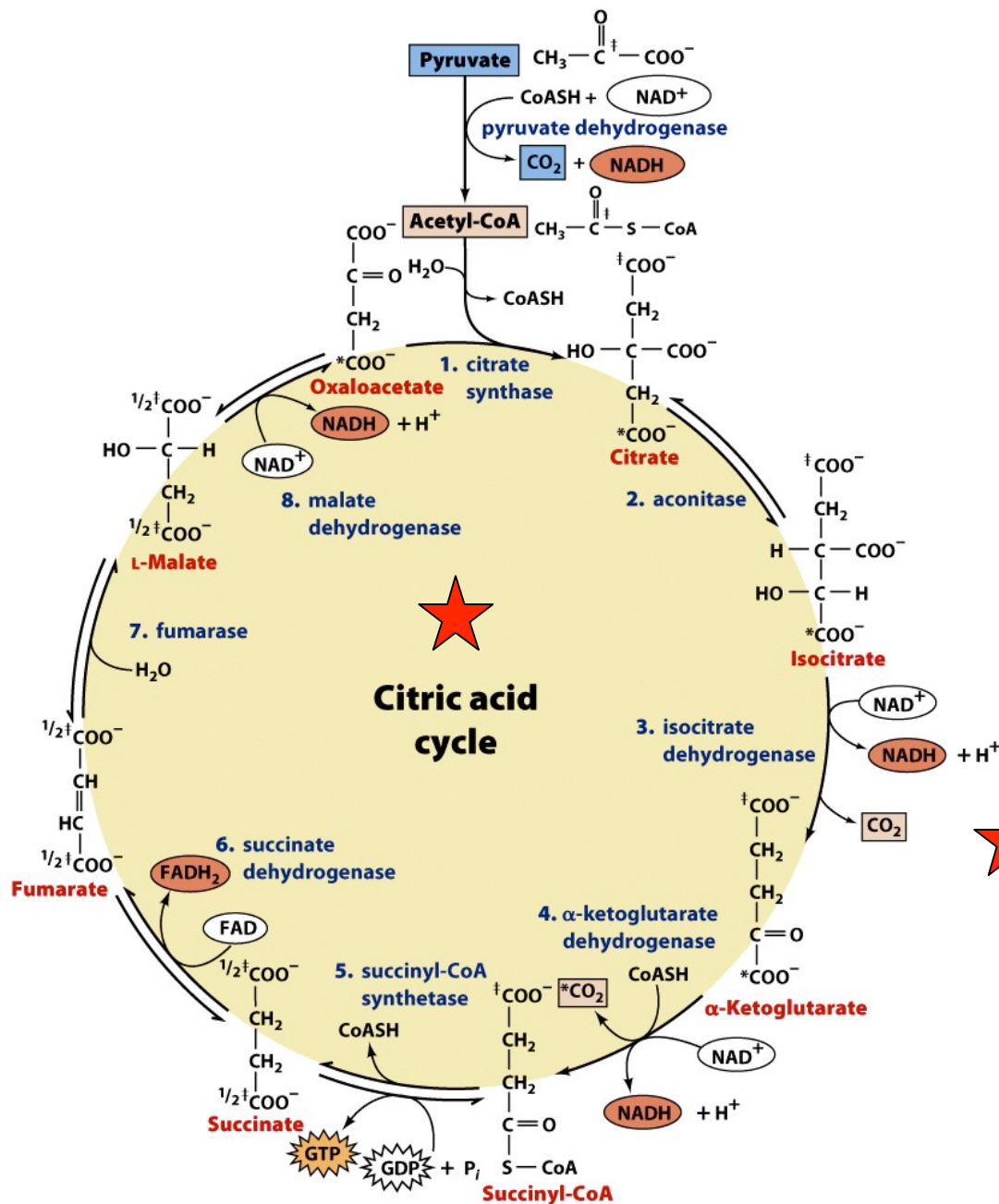


Chapter 17
Citric Acid Cycle (II)
(4/26/11)
Summary and Conclusion

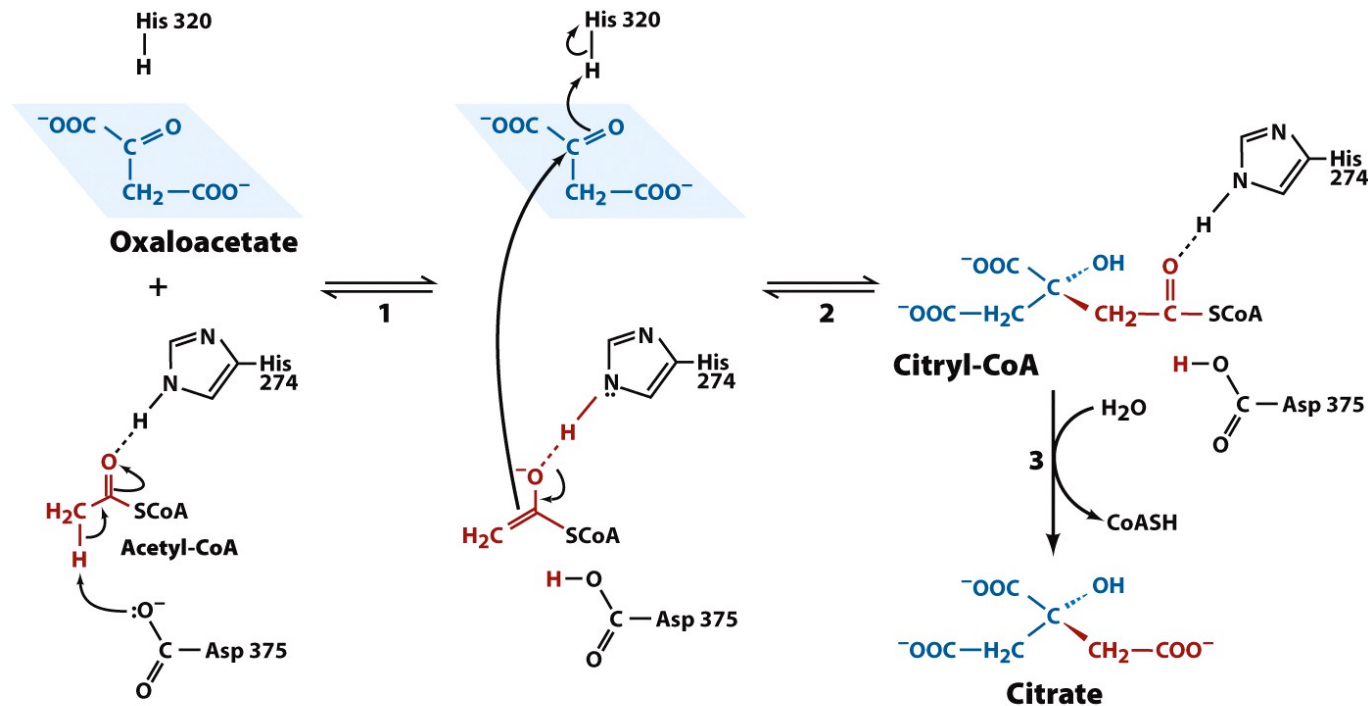
You **MUST** know this entire figure, including molecules, enzymes, and cofactors.

- In eukaryotes, all of the enzymes in the TCA are located in the **mitochondria**, so all substrates including NAD^+ and GDP must be generated or transported there

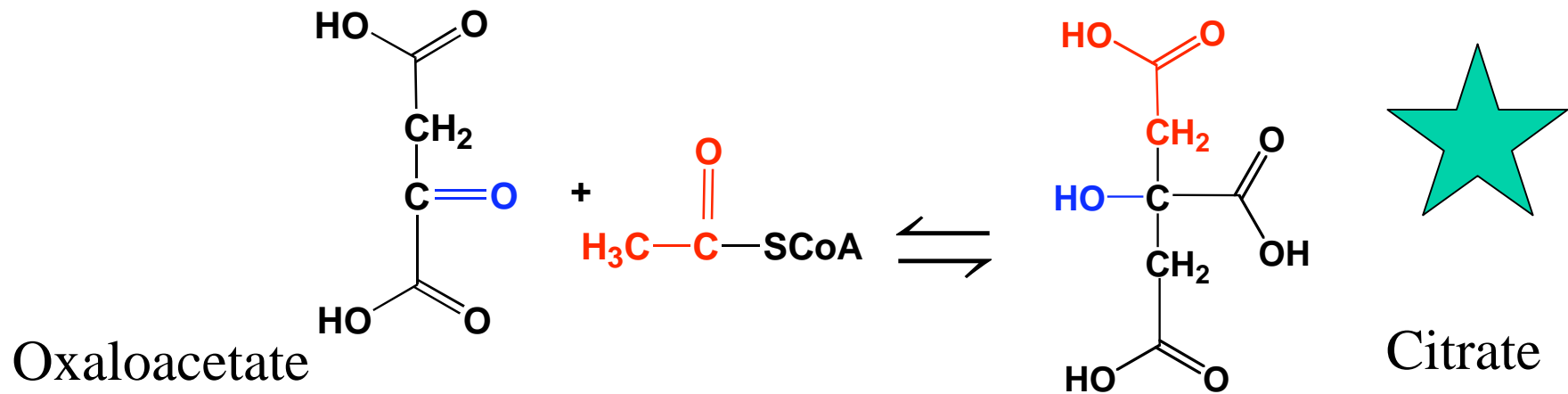
- ★ The carbon atoms of the 2CO_2 molecules generated in one turn of the cycle do not come from the acetyl group of acetyl-CoA but from oxaloacetate



