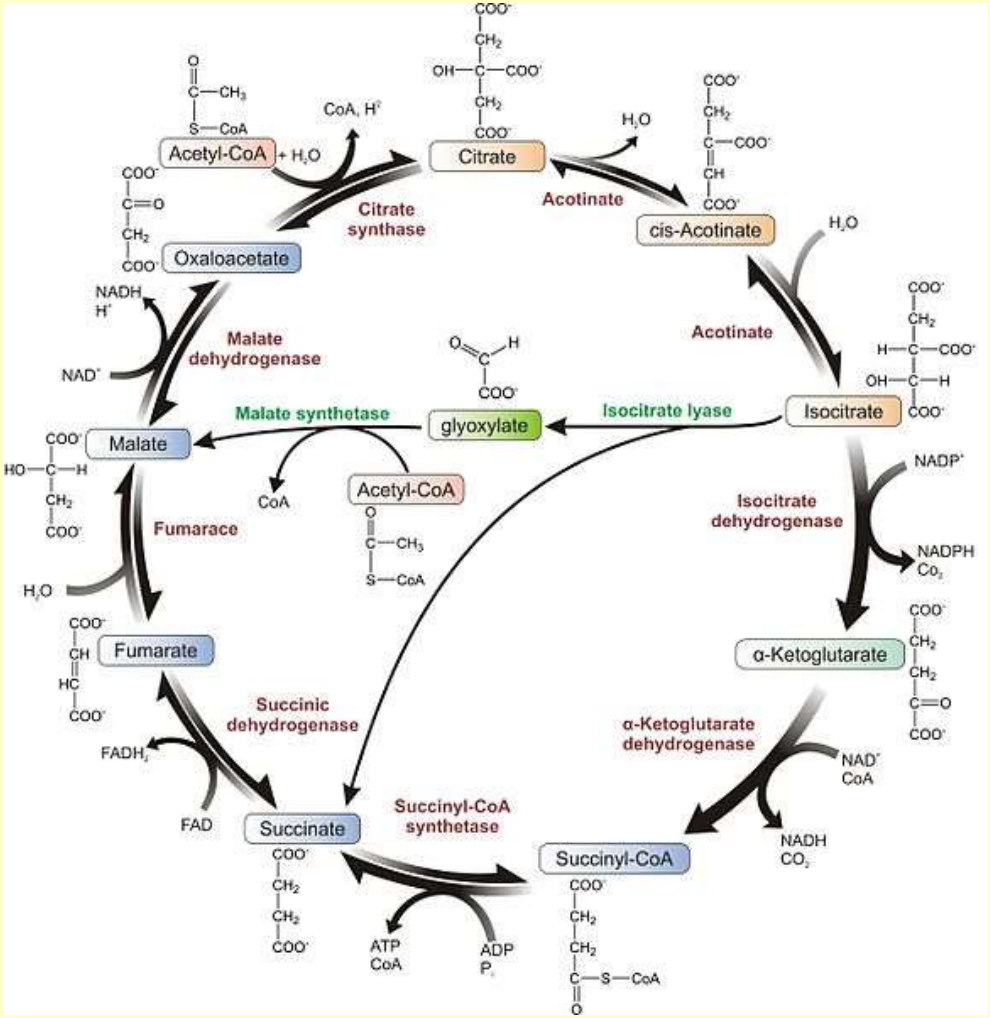
Fig. 2. The TCA cycle and human diseases: a schematized view of our present knowledge on the consequences of impairing TCA cycle enzyme activity (specific blockade indicated by dotted lines) on the clinical features observed in humans. AAT, aspartate amino transferase; ACO, aconitase; A-CoA, acetyl-CoA; asp, aspartate; cit, citrate; CS, citrate synthase; FUM, fumarase; fum, fumarate, glu, glutamate; IDH, isocitrate dehydrogenase; iso, isocitrate; α-KG, α-ketoglutarate; KGDH, α-ketoglutarate dehydrogenase; mal, malate; MDH, malate dehydrogenase; oaa, oxaloacetate; PDH, pyruvate dehydrogenase; Pyr, pyruvate; S-CoA, succinyl-CoA; SCA, succinyl-CoA synthase; SDH, succinate dehydrogenase; succ, succinate.

Briere et al., 2006.

