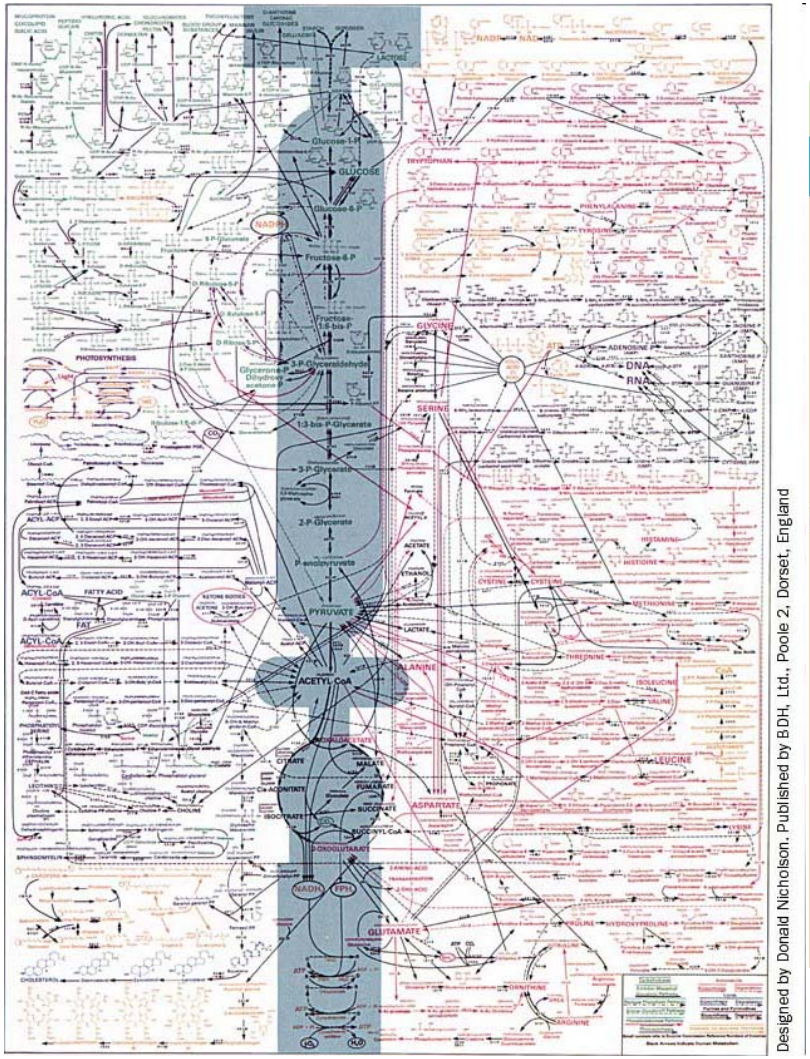
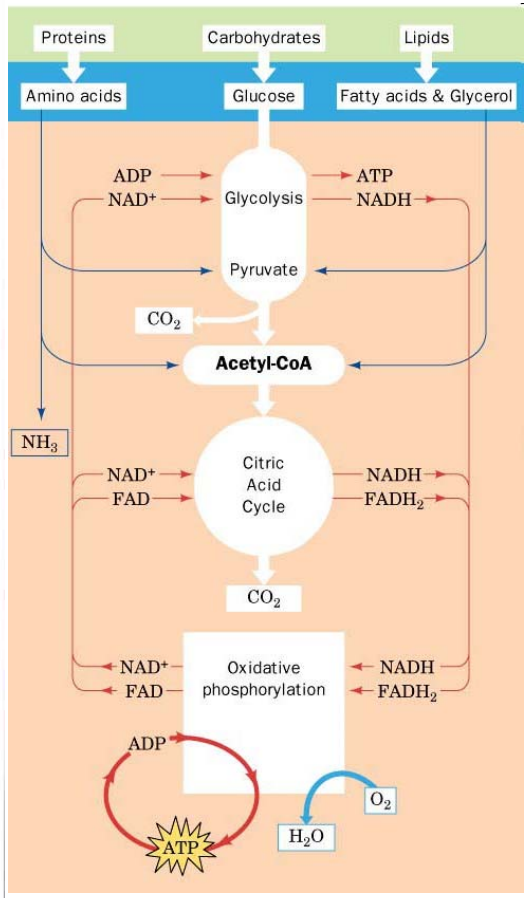
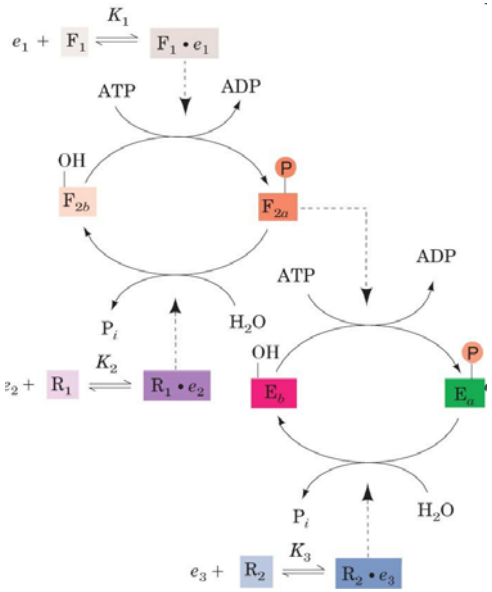
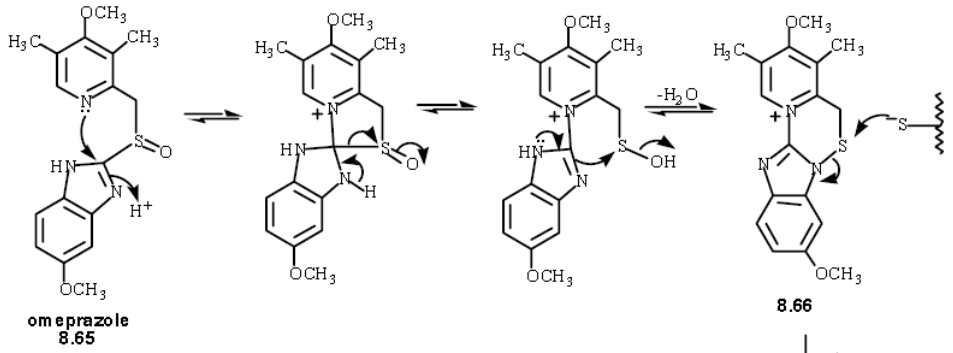
# Metabolic Pathways and Energy Metabolism

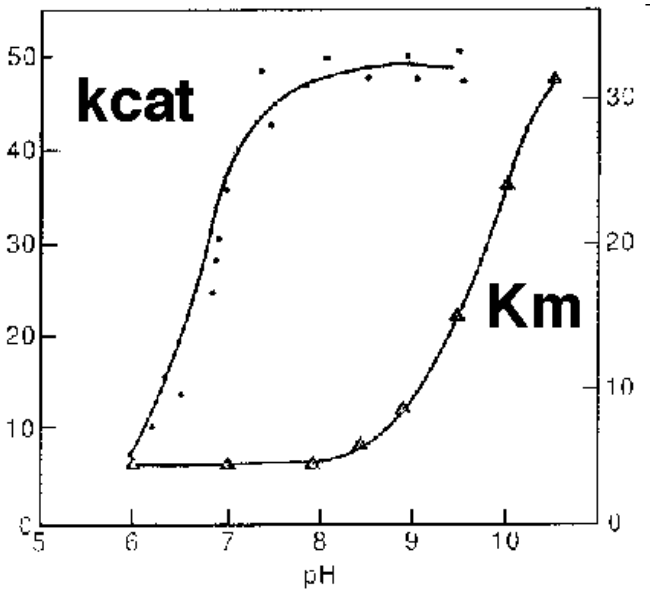


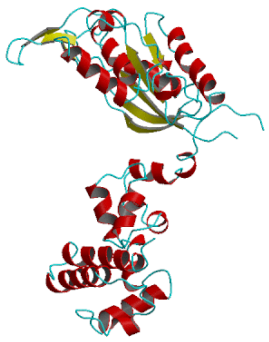



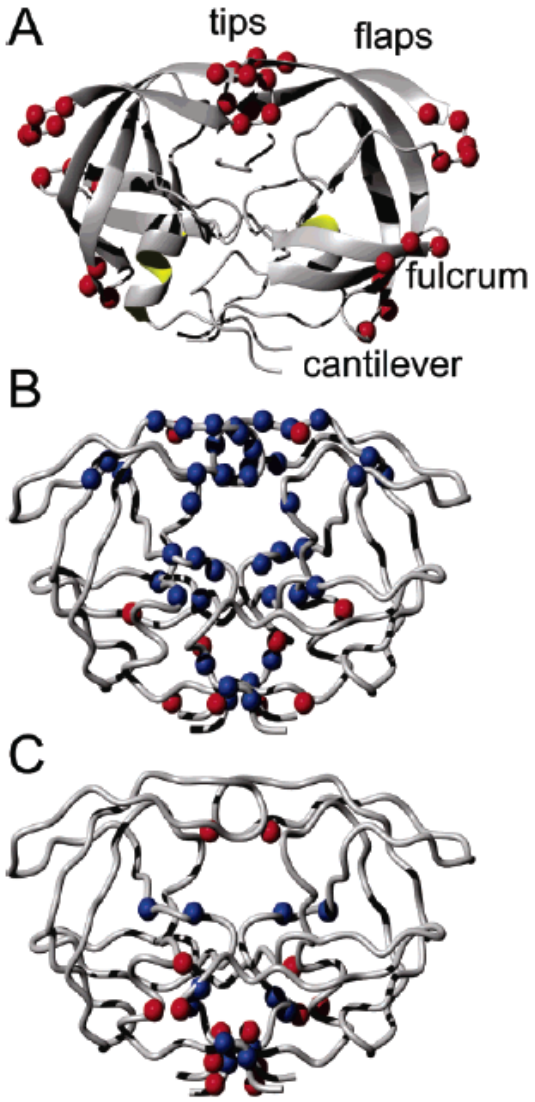



# Last Week...


omeprazole
8.65
-H2O
8.66
+ H+


kcat
Km
pH




A
tips
flaps
fulcrum
cantilever
B
C

# Energy Metabolism

- The first thing a living organism has got to be able to do is harness energy from the environment

- Plants do it by absorbing sunlight

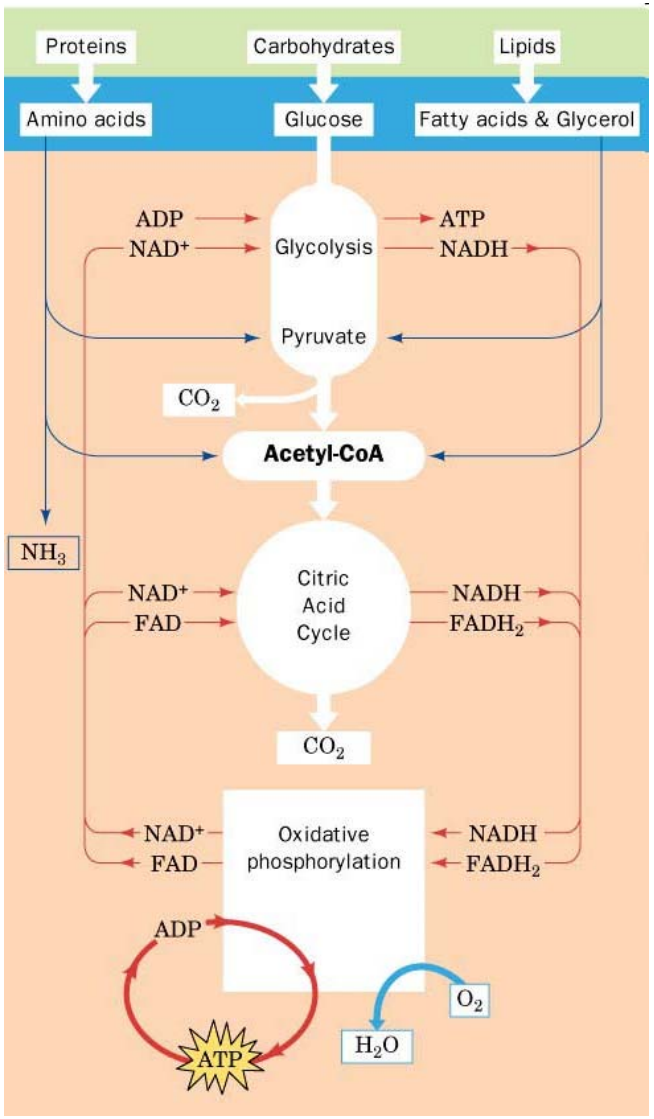
- We do it by eating food

- I suppose this explains the focus of every biochemistry textbook on the processes of energy metabolism:

1) Glycolysis

2) The citiric acid cycle

3) Oxidative Phosphorylation

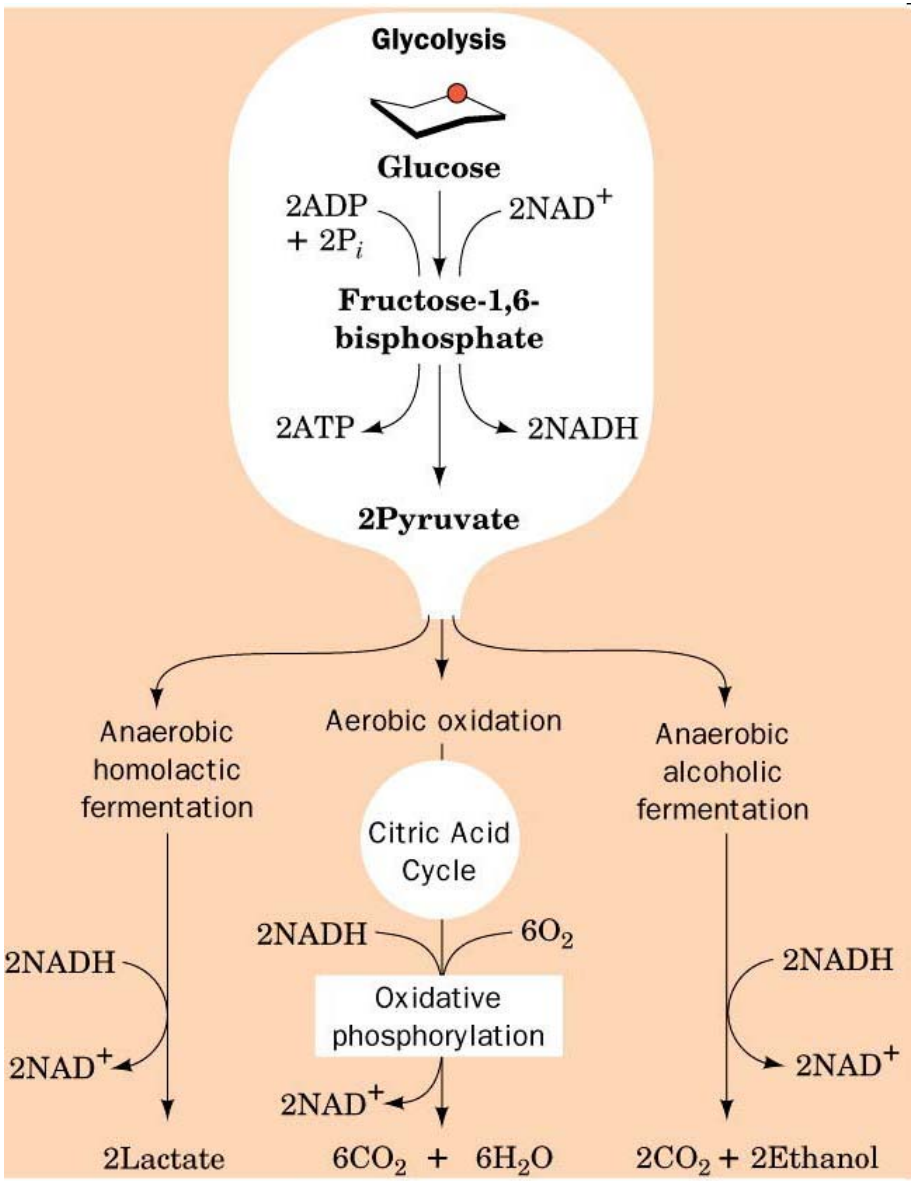


# Sugar to Energy Overview

- Glucose is the most direct input into the energetics metabolic pathway

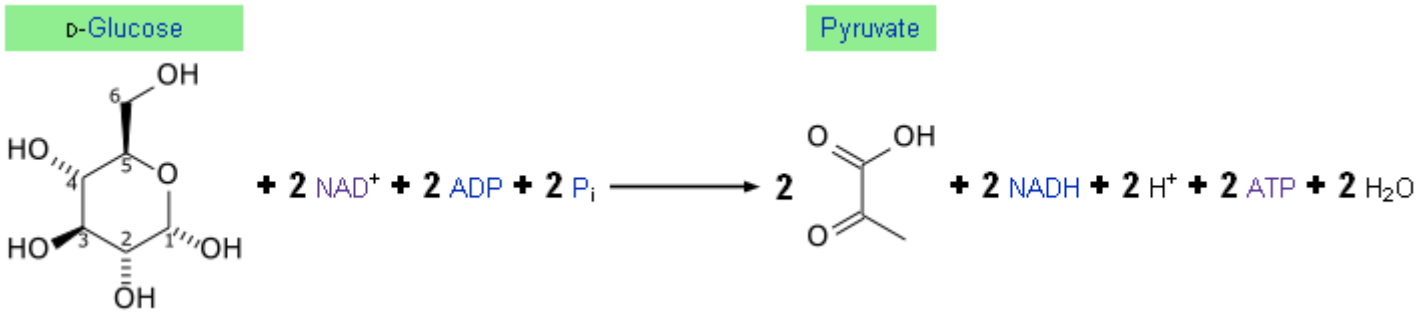
- As evidenced by the behavior of a typical 7 year old after he/she has a slushy.

- The first step is glycolysis, in which we directly break the sugar up.



# Glycolysis: The Playas!

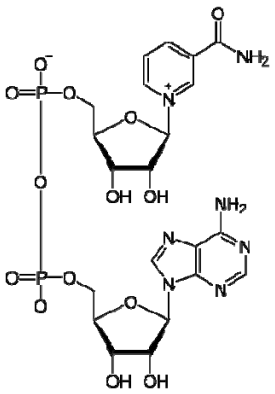
- The overall reaction



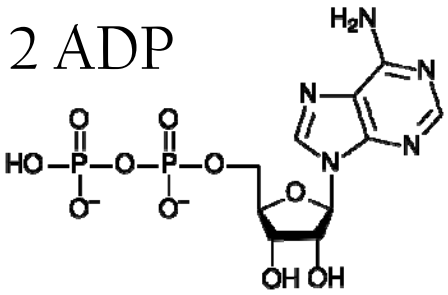

We start with:

α-D-Glucose

2 (Oxidized) $NAD^+$



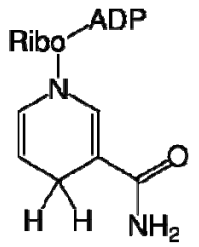
2 ADP



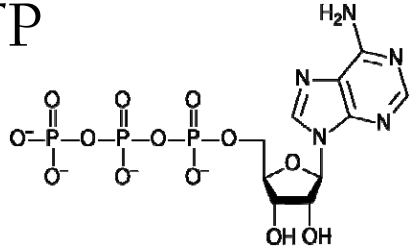
We end up with:

2 Pyruvate

2 NADH




2 ATP

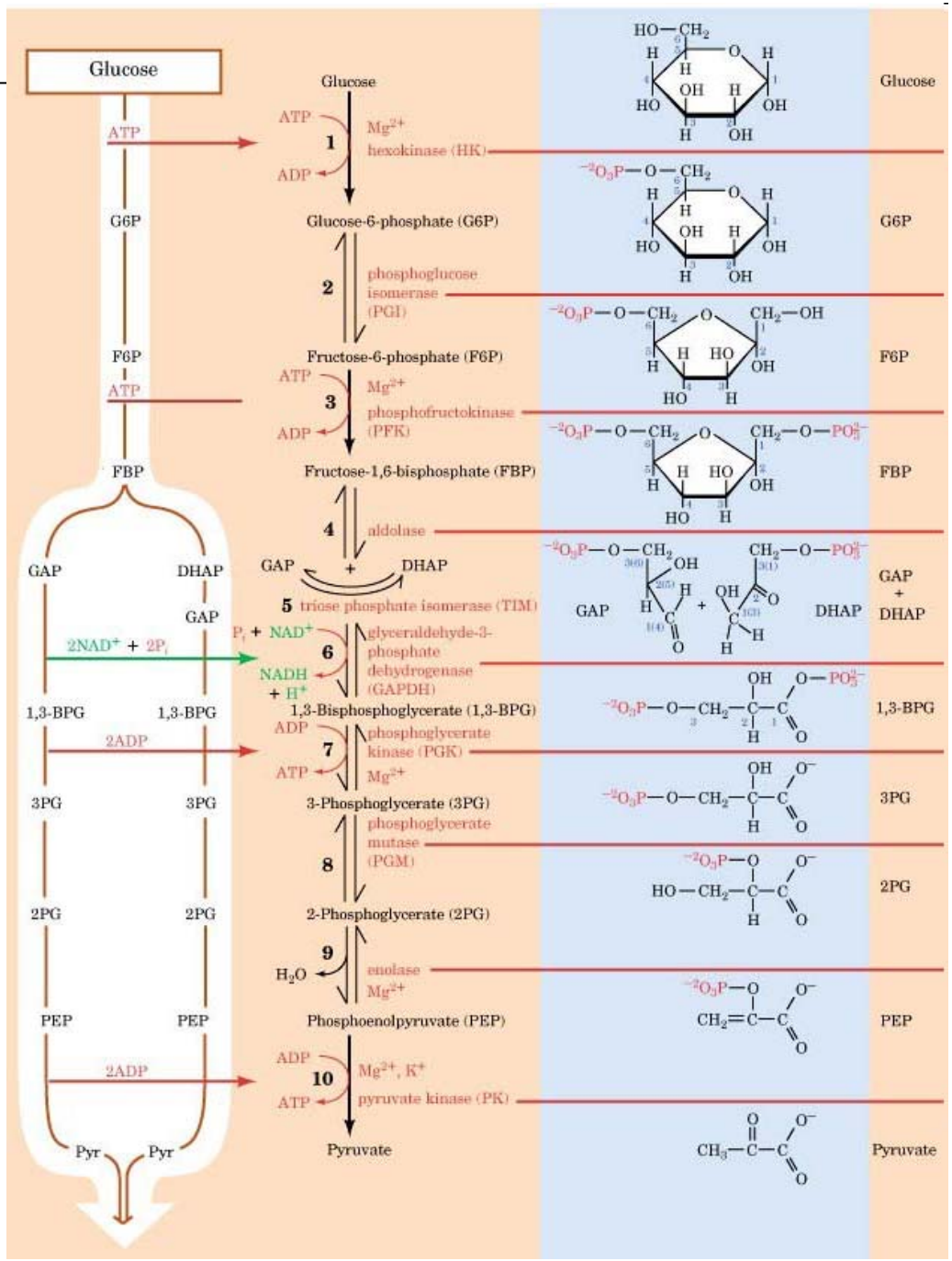


# The Whole Shebang!

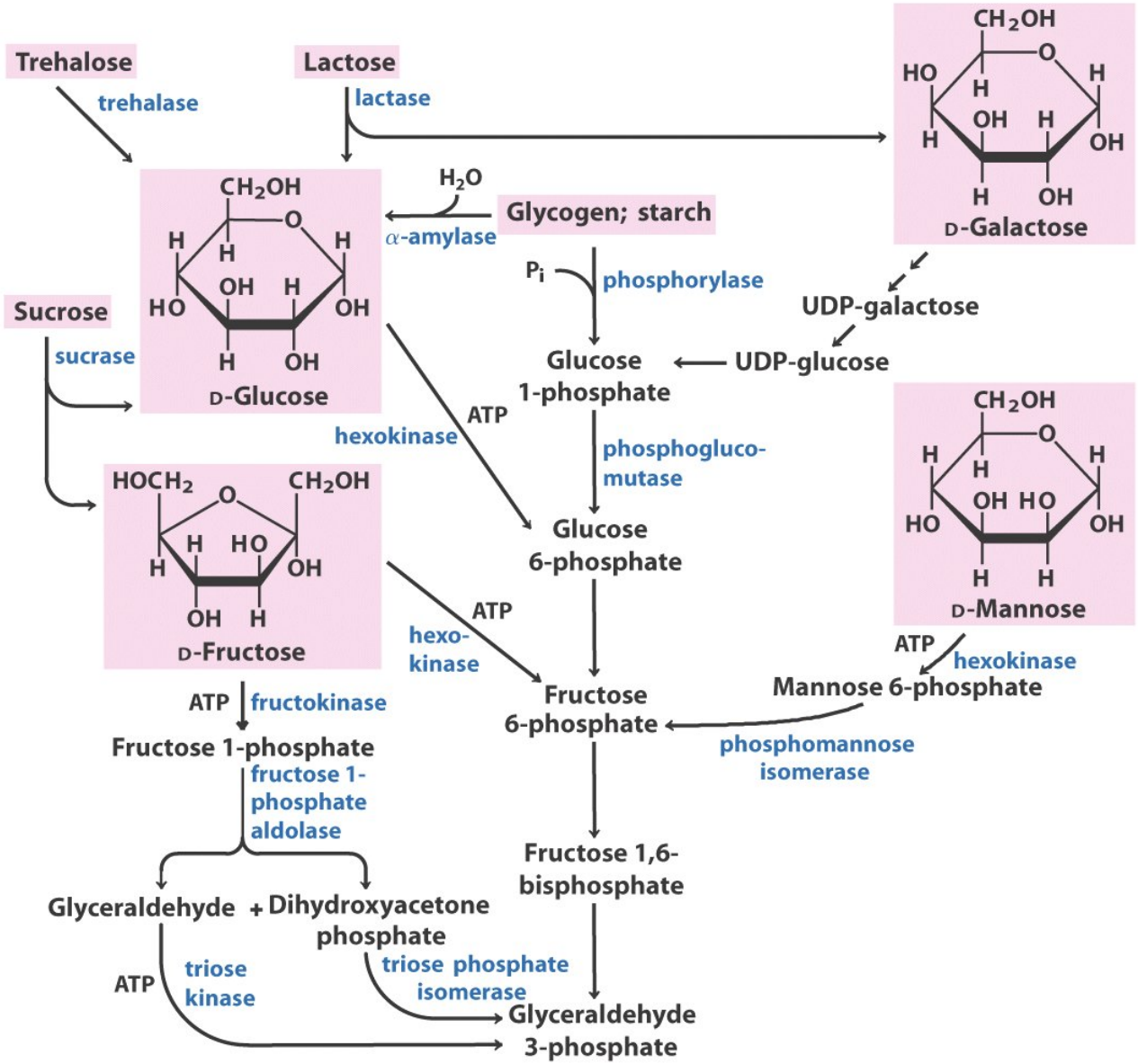
- The whole enchilada!

- El toto!

- The long and the short of it!

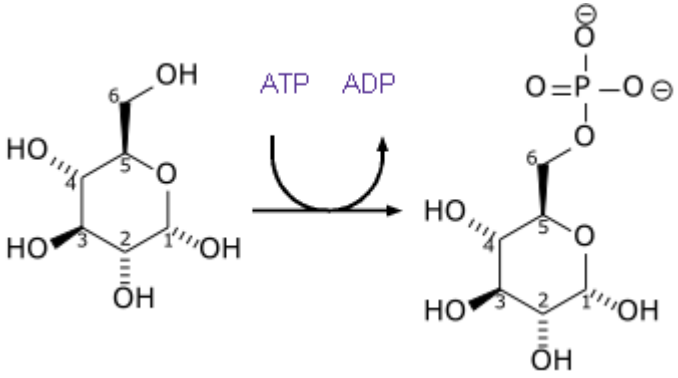


# Feeder Pathways: It Ain't Always Glucose!

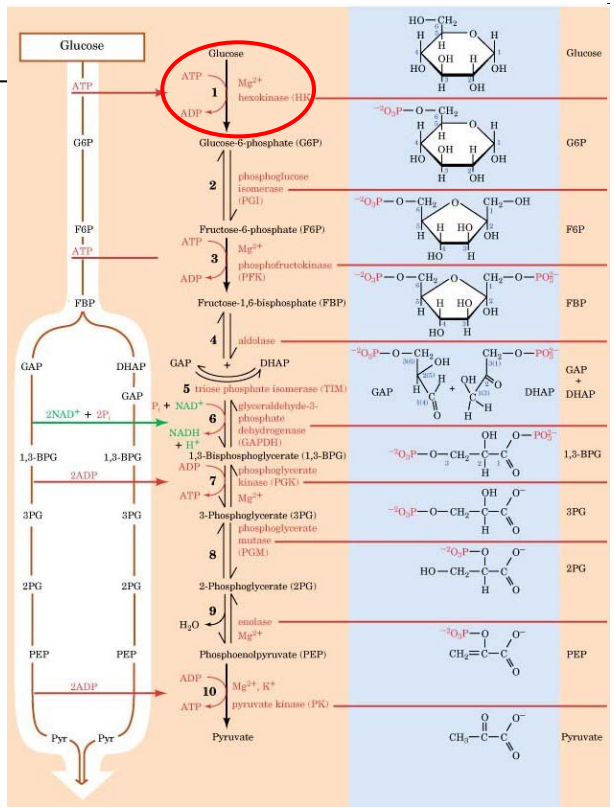


# Glycolysis Step 1

- Gluocose isn't going to hydrolyze itself! We need to activate it

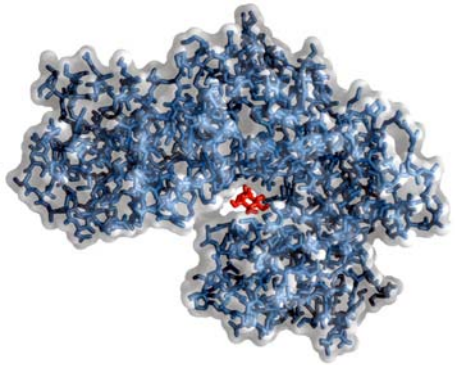



G6P

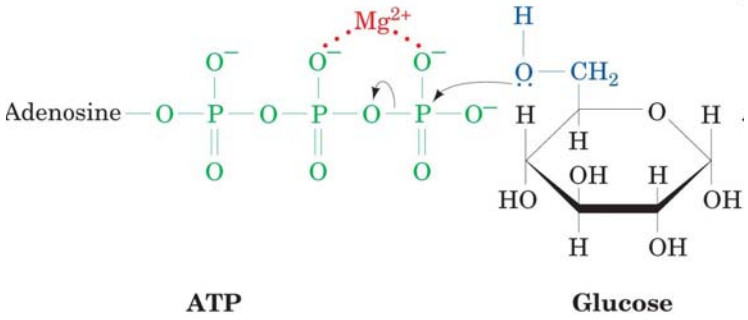



- Also prevents glucose from leaking out of the cell, promotes glucose uptake

- The enzyme? Hexokinase:

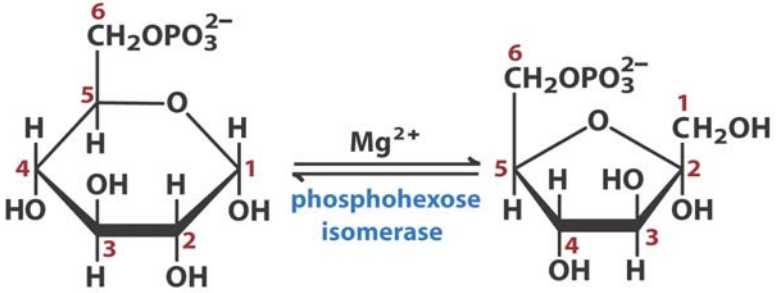


- The reaction is $Mg^{2+}$ dependent

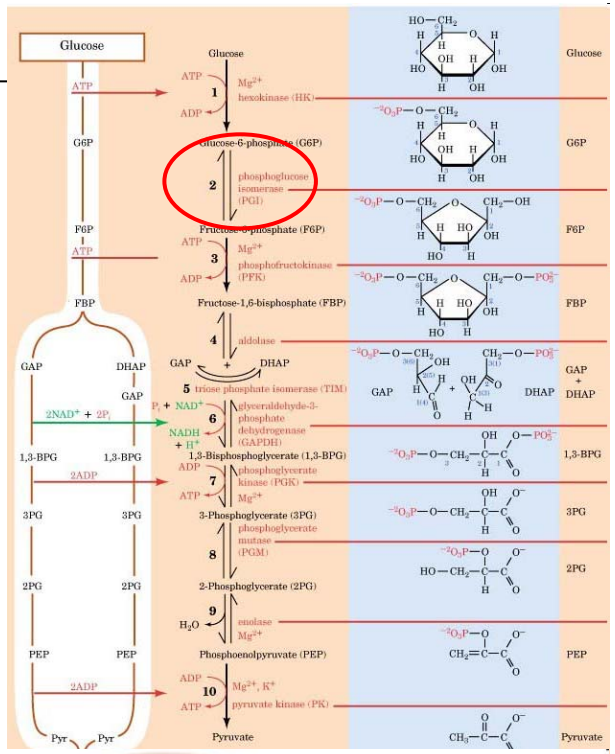


# Glycolysis Step 2

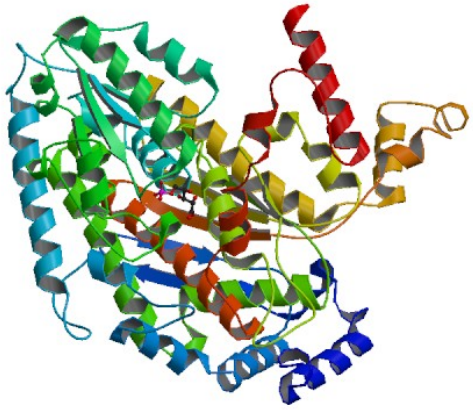
- Now we need to isomerize the five membered pyranose ring to a four membered furanose ring



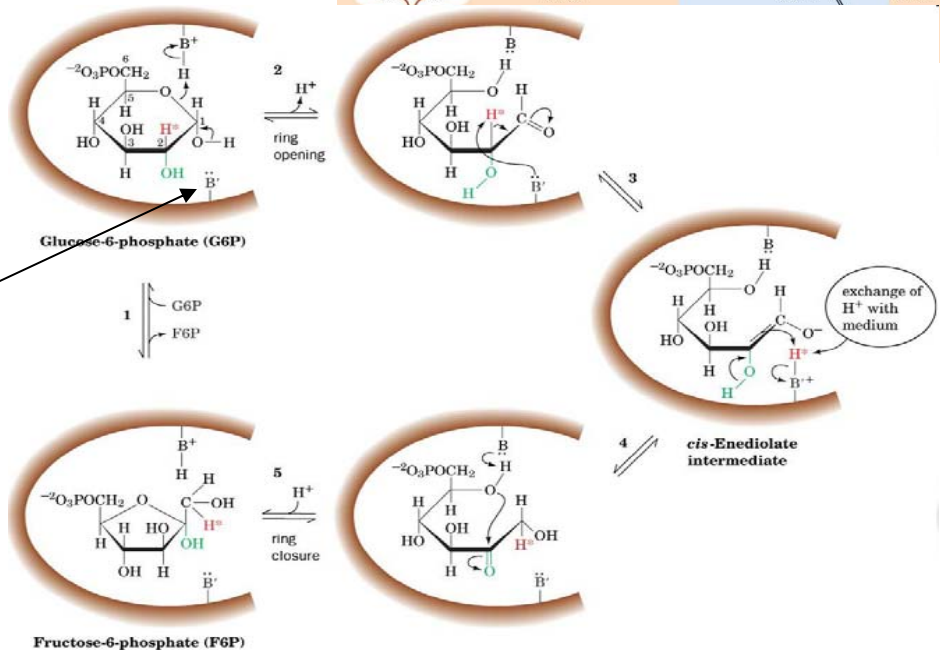

$\Delta G'^{\circ} = 1.7$ kJ/mol




- The enzyme: G6P Isomerase

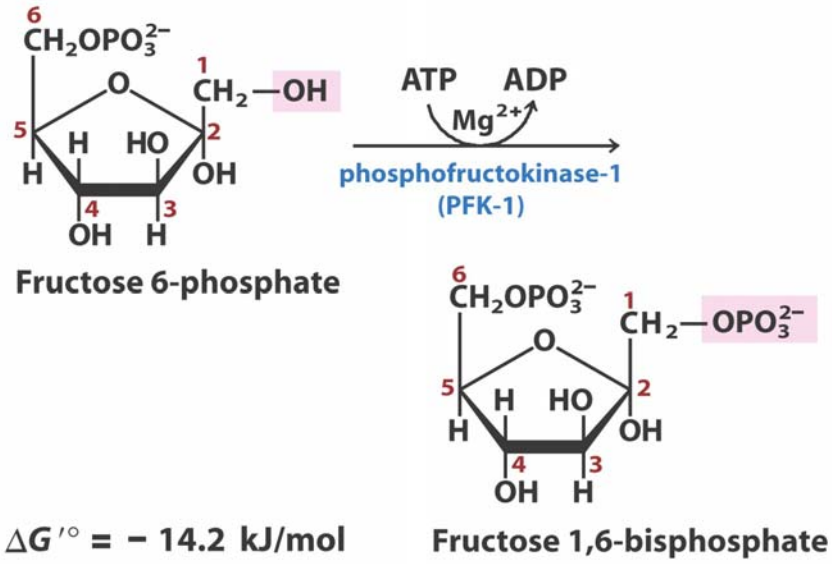


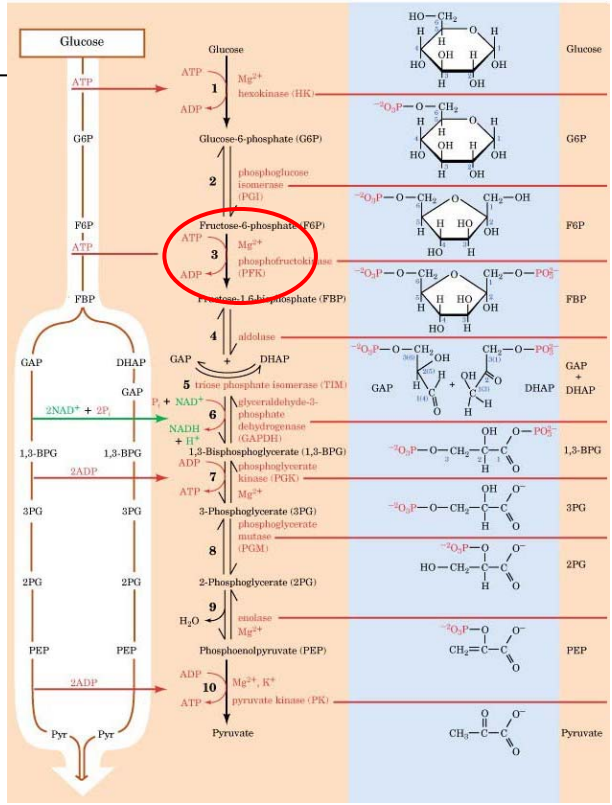
His388

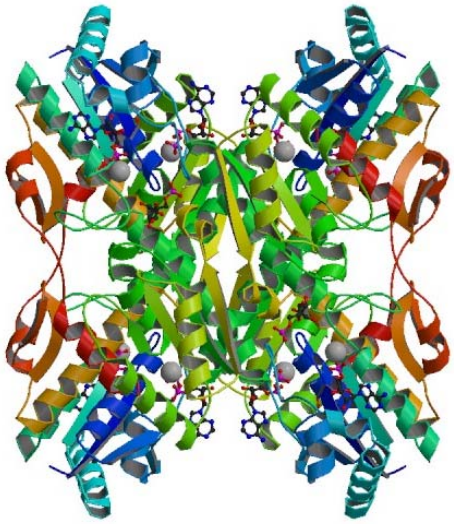


# Glycolysis Step 3

- How much glycolysis we gonna have?

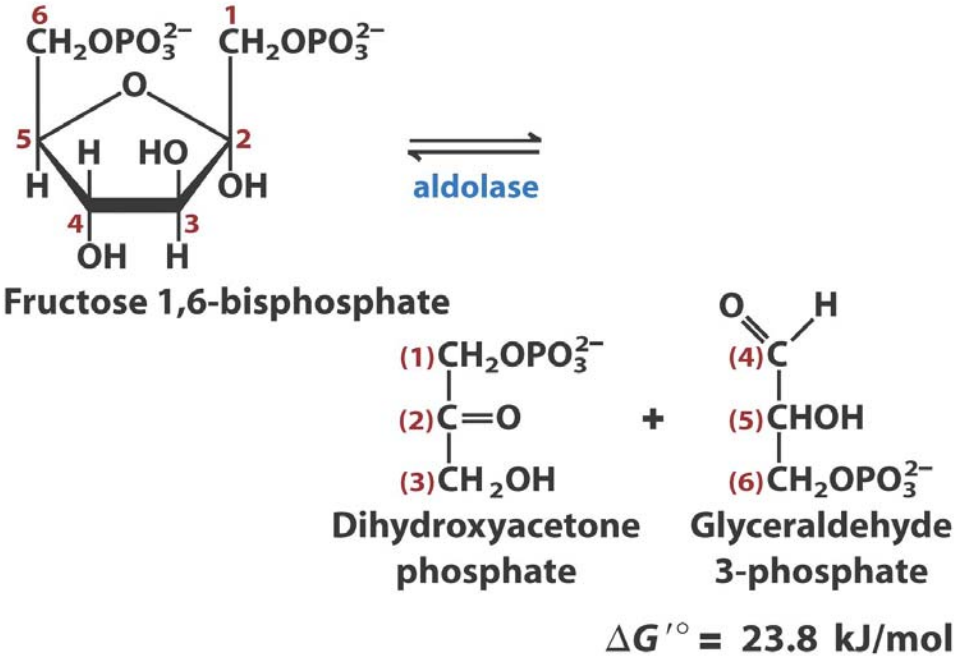


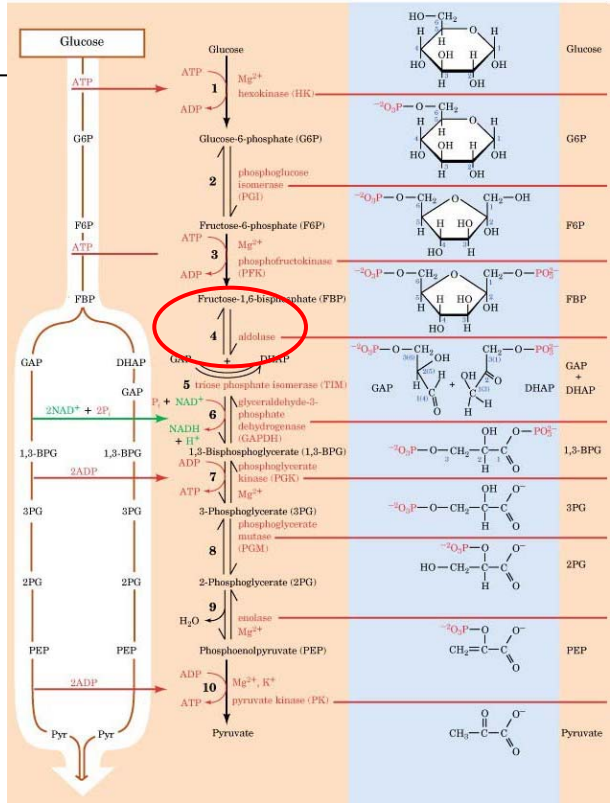



- As the first irreversible step in glycolysis, PFK-1 is tightly controlled.
- Allosterically inhibited by citrate, glucose 1,6-bisphosphate and ATP (two active sites)!
- Glucagon ↑ PFK-1 Expression ↓
- Allosteric activation: AMP (low energy)

# Glycolysis Step 4

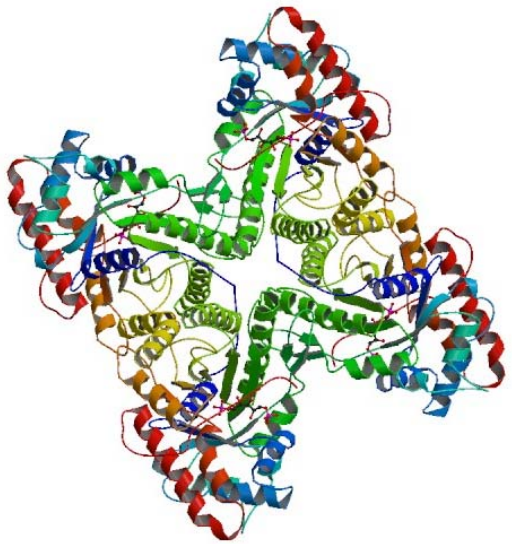



- Finally, we bust up the ring for good!!