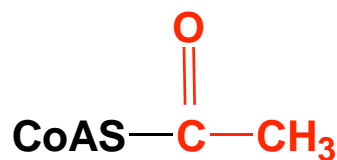
1. Citrate Synthase



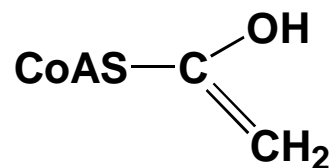
© 2008 John Wiley & Sons, Inc. All rights reserved.



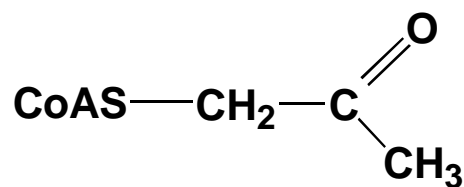
**Induced fit needs binding of oxaloacetate before
Acetyl-CoA can bind.**



Acetyl-CoA

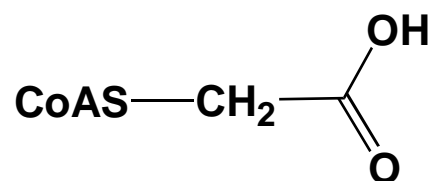


Proposed intermediate



Acetonyl-CoA

(ground-state analog)

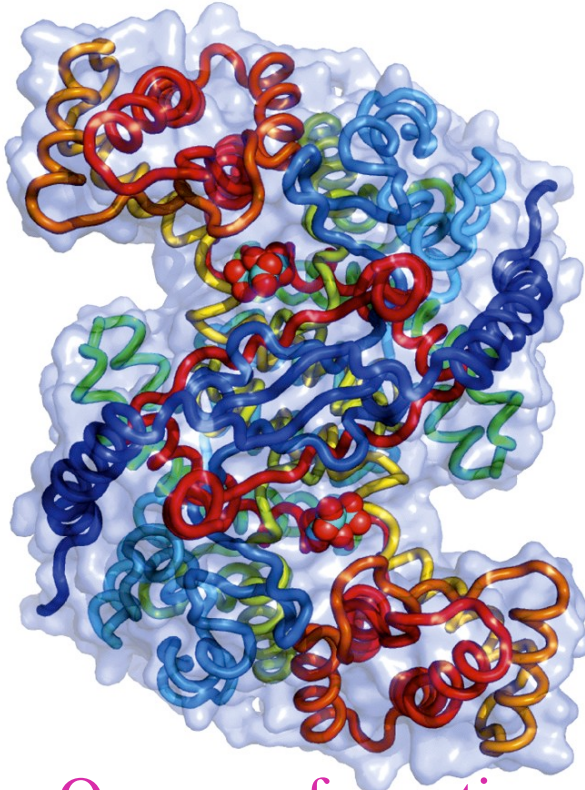


Carboxymethyl-CoA

(transition state analog)

Conformational changes in citrate synthase

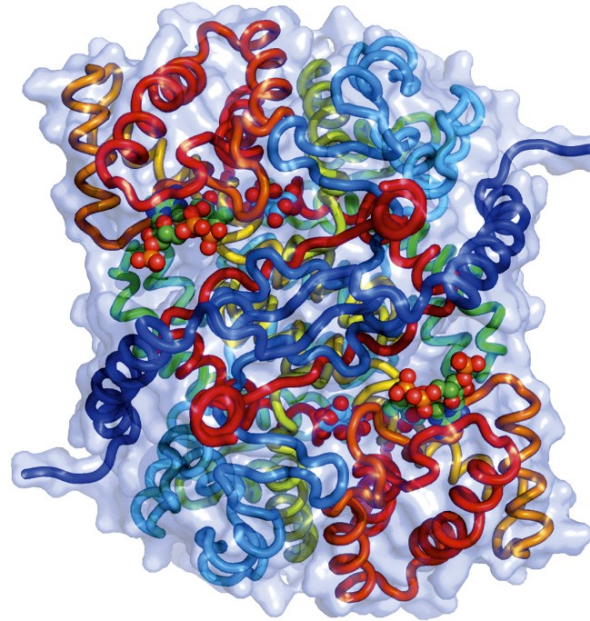
(a)



Open conformation

© 2008 John Wiley & Sons, Inc. All rights reserved.

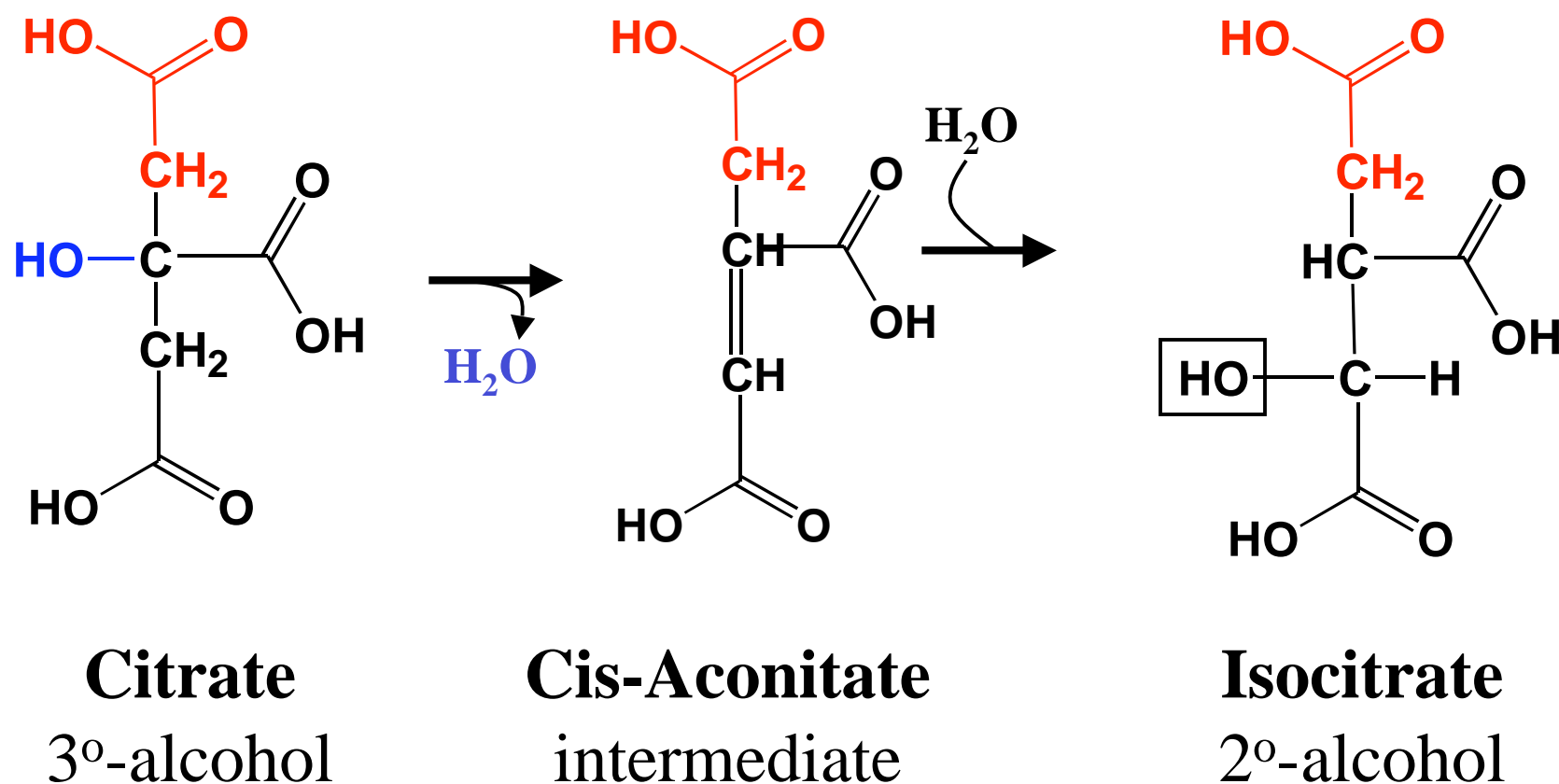
(b)