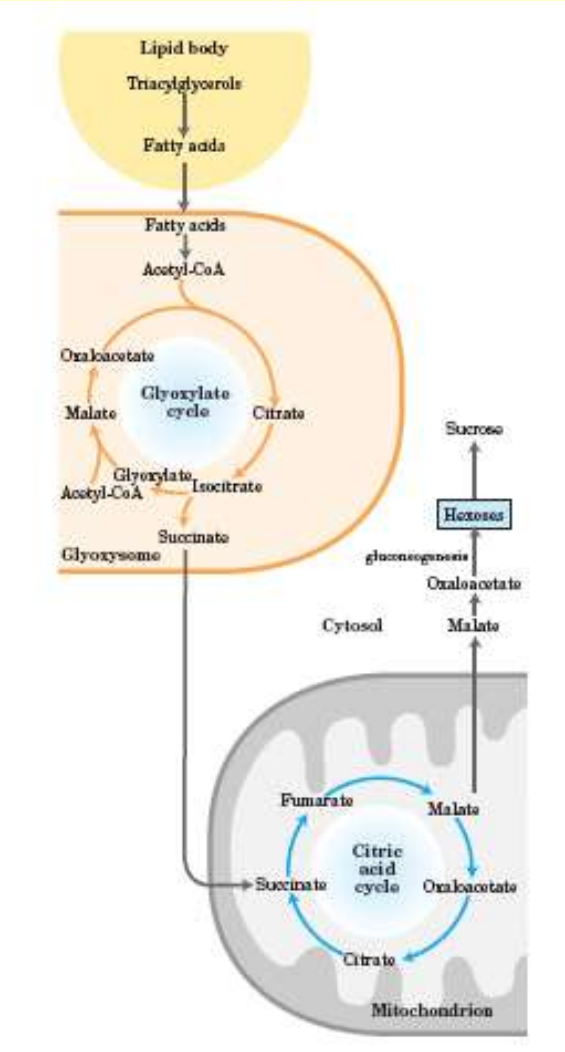
Ciclo de Krebs, conclusiones globales

- En eucariotes transcurre en la mitocondria.
- Vía central del metabolismo aerobio: es la vía oxidativa final en el catabolismo de los carbohidratos, ácidos grasos y aminoácidos.
- La acción acoplada del ciclo del ácido cítrico y la cadena de transporte de electrones son responsables de la mayoría de la energía producida.
- Fuente importante de intermediarios de vías biosintéticas.