- Aldolase works through a complex, multistep mechanism involving... two Schiff Bases!

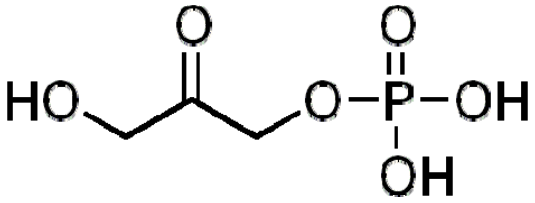
- Needs for there to be a carbonyl on $C_2$ and an alcohol on $C_4$ so that the Schiff base can do it's business!



# Aldolase mechanism (lyase)

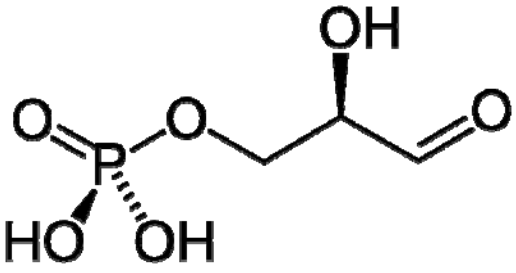
- Makes:

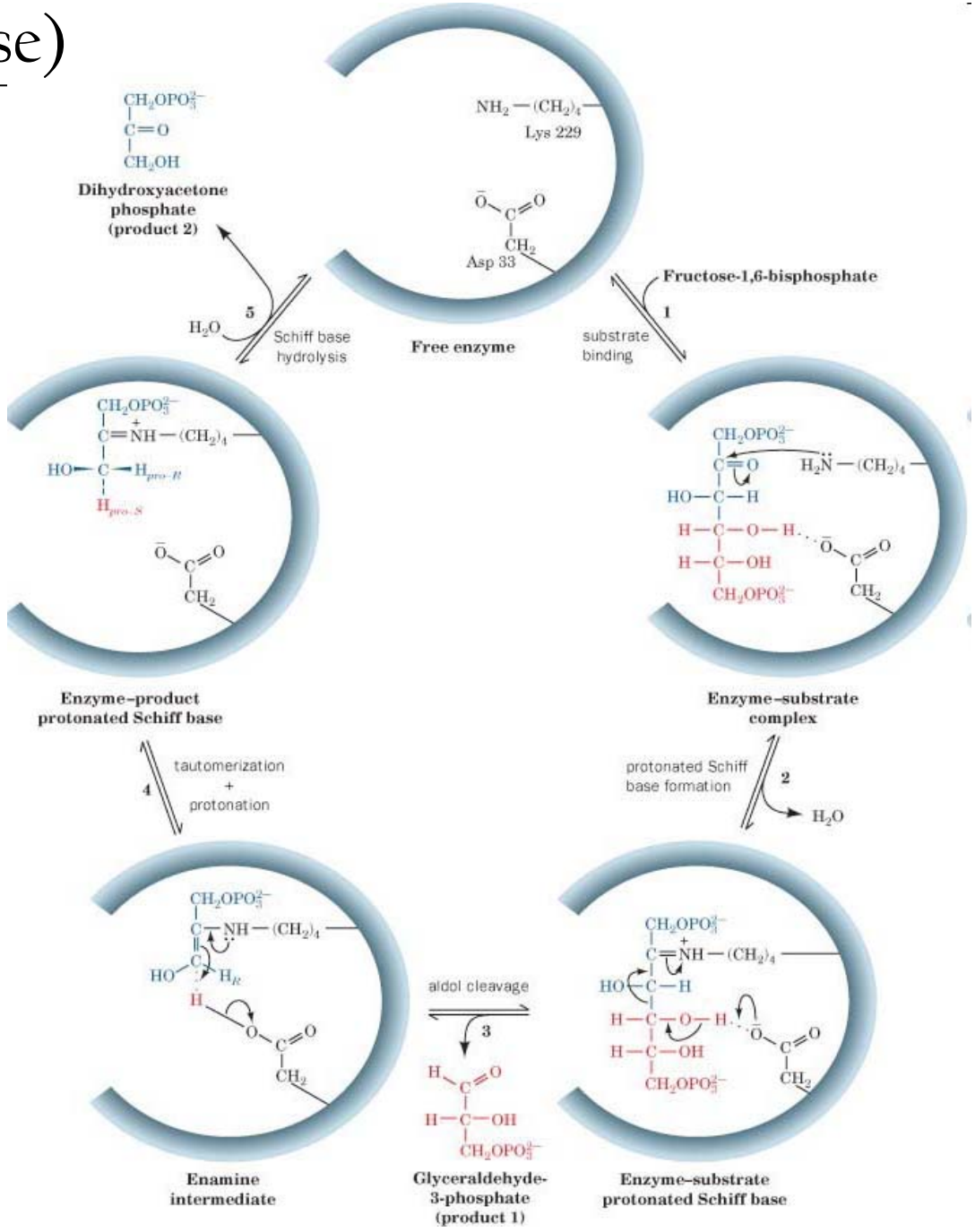
Dihydroxyacetone phosphate (DHAP)



and...

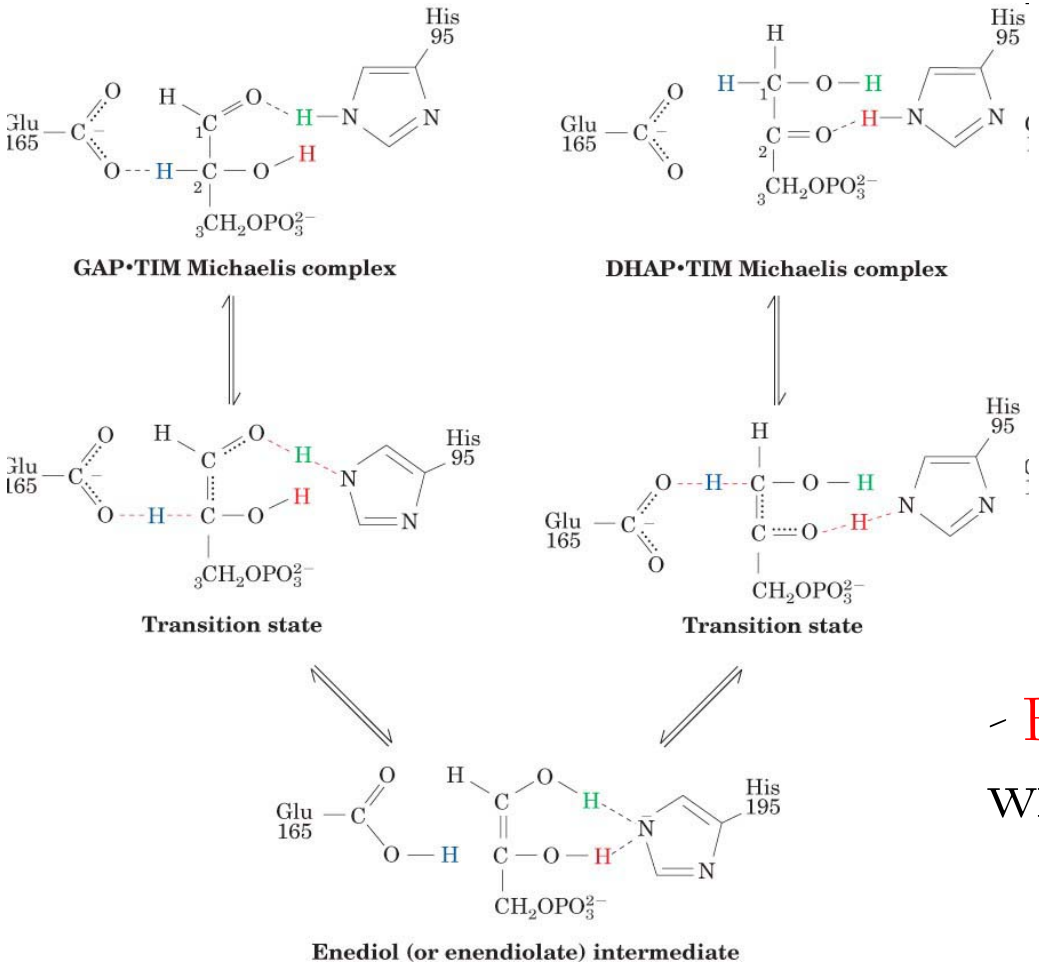
Glyceraldehyde-3-phophate

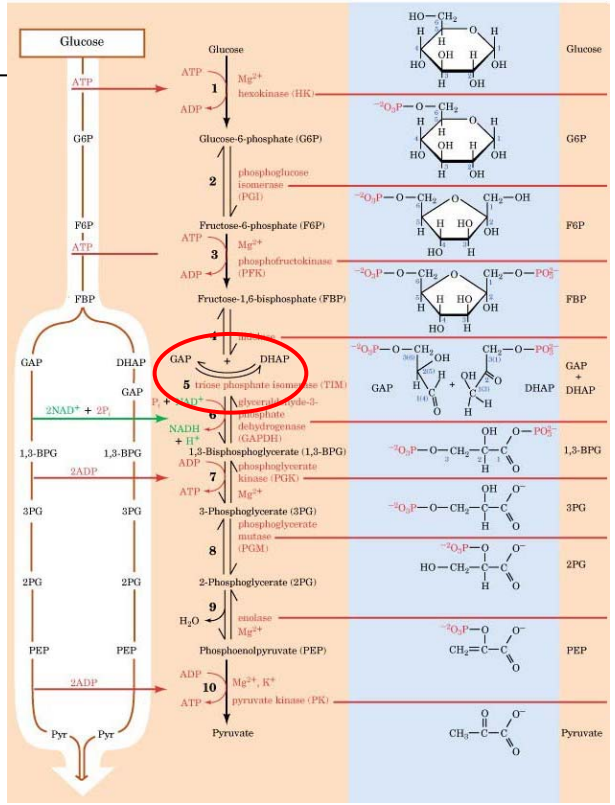




# Glycolysis Step 5

- GHAP → GAP with Triose Phosphate Isomerase (TIM). Diffusion controlled!



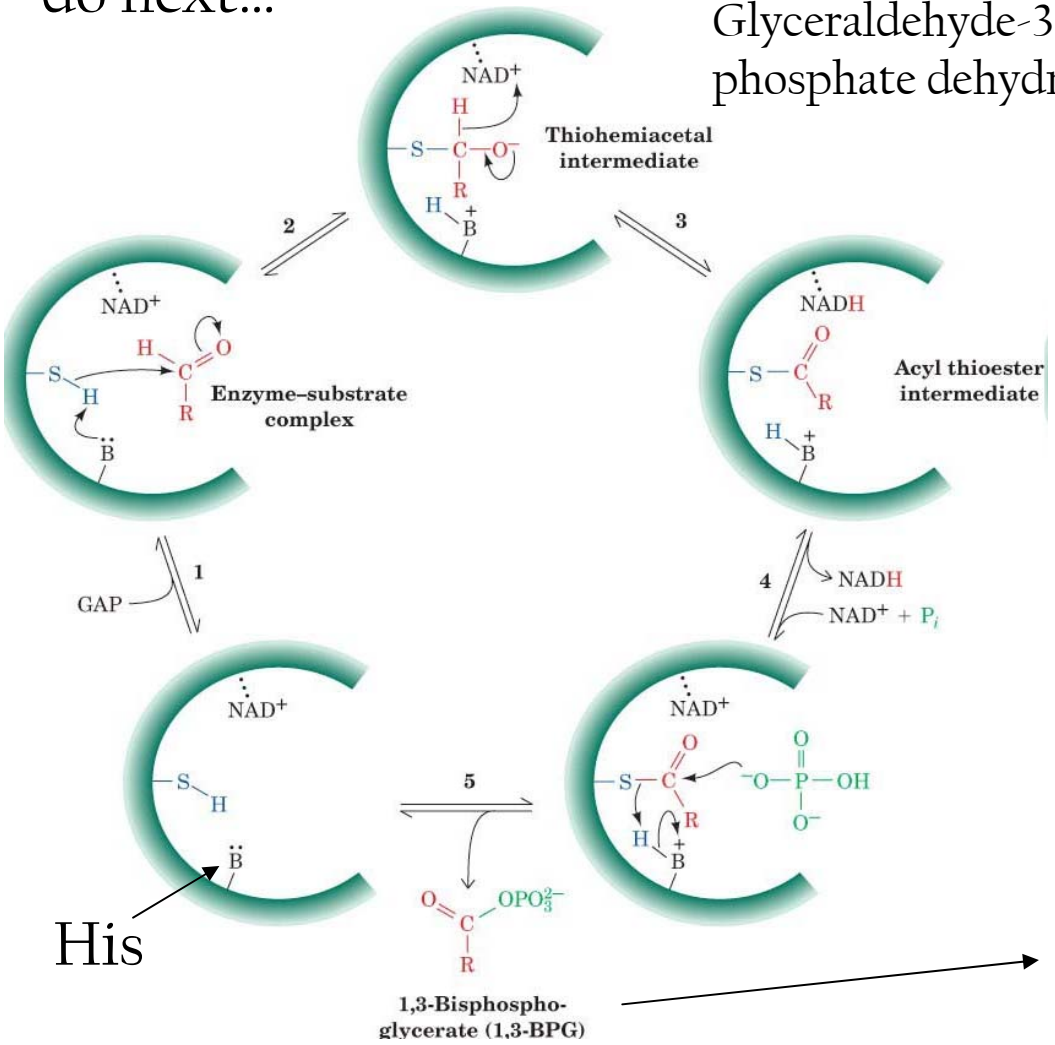



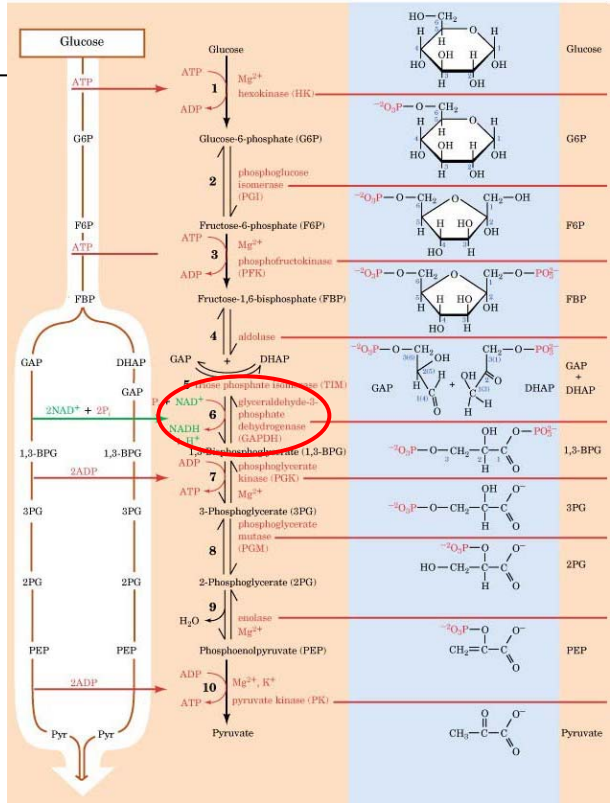

- Basically His and Glu playing with a couple of protons

# Glycolysis Step 6

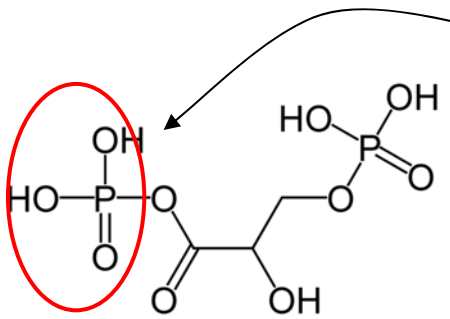
- We now have a conveniently placed carbonyl carbon… guess what we're going to do next…