Closed conformation

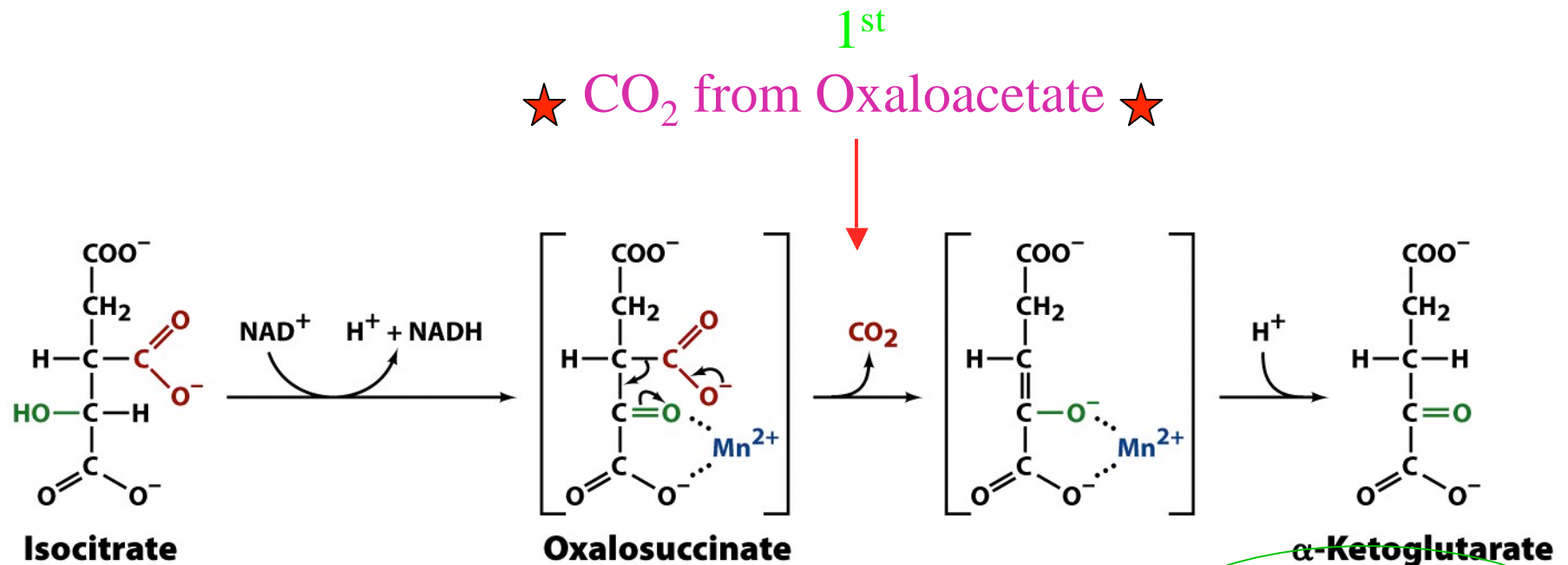
- Oxaloacetate binding causes a conformational change that creates the acetyl-CoA binding site and seals the oxaloacetate binding site so that solvent cannot reach the bound substrate
- Asp375 (base), His274 (acid), and His320 (acid) are the catalytic residues

2. Aconitase



Aconitase has an iron-sulfur cluster (Fe_4S_4) at the active site. These are normally involved in e^- -transfer but not in this case.

3. $\text{NAD}^+/\text{Mn}^{2+}$ -Dependent Isocitrate dehydrogenase



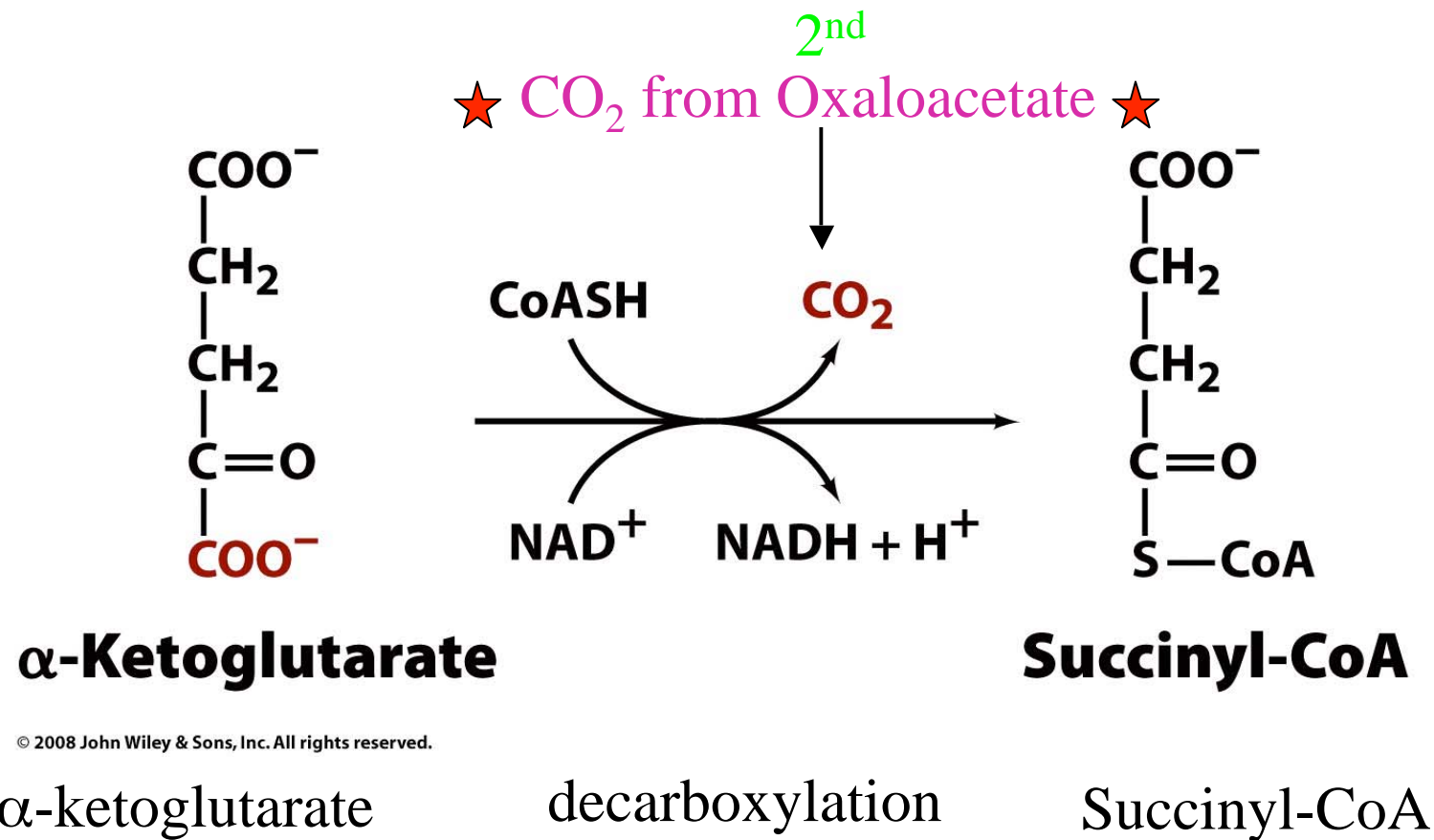
© 2008 John Wiley & Sons, Inc. All rights reserved.

Oxidative decarboxylation

Link to amino acid metabolism
Which one?