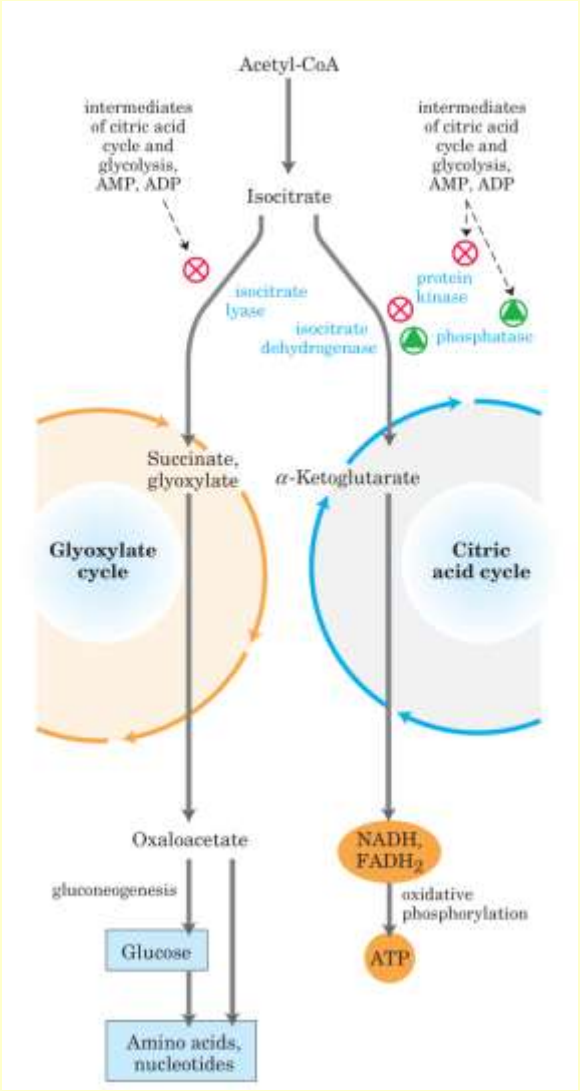
Ciclo del Glioxilato



Interconexión entre el ciclo de Krebs y el ciclo del Glioxilato



Regulación del ciclo del Glioxilato

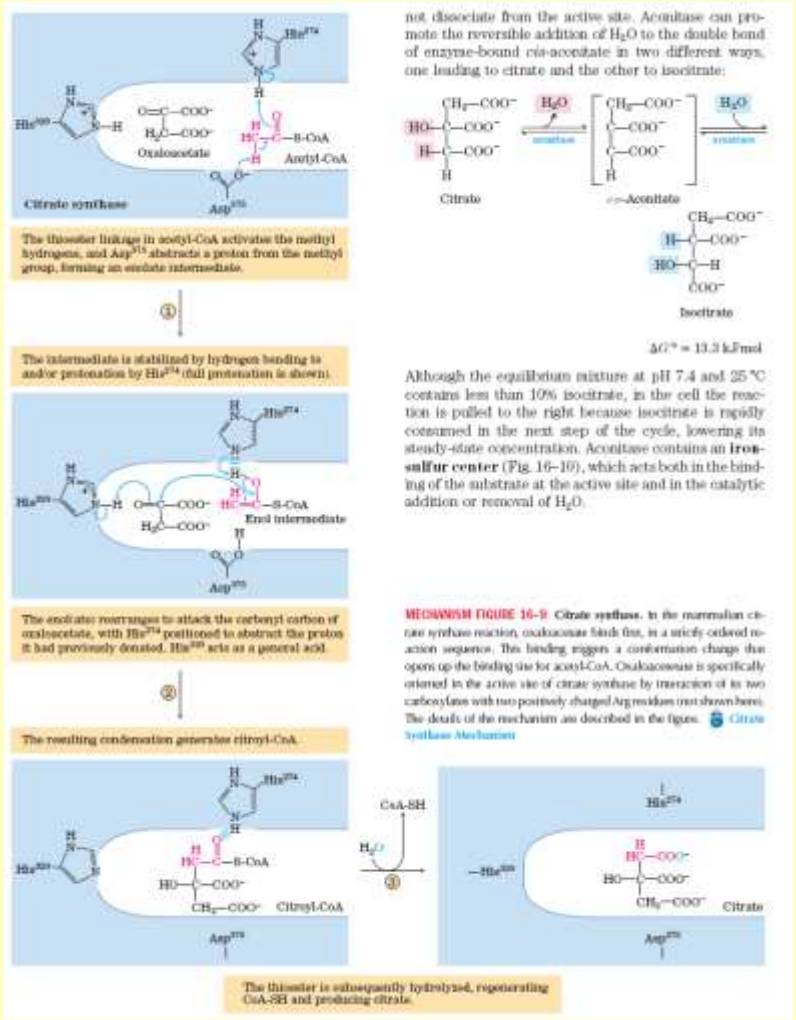


Fin

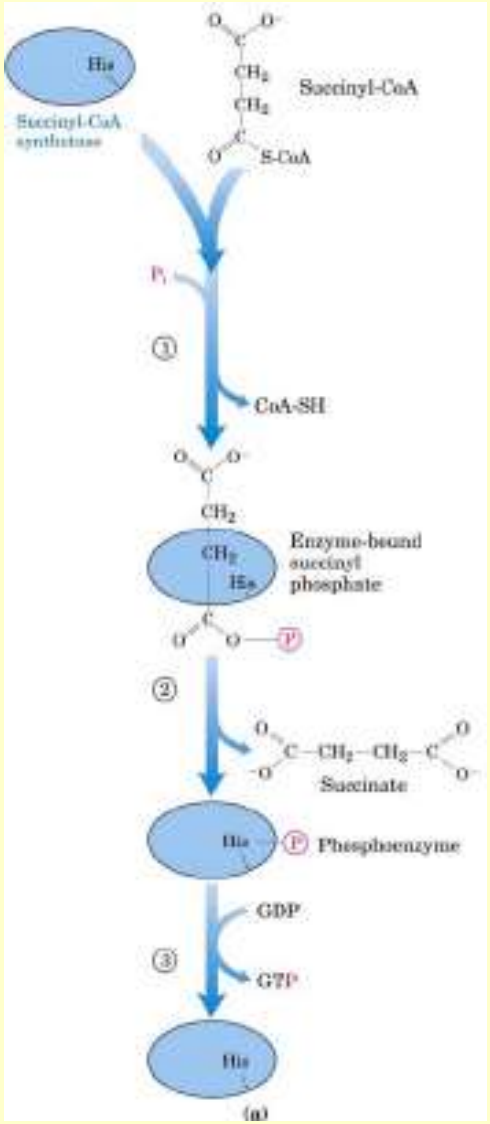
Anexos

Mecanismos de reacción

Síntesis de acetilCoA



Mecanismo de la Succinil CoA Sintetasa



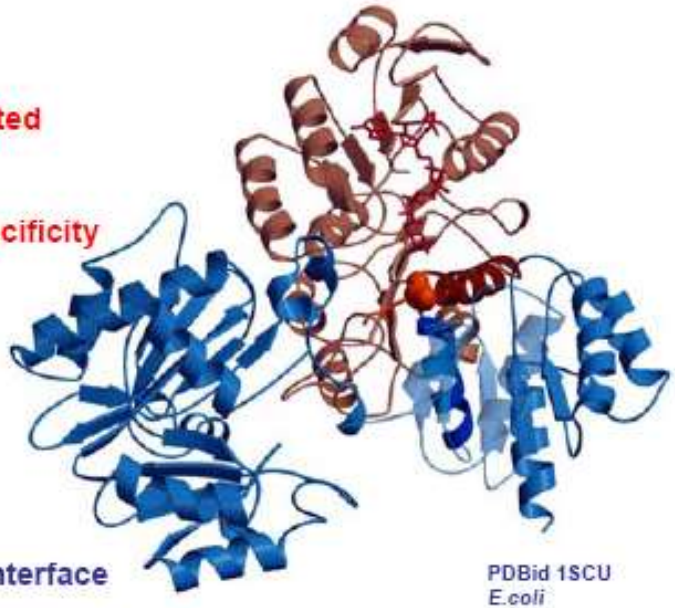
Reactions of the Citric Acid Cycle

Step 5 **Structure of succinyl-CoA synthetase**

Two subunits:

α SU (32 kDa)
His^{246} is phosphorylated

β SU (42 kDa)
confers ATP/GTP specificity



Active site is on the SU interface
→ "power helices"

PDBid 1SCU
E.coli

Historia

- El ciclo de Krebs recibe su nombre en honor a su descubridor Sir Hans Krebs, quien propuso los elementos clave de esta vía en 1937.

- La historia comienza a principios de la década de los 30´s con el descubrimiento de que al agregar succinato, fumarato y malato a músculos machacados incrementa la velocidad del consumo de Oxígeno.

- El oxaloacetato se incorporó a la lista de ácidos dicarboxílicos cuando se descubrió que se podía formar en condiciones aeróbicas a partir del piruvato. En 1935 A. Szent-Györgyi propuso que ciertos pares de ácidos dicarboxilicos eran interconvertidos por la acción de deshidrogenasas y que este proceso estaba relacionado con la respiración.

- Carl Martius y Franz Knoop mostraron que el ácido cítrico es convertido en alfa-cetoglutarato por medio del isocitrato. Se supo también que el alfa-cetoglutarato puede ser oxidado a succinato.

- La formación del citrato era la pieza faltante para poder armar completamente el rompecabezas metabólico.

- El descubrimiento que resolvió este rompecabezas y unificó el metabolismo fue hecho en 1937 por Sir Hans Krebs y W.A. Johnson: ellos mostraron que el citrato es derivado del piruvato y del oxaloacetato completando lo que se conoce como el ciclo del ácido cítrico.

By 1930 it was established that the addition of lactate, acetate succinate, malate, α-ketoglutaric acid (dicarboxylic acids) and citrate and isocitrate (tricarboxylic acids) when added to muscle mince that they stimulated oxygen consumption and release of CO_2

1935 Albert Szent-Györgyi showed that