Glyceraldehyde-3-phosphate dehydrogenase




- We get our first reduced NAD! (NADH)

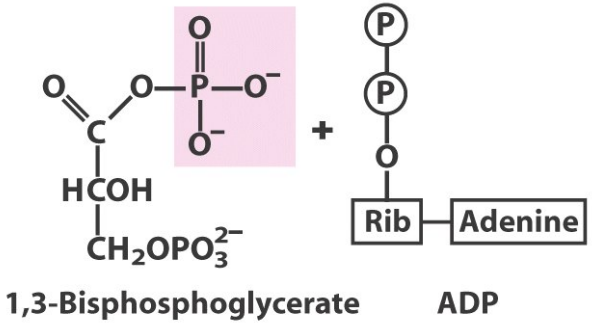


Quite Unstable!

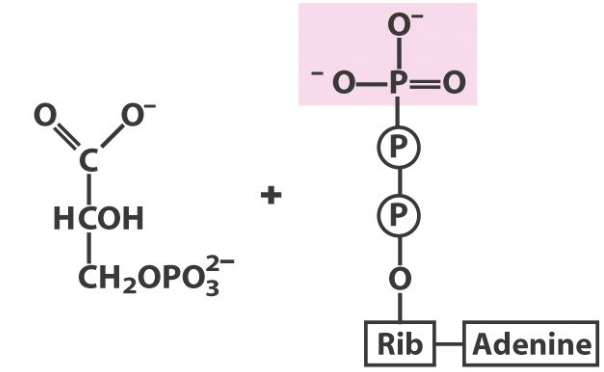
Is going to move on

# Glycolysis Step 7

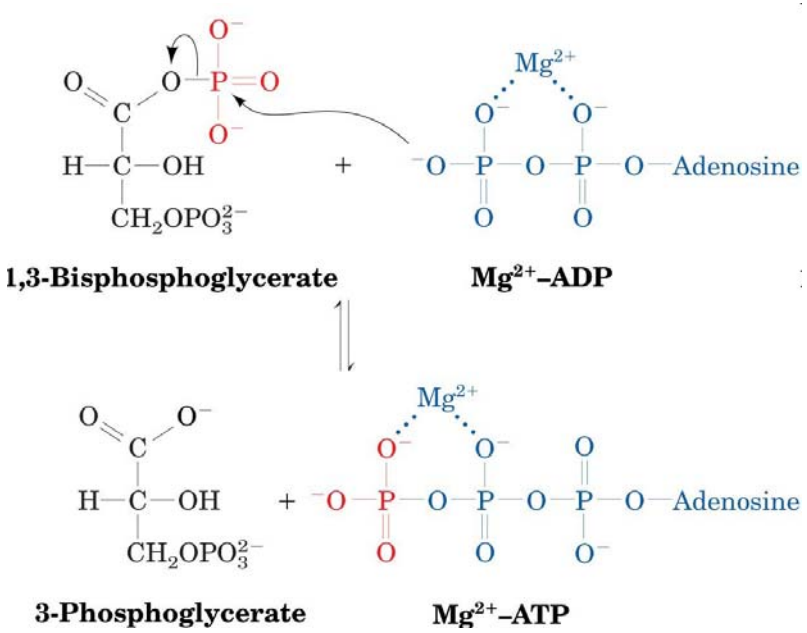
- We've got our high energy phopshoester linkage, now we can use it to make ATP!



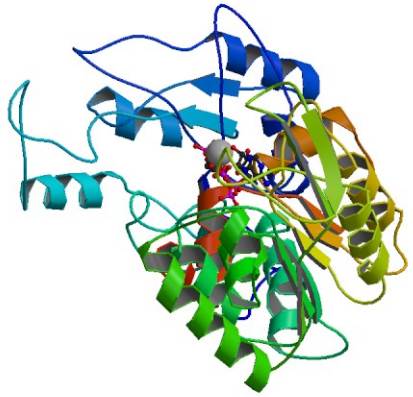

$Mg^{2+}$ phosphoglycerate kinase




$\Delta G'^{\circ} = -18.5$ kJ/mol

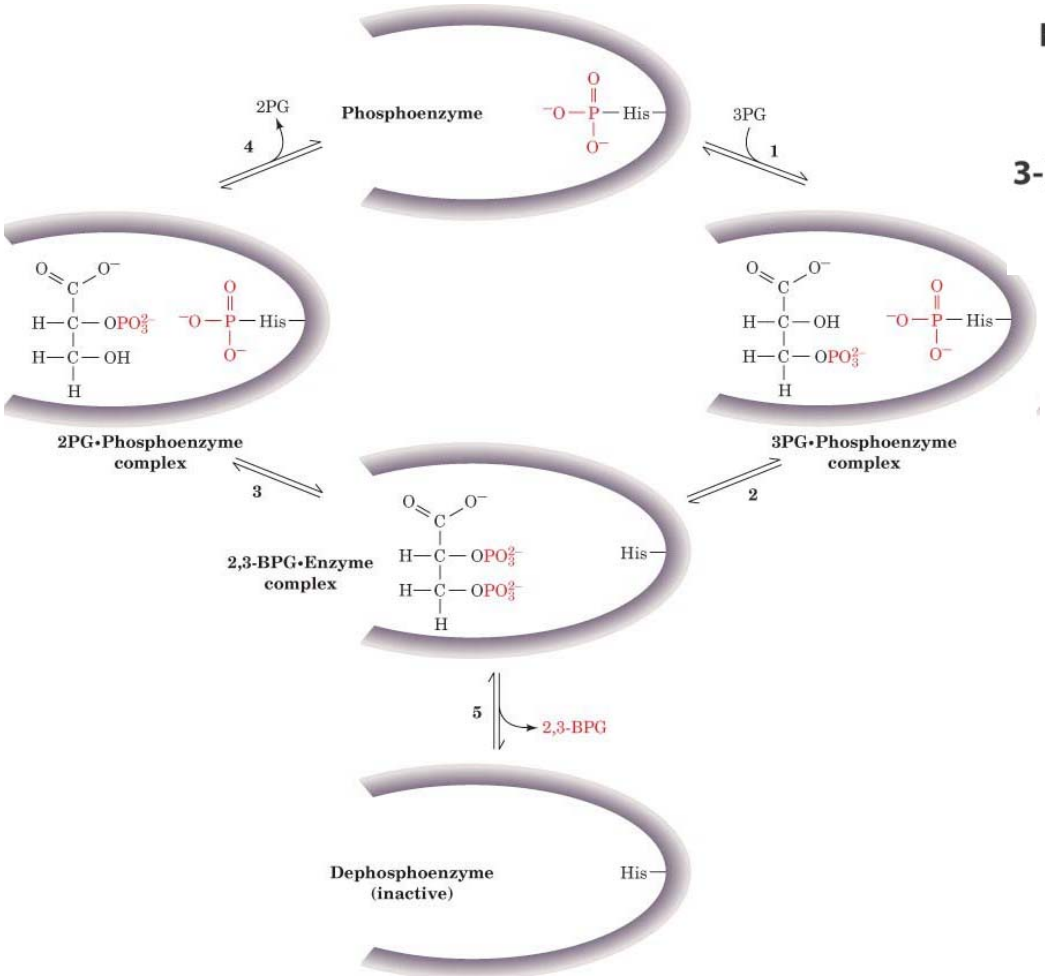


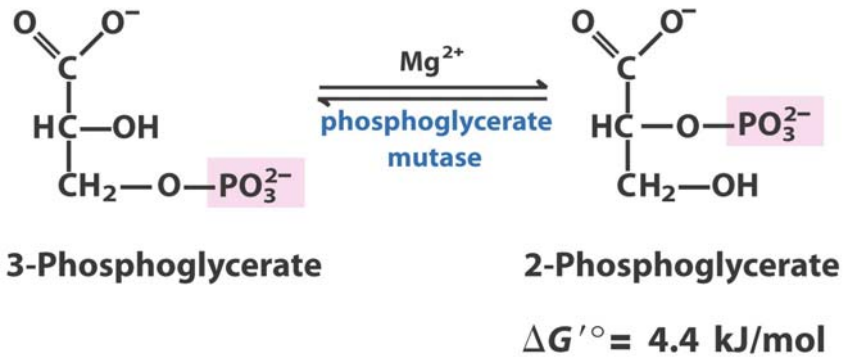

- Nucleophilic attack by $Mg^{2+}$ activated oxygen



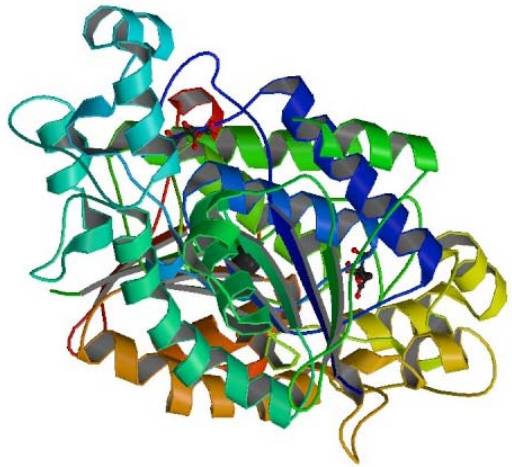
# Glycolysis Step 8

- We need to activate the phosphate group on 3-phosphoglcerate by putting it near some 'mobile' electrons



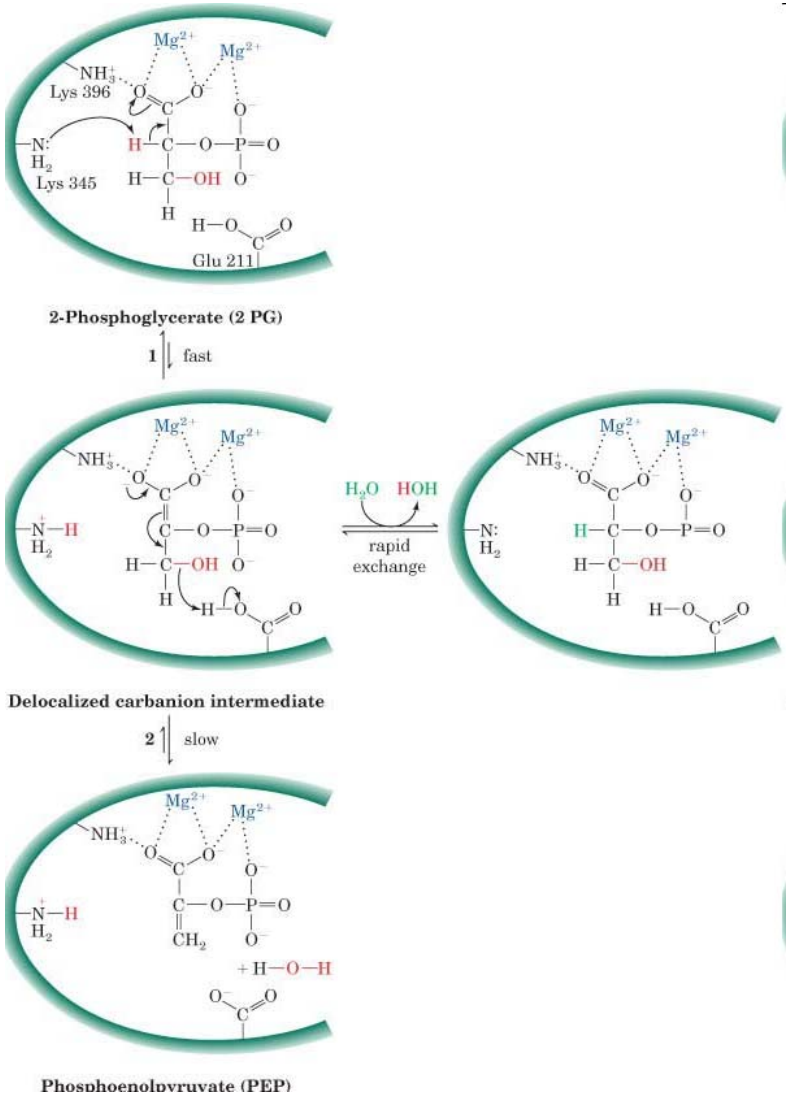



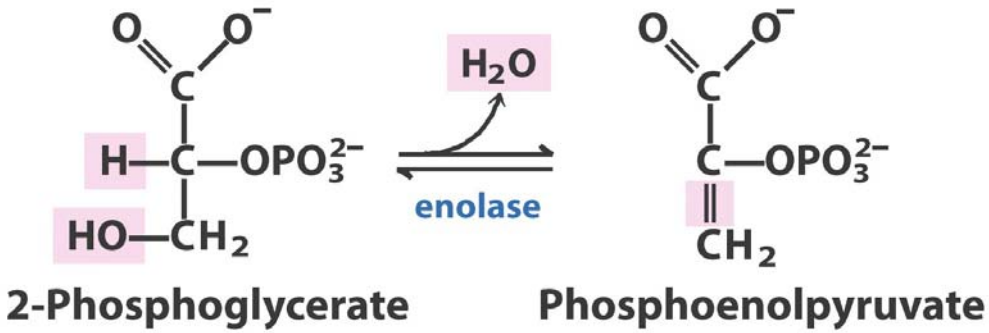

- Mechanism requires phosphohistidine! (His 8).