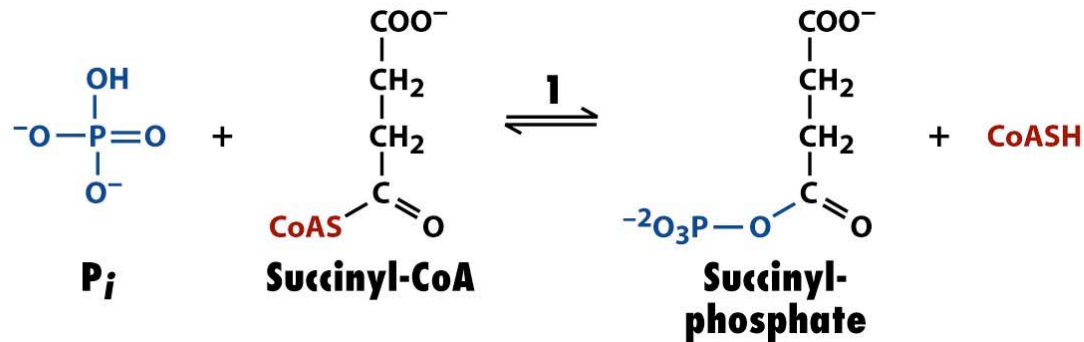


4. α -Ketoglutarate dehydrogenase

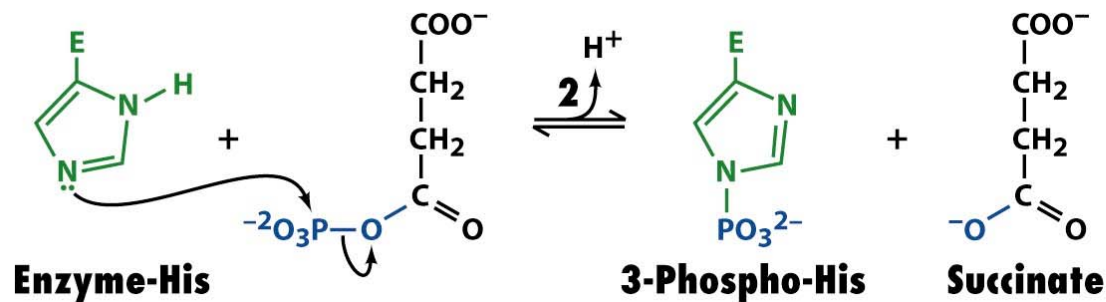


This enzyme is just like **pyruvate dehydrogenase** - a multienzyme complex that is specific for longer CoA derivatives

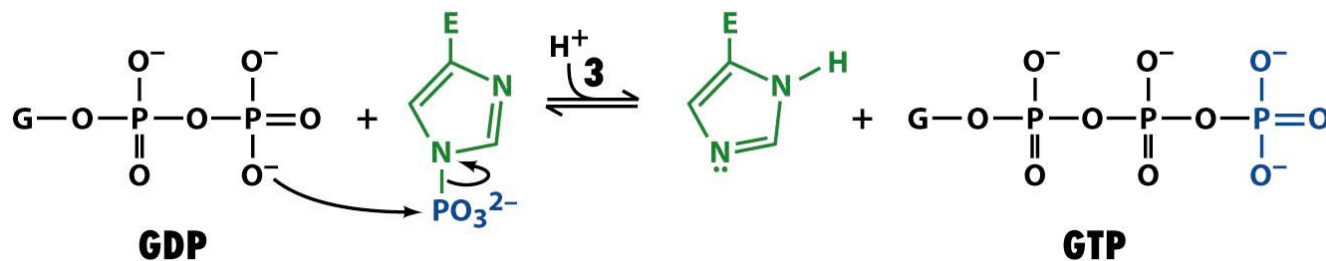
5. Succinyl-CoA Synthetase or Succinate Thiokinase



High energy compound
: Succinyl-CoA,
then 3-phospho-His,
Then GTP

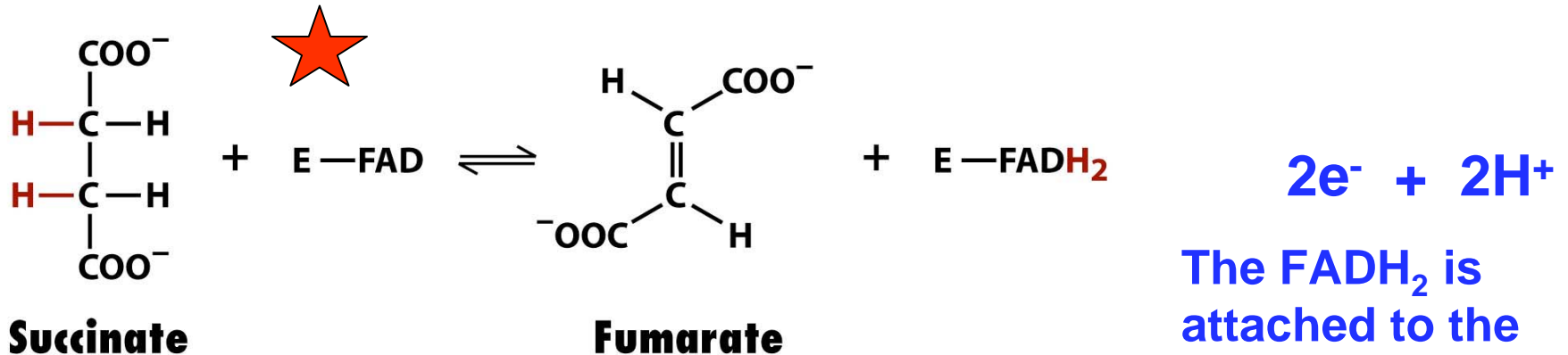


In the rest of the cycle,
succinate will be converted
To oxaloacetate

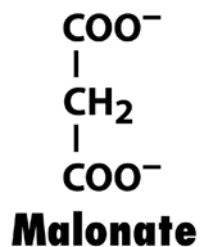


High-energy
compound

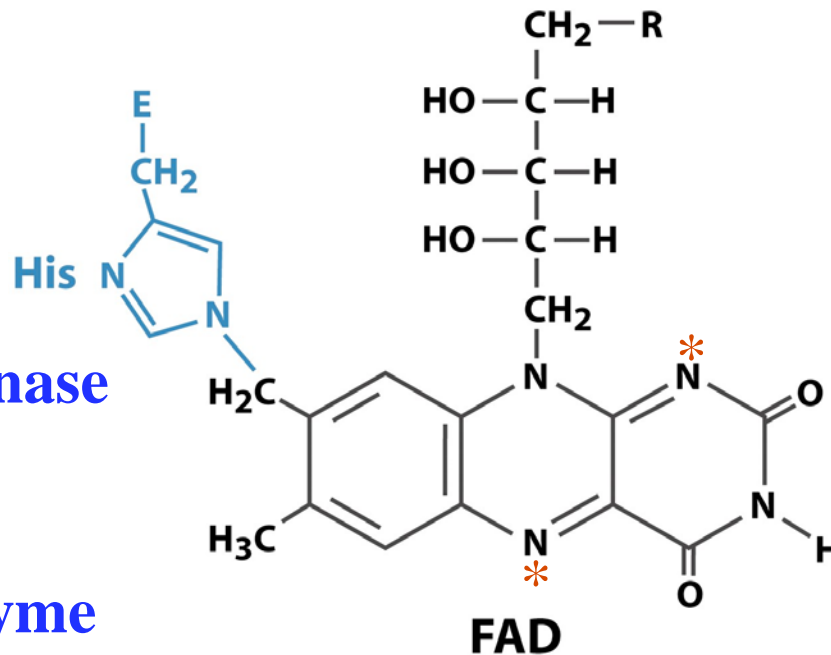
6. Succinate dehydrogenase



The FADH₂ is attached to the enzyme and must be re-oxidized before next rxn. This liberates 2e⁻ and 2H⁺