succinate → fumarate → malate → oxaloacetate

Carl Martius and Franz Knoop showed

citrate → cis-aconitate → isocitrate → α ketoglutarate

succinate → fumarate → malate → oxaloacetate

Martius and Knoop showed that pyruvate and oxaloacetate could form citrate non-enzymatically by the addition of peroxide under basic conditions.

Krebs showed that succinate is formed from fumarate, malate or oxaloacetate. This is interesting since it was shown that the other way worked as well!!

Pyruvate can form citrate enzymatically

Pyruvate + oxaloacetate → citrate + CO_2

The interconversion rates of the intermediates was fast enough to support respiration rates.

How did the citric acid cycle come into being? Although definitive answers are elusive, it is nevertheless instructive to speculate how this complicated central hub of metabolism developed. We can perhaps begin to comprehend how evolution might work at the level of biochemical pathways.

The manuscript proposing the citric acid cycle was submitted for publication to *Nature* but was rejected. It was subsequently published in *Enzymologia*. Dr. Krebs proudly displayed the rejection letter throughout his career as encouragement for young scientists.

"June 1937

The editor of NATURE presents his compliments to Dr. H. A. Krebs and regrets that as he has already sufficient letters to fill the correspondence columns of NATURE for seven or eight weeks, it is undesirable to accept further letters at the present time on account of the time delay which must occur in their publication.

If Dr. Krebs does not mind much delay the editor is prepared to keep the letter until the congestion is relieved in the hope of making use of it.

He returns it now, in case Dr. Krebs prefers to submit it for early publication to another periodical."

If citrate is added the rate of respiration is often increased . . . the extra oxygen uptake is by far greater than can be accounted for by the complete oxidation of citrate . . . Since citric acid reacts catalytically in the tissue it is probable that it is removed by a primary reaction but regenerated by a subsequent reaction.

—*H. A. Krebs and W. A. Johnson, article in* Enzymologia, *1937*