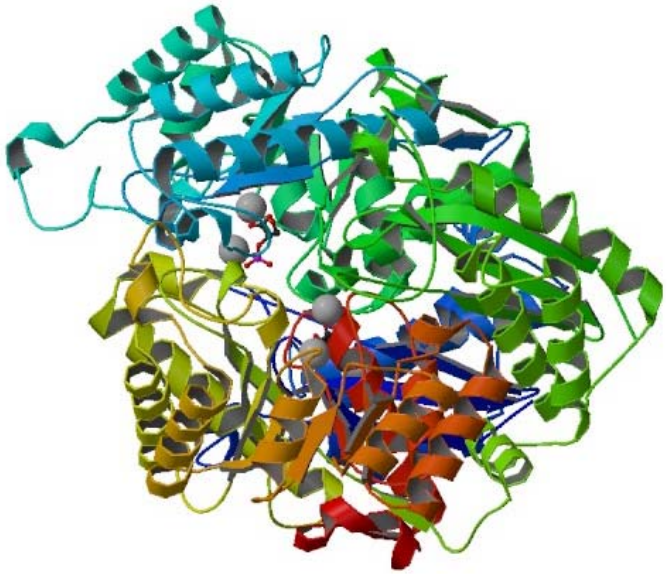
# Glycolysis Step 9

- In case the carbonyl wasn't enough, we're going to add a Carbon-Carbon double bond adjacent to the phosphate:

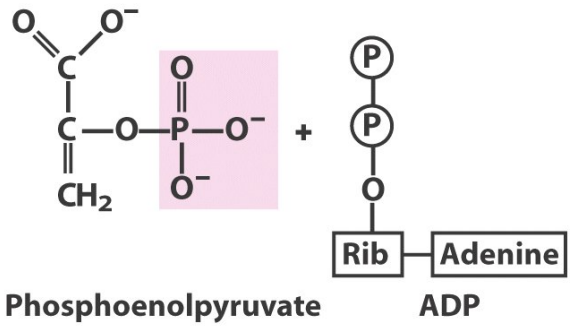


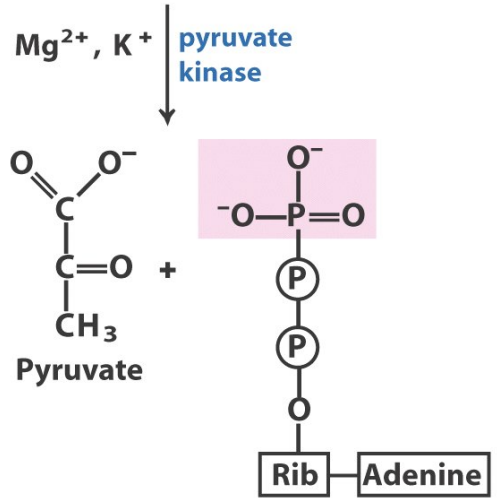




$\Delta G'^{\circ} = 7.5$ kJ/mol

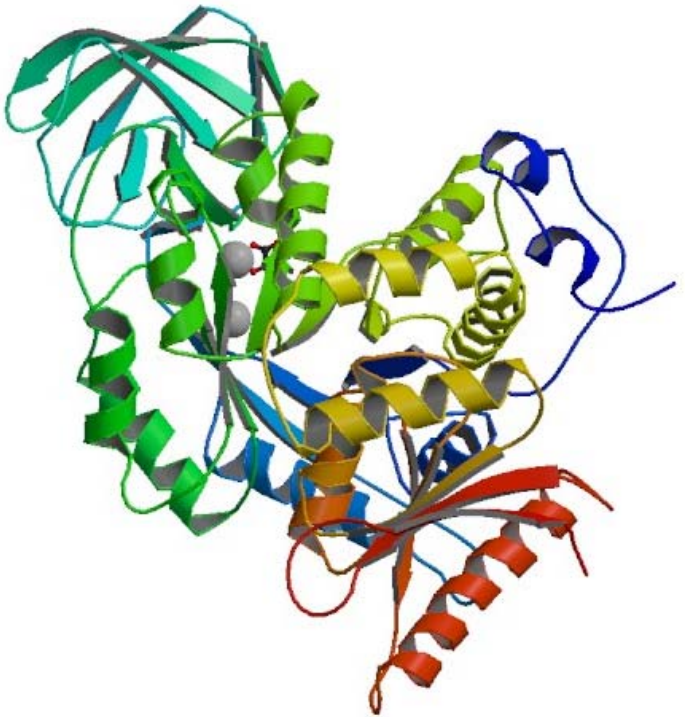


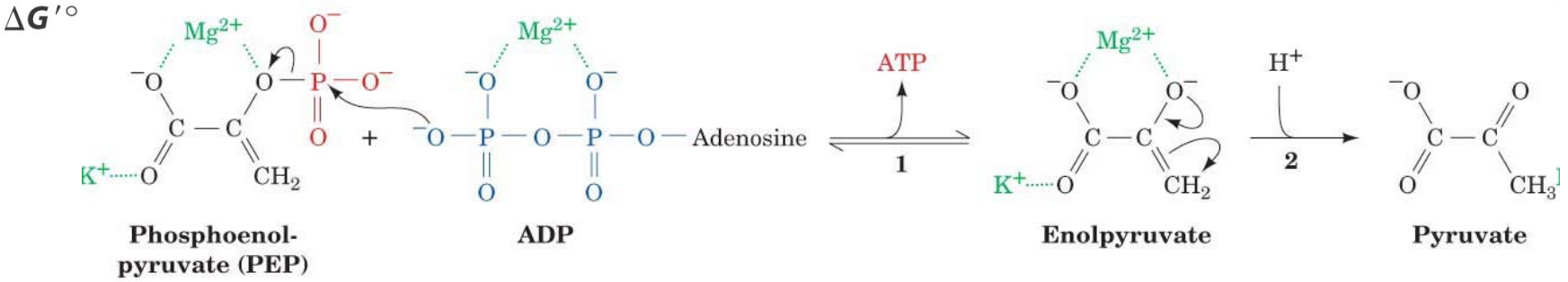
# Glycolysis Step 10




- Time to reap the reward!

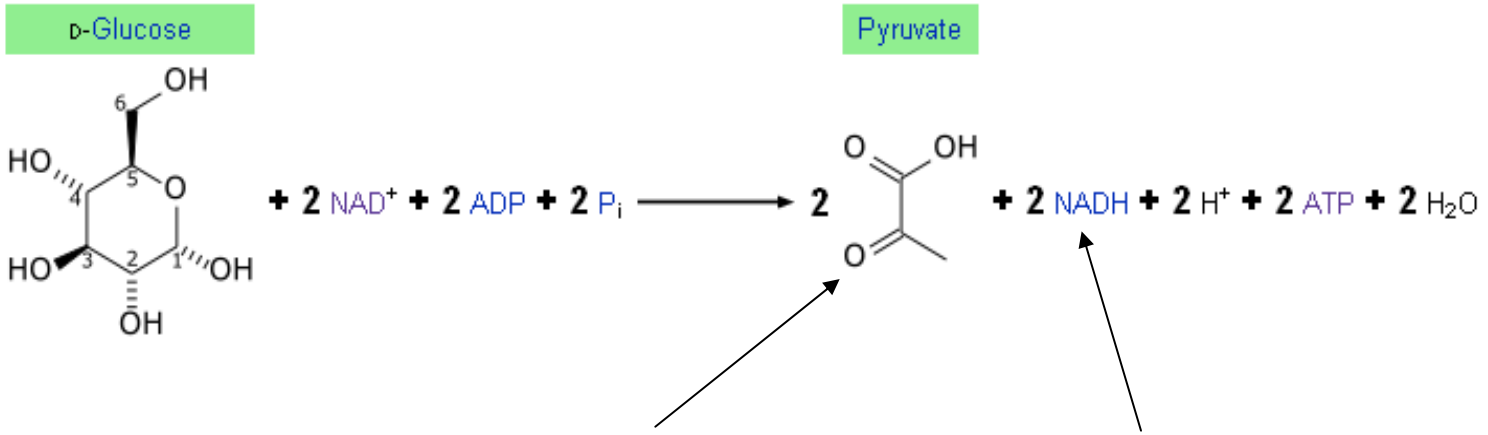





$\Delta G^{\circ\prime} = +14.4\ \text{kJ}\cdot\text{mol}^{-1}$ $\Delta G^{\circ\prime} = -46\ \text{kJ}\cdot\text{mol}^{-1}$

Overall $\Delta G^{\circ\prime} = -31.4\ \text{kJ}\cdot\text{mol}^{-1}$

# Glycolysis: The end?... Of Course Not!

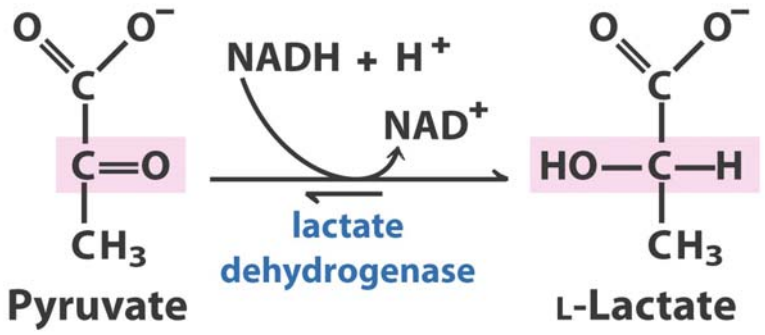


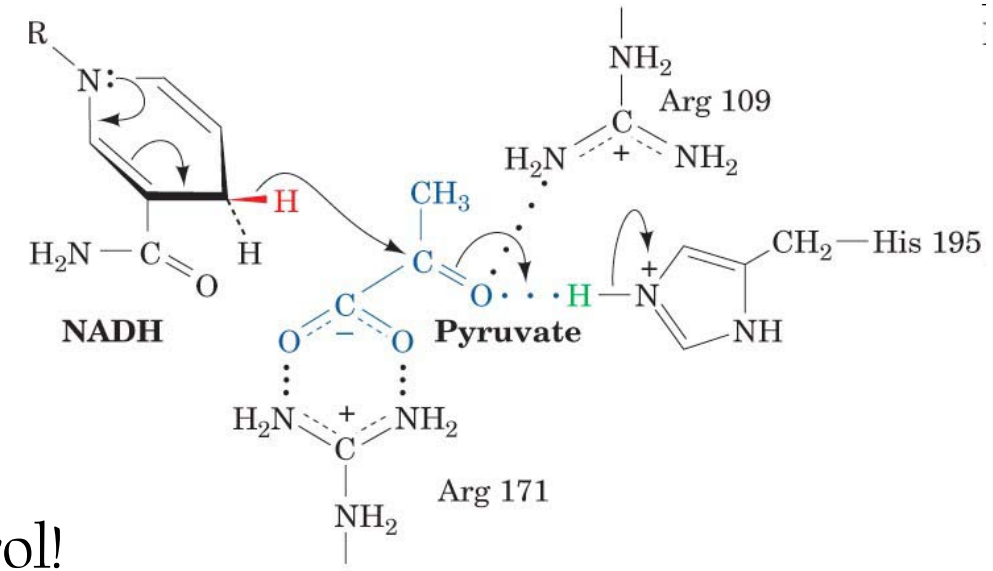

- And what do we do with this?

- Sure, as long as we have a way to get rid of this!

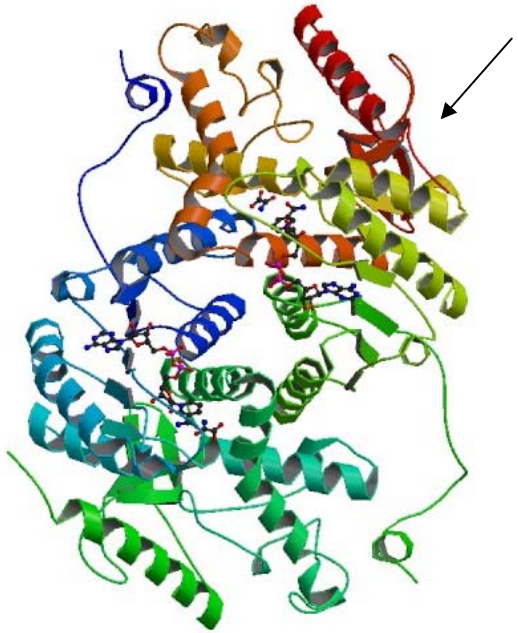
- Well, of we need to, we can oxidize NADH back to $NAD^+$ by reducing pyruvate to lactate

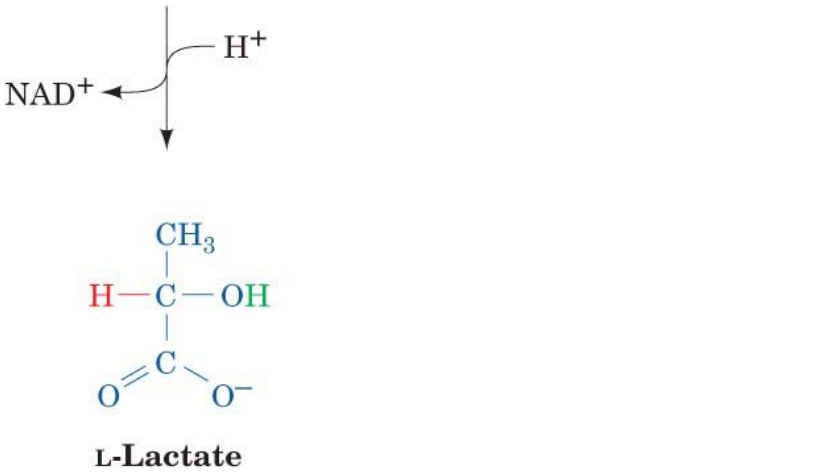
# Lactate dehydrogenase (Homolactic Fermentation)