Succinate dehydrogenase is the only membrane bound enzyme of the TCA

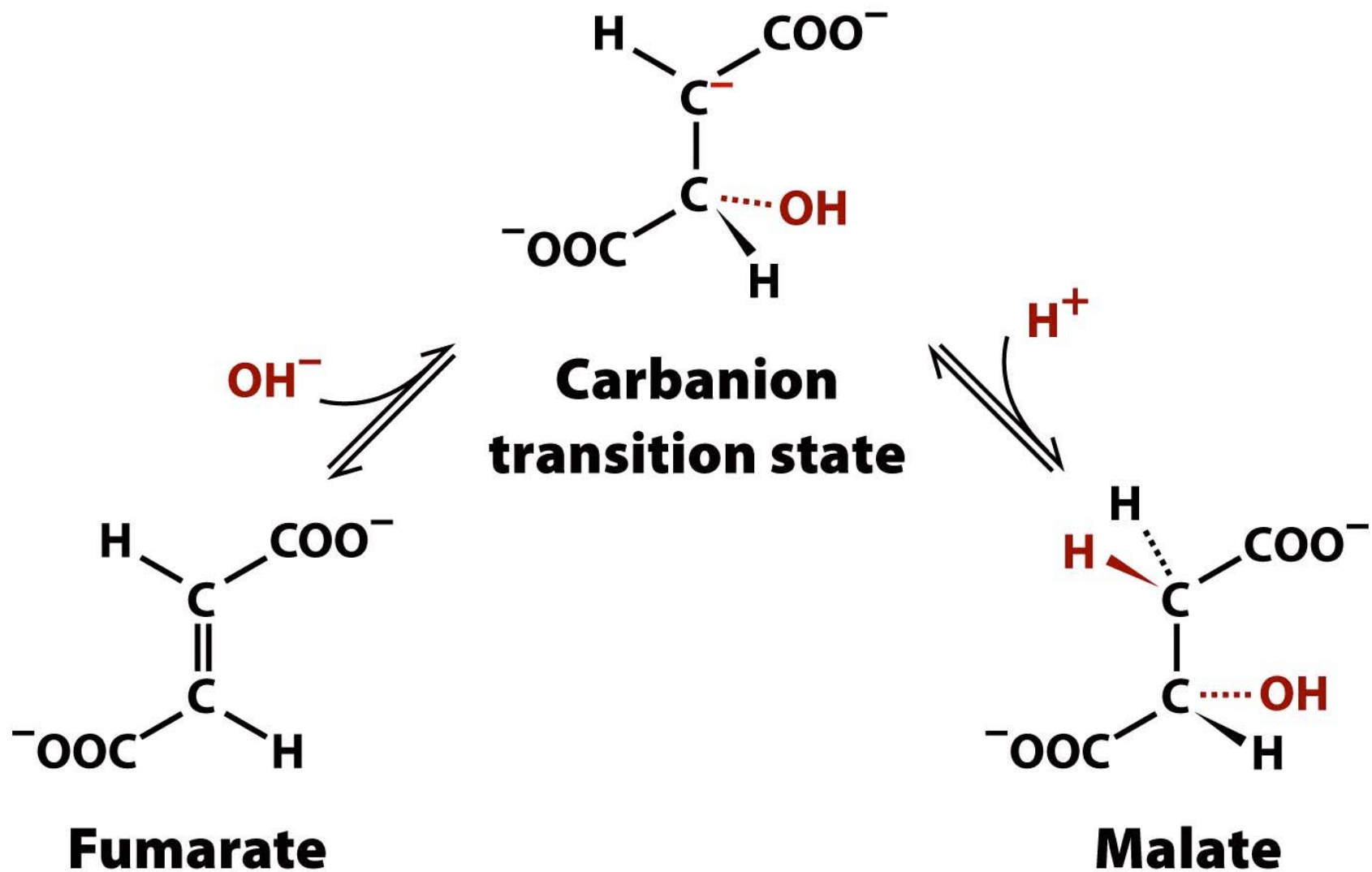


The FAD on the enzyme itself is reduced to FADH₂(*)

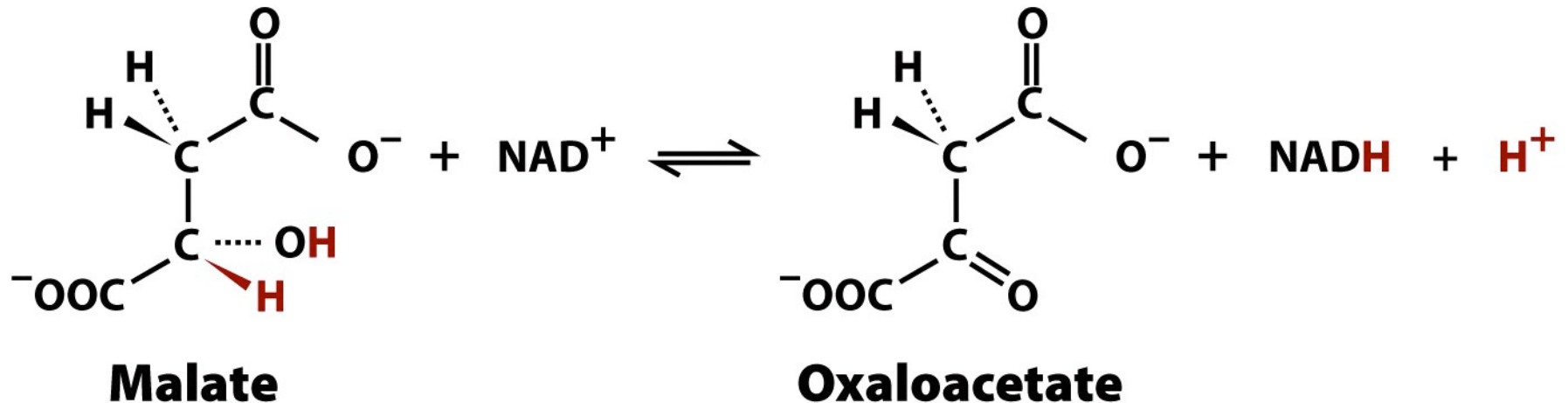
Succinate dehydrogenase is the only membrane bound enzyme in the citrate cycle

- **FAD** functions to oxidize alkanes to alkenes (as in step 6)
- **NAD⁺** functions to oxidize alcohols to aldehydes or ketones (as in step 8)
- Reoxidation of FADH₂ to FAD occurs in the mitochondrial electron transport chain
- This occurs via Succinate DH because it is membrane bound as is the electron transport chain

7. Fumarase



8. Malate dehydrogenase

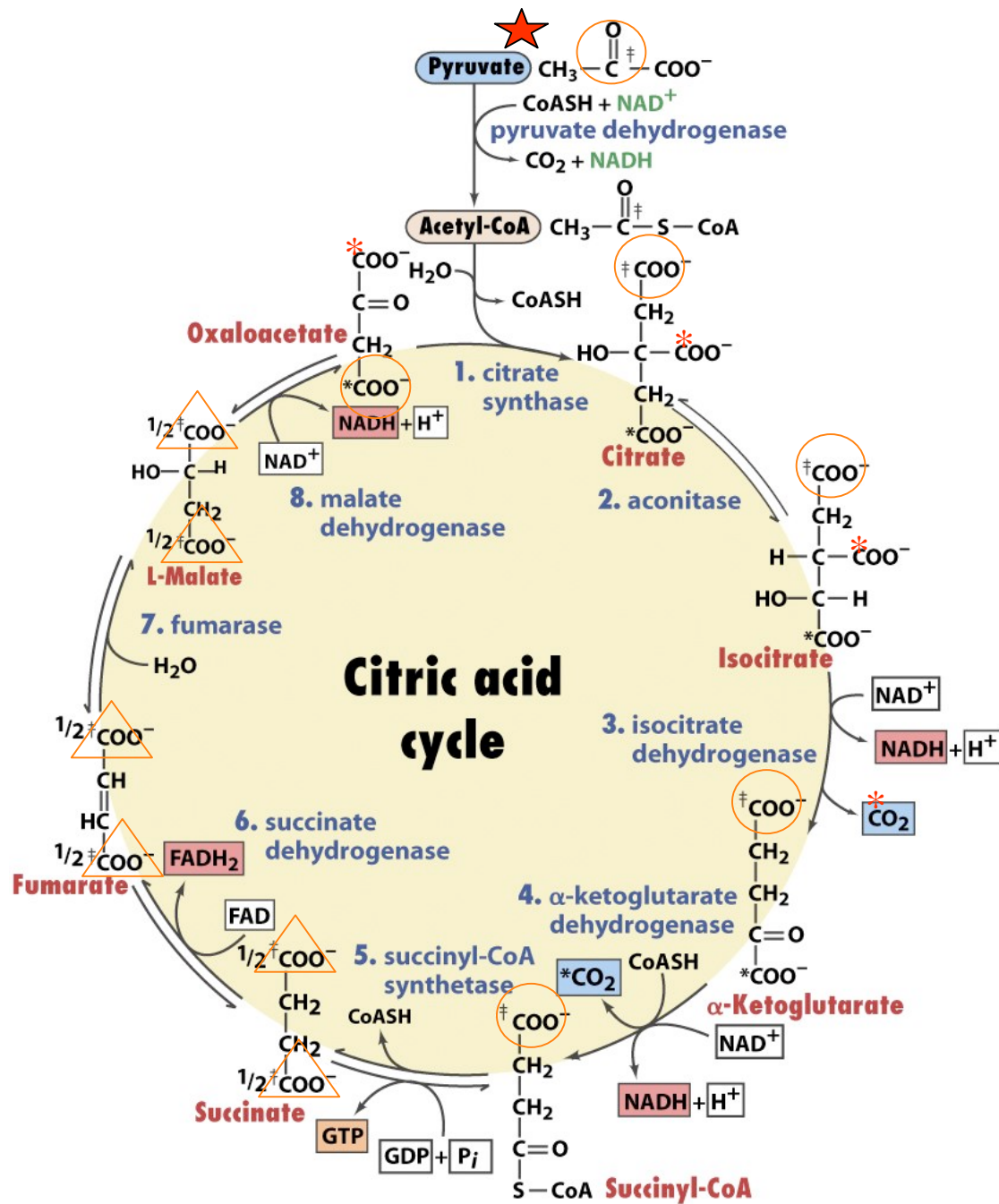


© 2008 John Wiley & Sons, Inc. All rights reserved.