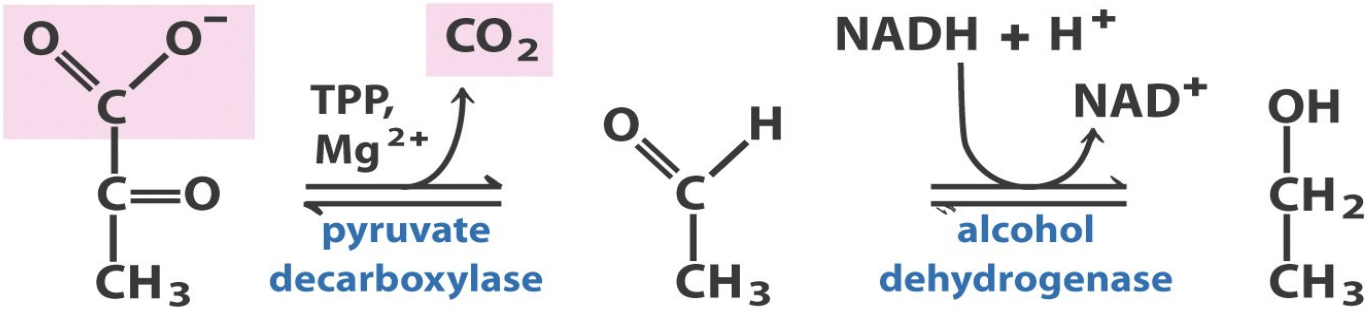



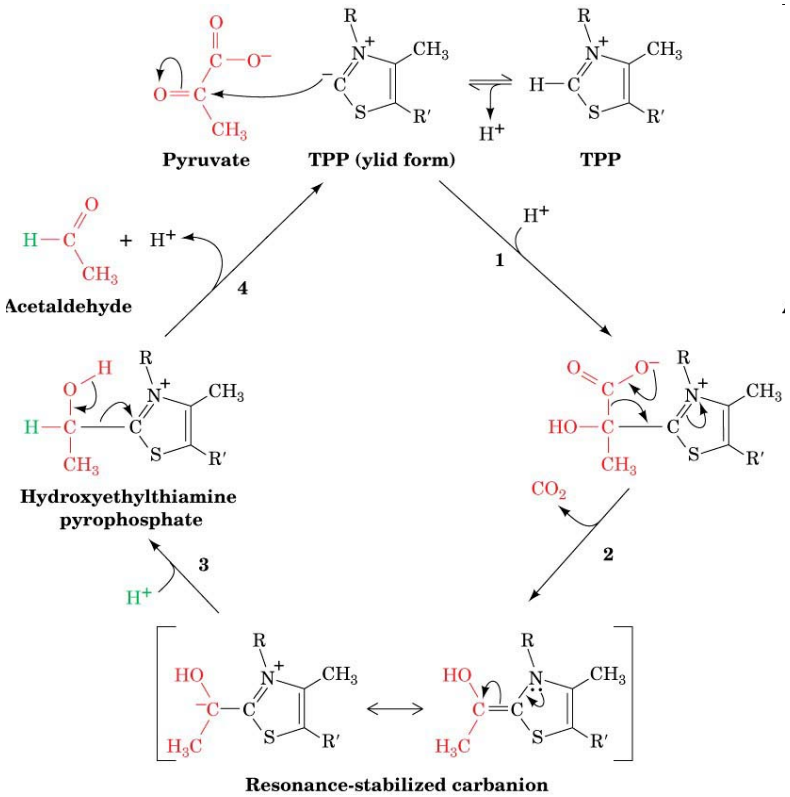

Isozyme Control!

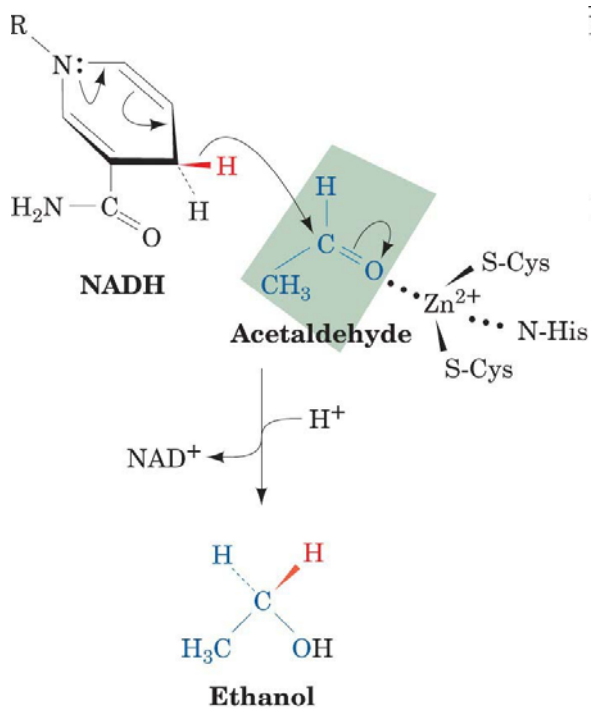




# Alcoholic Fermentation



# Summing up Glycolysis

Free energy change with conc.

Standard free energy change

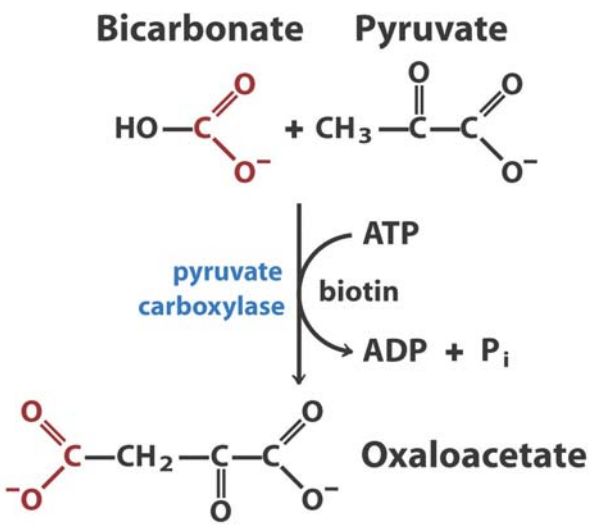
**TABLE 14–2** Free-Energy Changes of Glycolytic Reactions in Erythrocytes

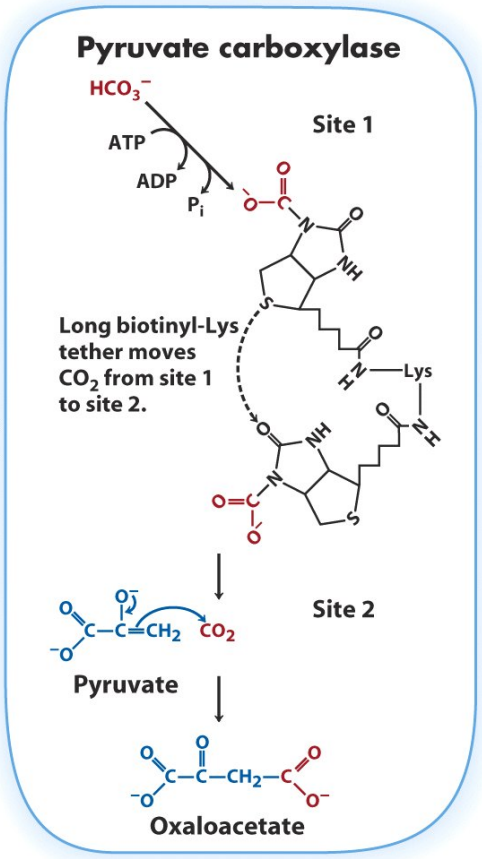
| *Glycolytic reaction step* | $\Delta G'^{\circ}$ *(kJ/mol)* | $\Delta G$ *(kJ/mol)* |
|---|---|---|
| (1) Glucose + ATP ⟶ glucose 6-phosphate + ADP | −16.7 | −33.4 |
| (2) Glucose 6-phosphate ⇌ fructose 6-phosphate | 1.7 | 0 to 25 |
| (3) Fructose 6-phosphate + ATP ⟶ fructose 1,6-bisphosphate + ADP | −14.2 | −22.2 |
| (4) Fructose 1,6-bisphosphate ⇌ dihydroxyacetone phosphate + glyceraldehyde 3-phosphate | 23.8 | 0 to −6 |
| (5) Dihydroxyacetone phosphate ⇌ glyceraldehyde 3-phosphate | 7.5 | 0 to 4 |
| (6) Glyceraldehyde 3-phosphate + $P_i$ + $NAD^+$ ⇌ 1,3-bisphosphoglycerate + NADH + $H^+$ | 6.3 | −2 to 2 |
| (7) 1,3-Bisphosphoglycerate + ADP ⇌ 3-phosphoglycerate + ATP | −18.8 | 0 to 2 |
| (8) 3-Phosphoglycerate ⇌ 2-phosphoglycerate | 4.4 | 0 to 0.8 |
| (9) 2-Phosphoglycerate ⇌ phosphoenolpyruvate + $H_2O$ | 7.5 | 0 to 3.3 |
| (10) Phosphoenolpyruvate + ADP ⟶ pyruvate + ATP | −31.4 | −16.7 |

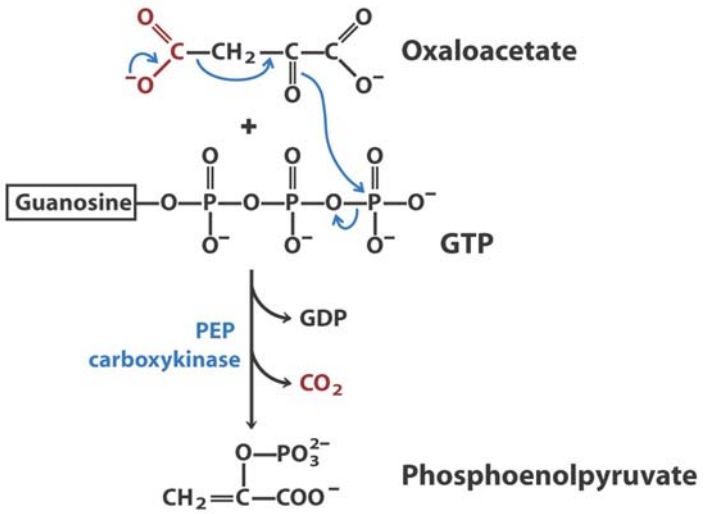
Note: $\Delta G'^{\circ}$ is the standard free-energy change, as defined in Chapter 13 (p. 491). $\Delta G$ is the free-energy change calculated from the actual concentrations of glycolytic intermediates present under physiological conditions in erythrocytes, at pH 7. The glycolytic reactions bypassed in gluconeogenesis are shown in red. Biochemical equations are not necessarily balanced for H or charge (p. 506).

# Gluconeogenesis

- Alternative pathway for last step of glycolysis




- This step occurs in mitochondria PEP then needs to be transported out

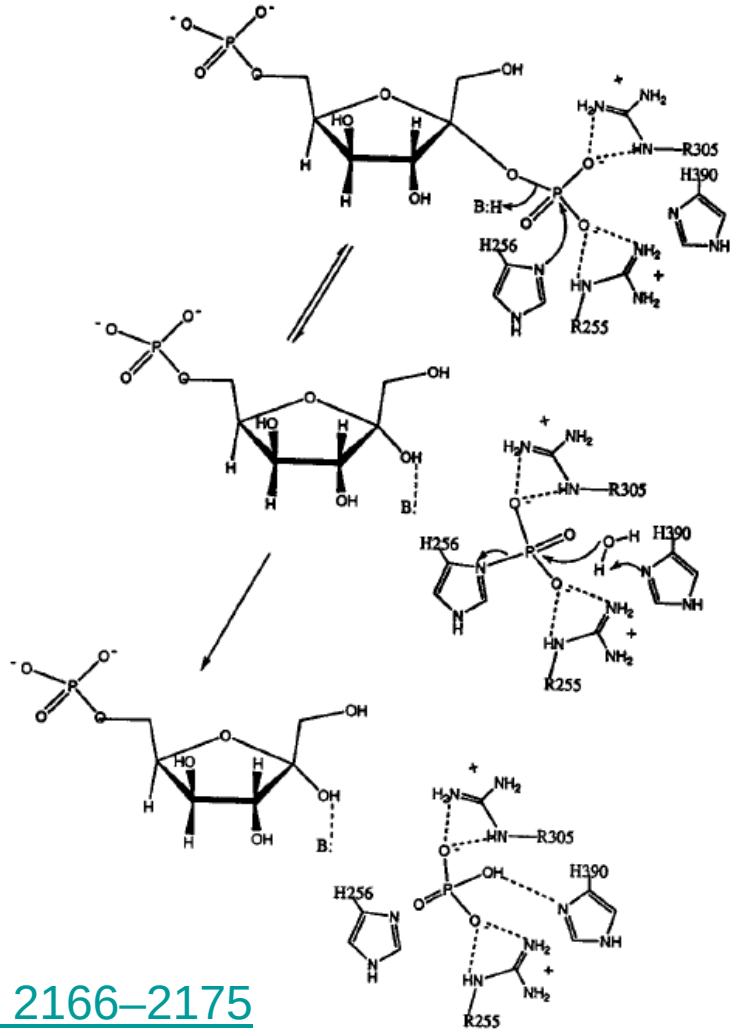
# Gluconeogenesis

- Bypassing step 3...

- Mediated by Fructose 1,6-bisphosphatase

Fructose 1,6-bisphosphate + H2O →
Fructose 6-phosphate + Pi

$\Delta G'^{o}$ = -16.3 kJ mol-1

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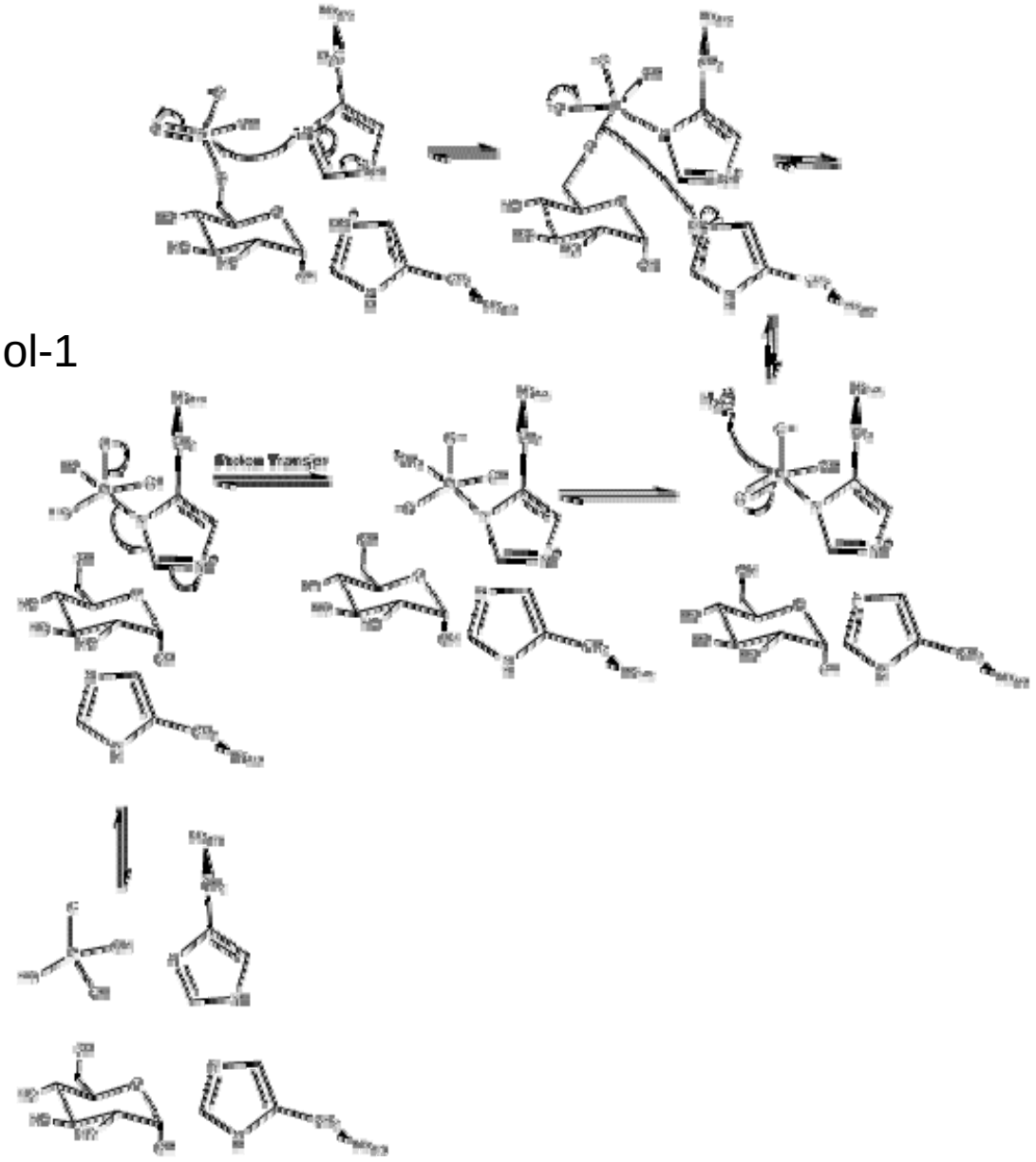
# Linearized Michaelis-Menten Kinetics

- Bypassing step 1...