Oxaloacetate is regenerated for the next turn of the TCA cycle.

This reaction is very unfavorable (+29.7 kJ/mol) so very little oxaloacetate is made.

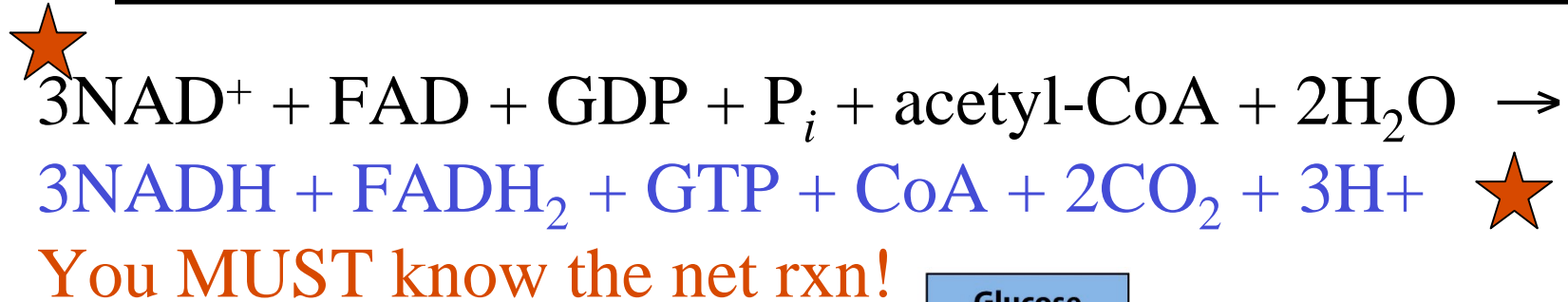
However, remember that the first rxn. of the cycle is *highly* exergonic (-31.5 kJ/mol). Citrate synthase requires oxaloacetate and so drives the creation of oxaloacetate by malate dehydrogenase.



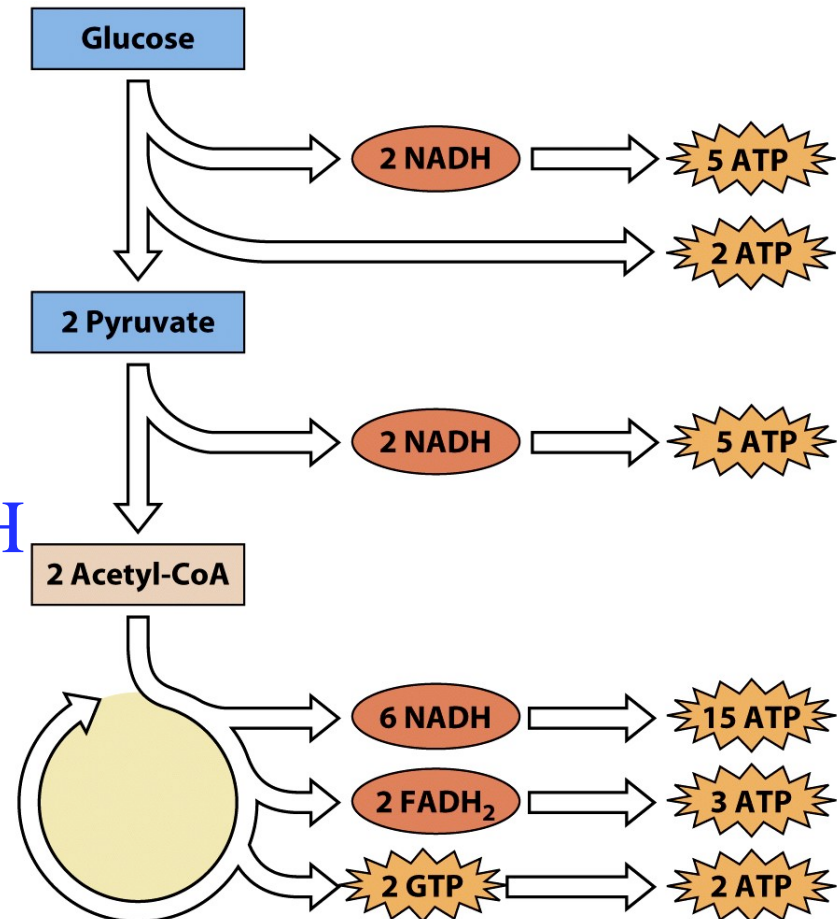
You **MUST** know this entire figure, including molecules, enzymes, and cofactors.

- In eukaryotes, all of the enzymes in the TCA are located in the **mitochondria**, so all substrates including NAD^+ and GDP must be generated or transported there
- The carbon atoms of the 2CO_2 molecules generated in **one turn of the cycle** do not come from the acetyl group of acetyl-CoA but from oxaloacetate

Overall net TCA reaction

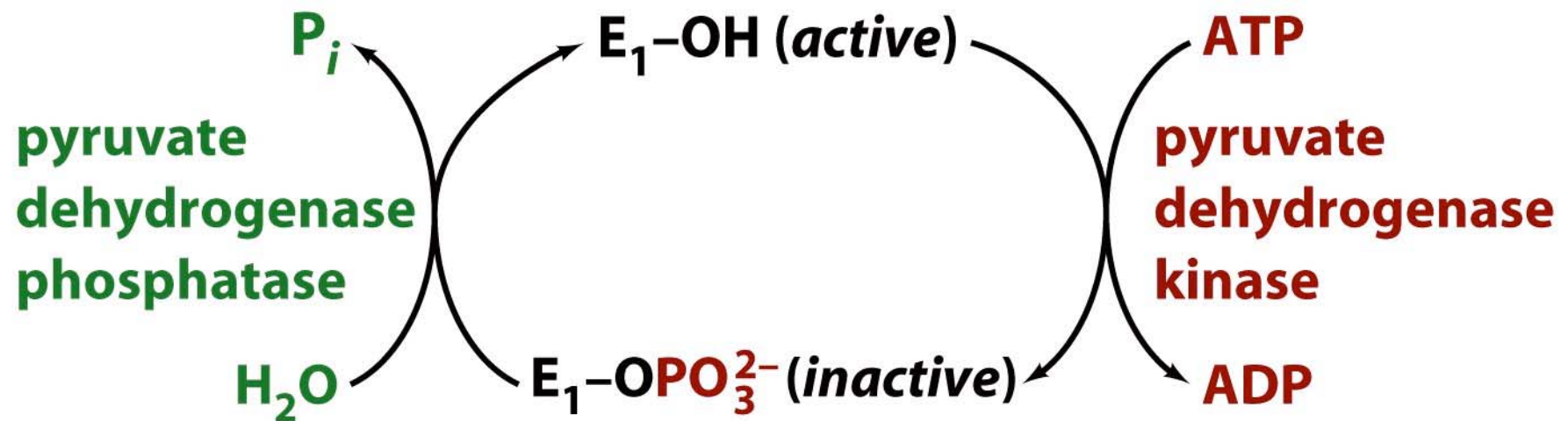


Energy-generating capacity of the cycle:
 ~32ATPs can be generated from one molecule of glucose!! (~2.5ATP per NADH reoxidation and ~1.5ATP per FADH_2 reox.)



Regulation of Pyruvate Dehydrogenase

1. Product inhibition by NADH and acetyl-CoA
 - NADH and acetyl-CoA compete for binding with NAD⁺ and CoA
 - They also drive the reversible transacetylase (E₂) and dihydrolipoyl dehydrogenase (E₃) reactions in the backward directions.
2. Covalent modification by phosphorylation/dephosphorylation of E₁
 - The products NADH and acetyl-CoA can activate the pyruvate dehydrogenase kinase associated with the enzyme complex which deactivates PDH
 - Insulin (which is a signal of fuel abundance) turns on the pyruvate dehydrogenase phosphatase which reactivates PDH



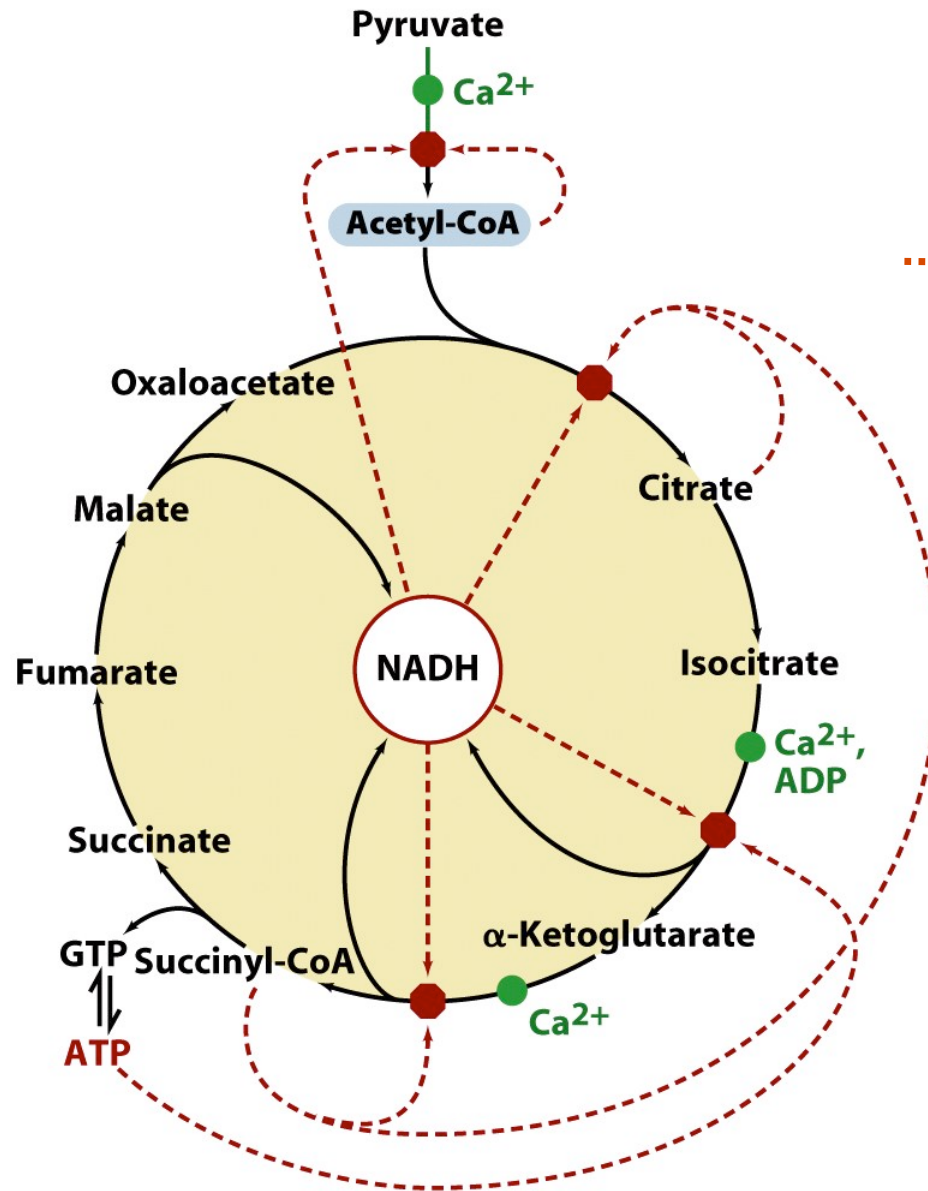
Regulation of the citric acid cycle

Table 17-2


Standard Free Energy Changes ($\Delta G^{\circ'}$) and Physiological Free Energy Changes (ΔG) of Citric Acid Cycle Reactions

Reaction	Enzyme	$\Delta G^{\circ'}$ (kJ · mol ⁻¹)	ΔG (kJ · mol ⁻¹)
1	Citrate synthase	-31.5	Negative
2	Aconitase	~5	~0
3	Isocitrate dehydrogenase	-21	Negative
4	α -Ketoglutarate dehydrogenase	-33	Negative
5	Succinyl-CoA synthetase	-2.1	~0
6	Succinate dehydrogenase	+6	~0
7	Fumarase	-3.4	~0
8	Malate dehydrogenase	+29.7	~0

The points of regulation of the cycle



 = point of inhibition

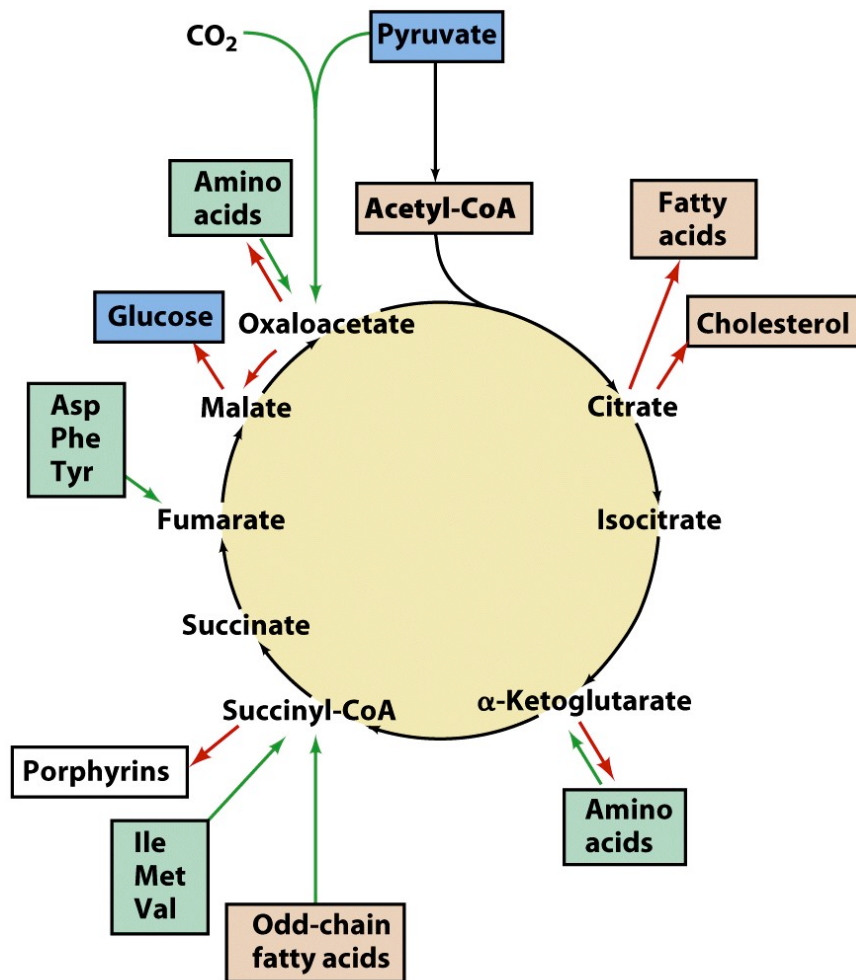
 = pathway intermediates that act as inhibitors

 = activators (ADP, Ca^{2+})

Flux controlled by 3 mech.

1. Substrate availability
2. Product inhibition
3. Competitive feedback inhibition

Citric acid cycle intermediates are always in flux



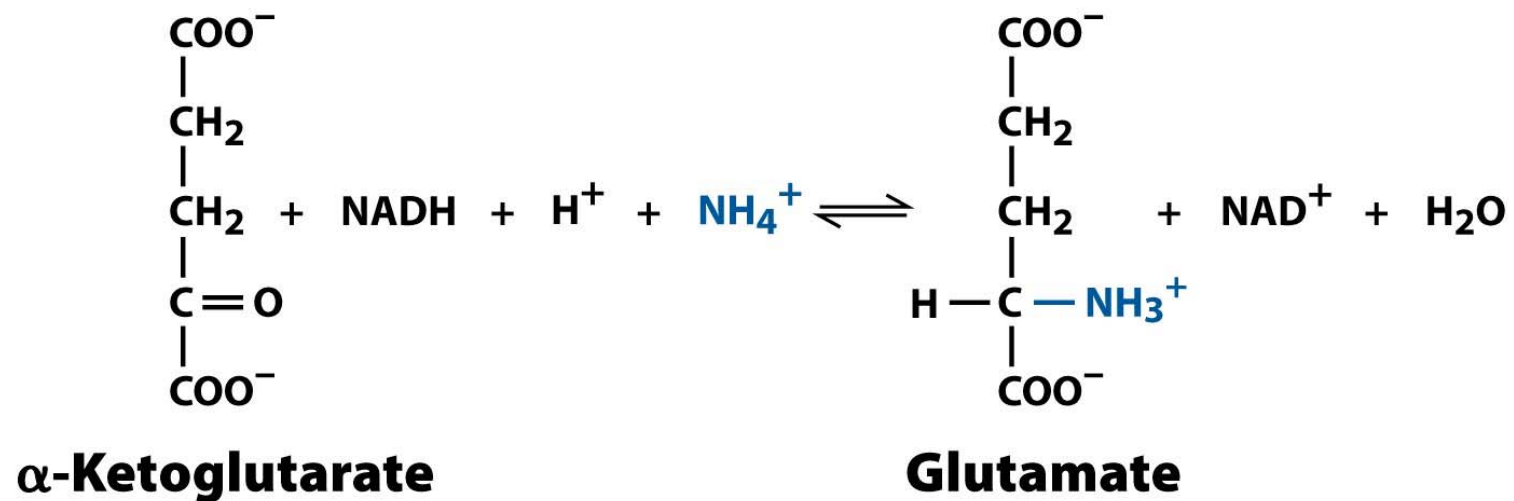
© 2008 John Wiley & Sons, Inc. All rights reserved.

Pathways using intermediates

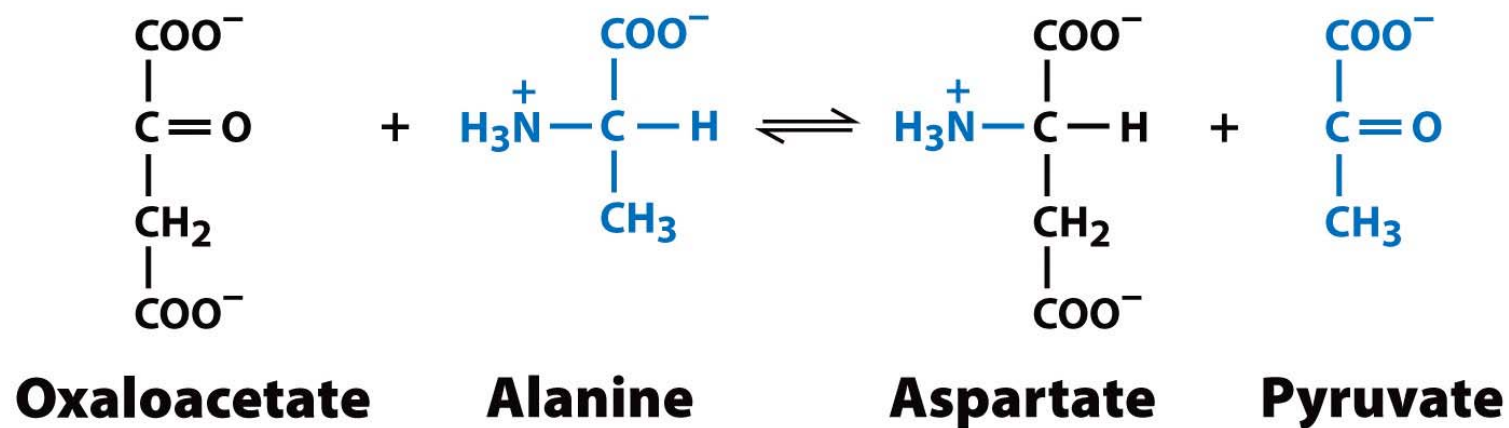
1. Gluconeogenesis
2. Fatty acid biosynthesis
3. Amino acid biosynthesis
4. Porphyrin biosynthesis

Reactions replenishing intermediates

1. Oxidation of odd-chain fatty acids
2. Breakdown of Ile, Met, Val, Asp, Phe, Tyr
3. Transamination/deamination of amino acids

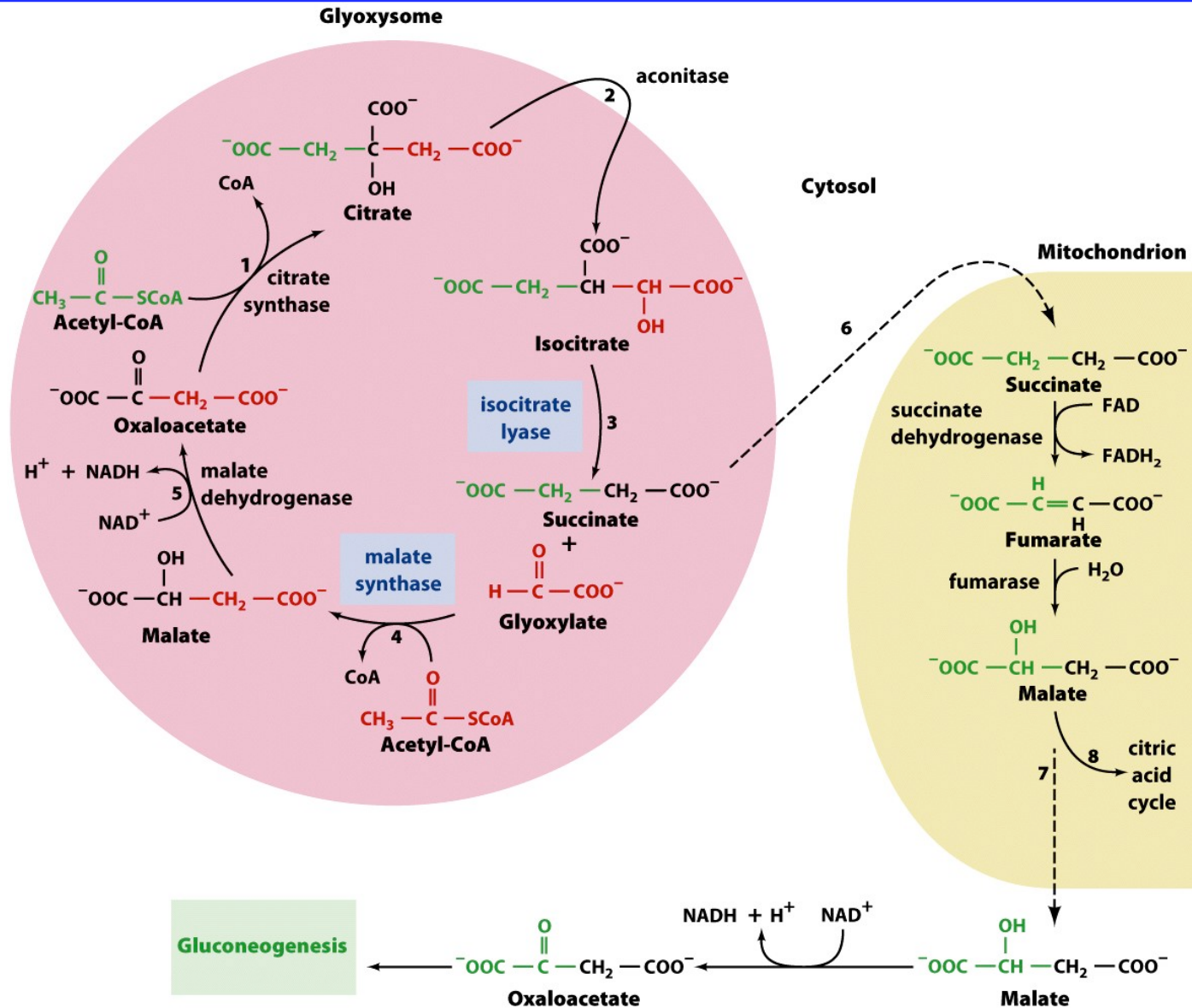


© 2008 John Wiley & Sons, Inc. All rights reserved.



© 2008 John Wiley & Sons, Inc. All rights reserved.

Glyoxalate cycle vs. Citric acid cycle



Questions:

1 (Companion#7). Which of the following labeled glucose molecule would yield $^{14}\text{CO}_2$ following glycolysis and the pyruvate dehydrogenase reaction?

- (a) 1- ^{14}C -glucose
- (b) 3- ^{14}C -glucose
- (c) 4- ^{14}C -glucose
- (d) 6- ^{14}C -glucose

2 (Book#5). The CO_2 produced in one round of the citric acid cycle does not originate in the acetyl carbons that entered that round.

- (a) If acetyl-CoA is labeled with ^{14}C at its carbonyl carbon, how many rounds of the cycle are required before $^{14}\text{CO}_2$ is released?
- (b) How many rounds are required if acetyl-CoA is labeled at its methyl group?