Glucose 6-phosphate + H2O → glucose + Pi

$\Delta G'^{o}$ = -13.8 kJ mol-1

- Mediated by Glucose 6-phosphatase



# Gluconeogenesis Overall

Overall:

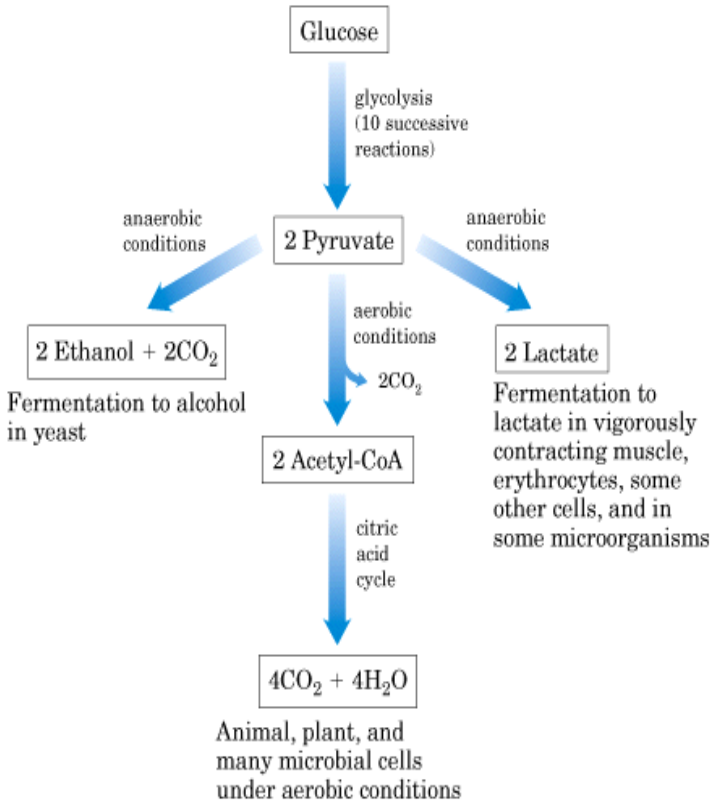
2 pyruvate + 4 ATP + 2 GTP + 2 NADPH + $2H^+$ + 4 $H_2O$ → 
Glucose + 4 ADP + 2 GDP + 6 $P_i$ + $2NAD^+$

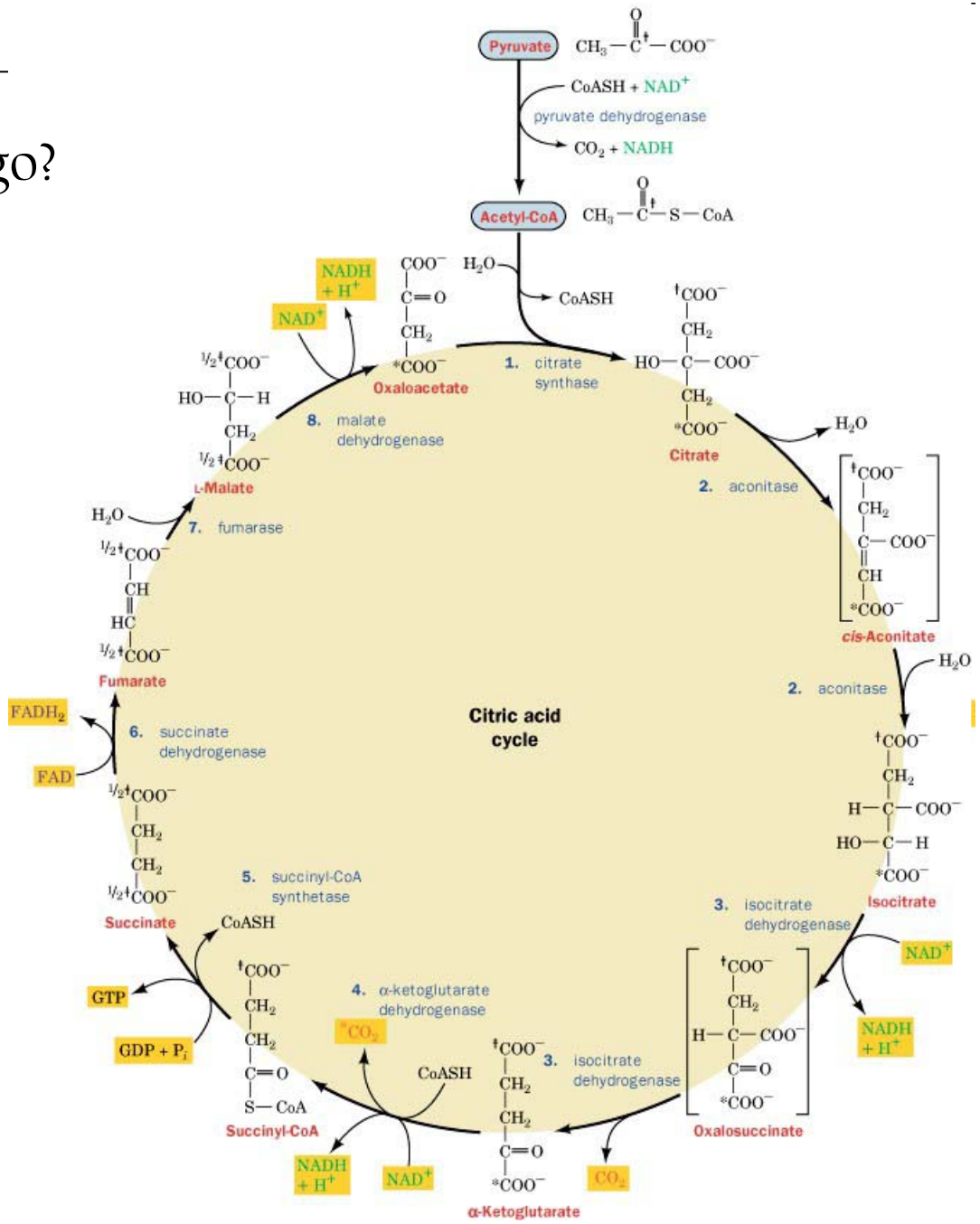
$\Delta G'^o = -16$ kJ $mol^{-1}$

- Gluconeogenesis happens mostly in the Liver when there's lots of ATP around.
- Excess sugar is stored as glycogen

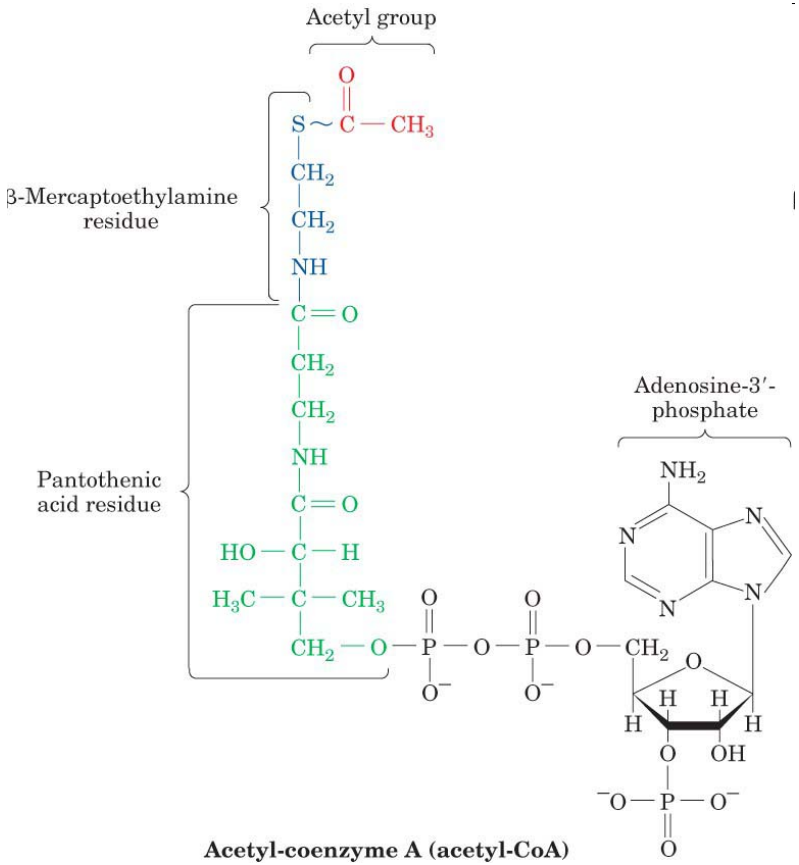
# The Citric Acid Cycle

- Where does the pyruvate go?



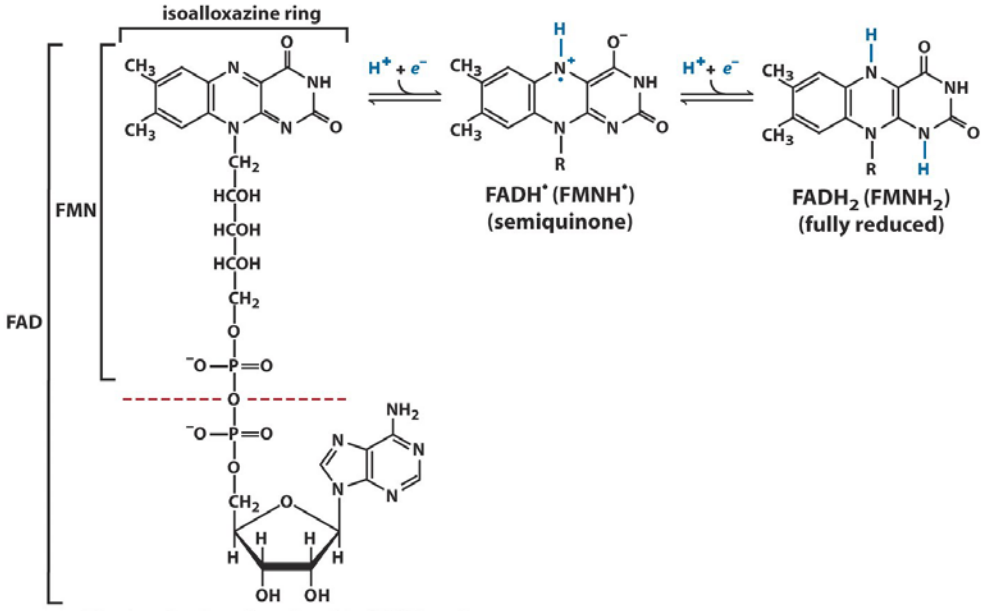



# Some New Playas!



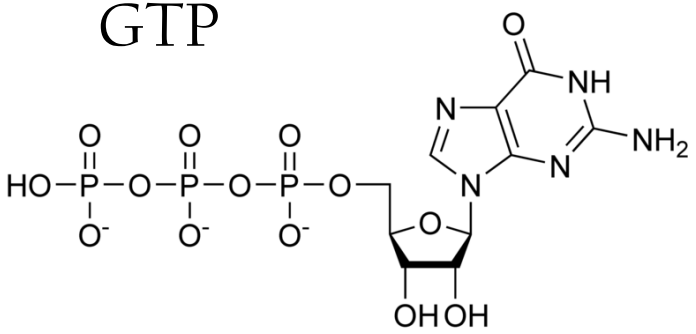
Acetyl-coenzyme A (acetyl-CoA)

## Flavin Adenine Dinucleotide

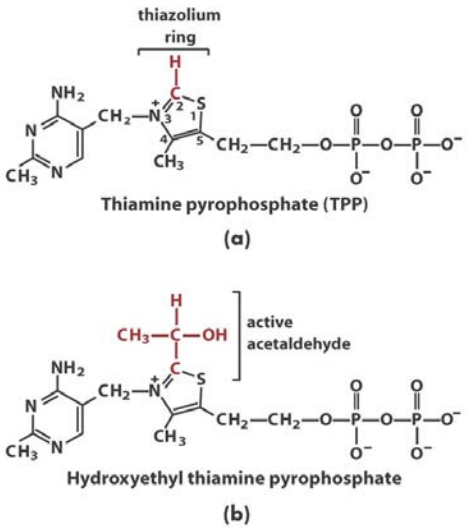


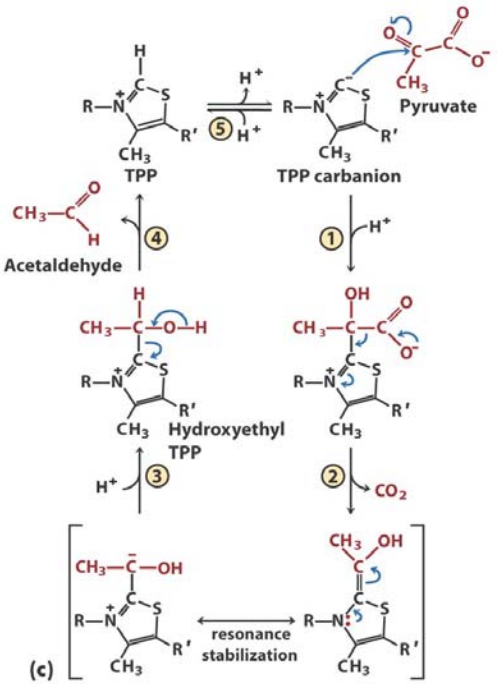
Flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN)

## GTP



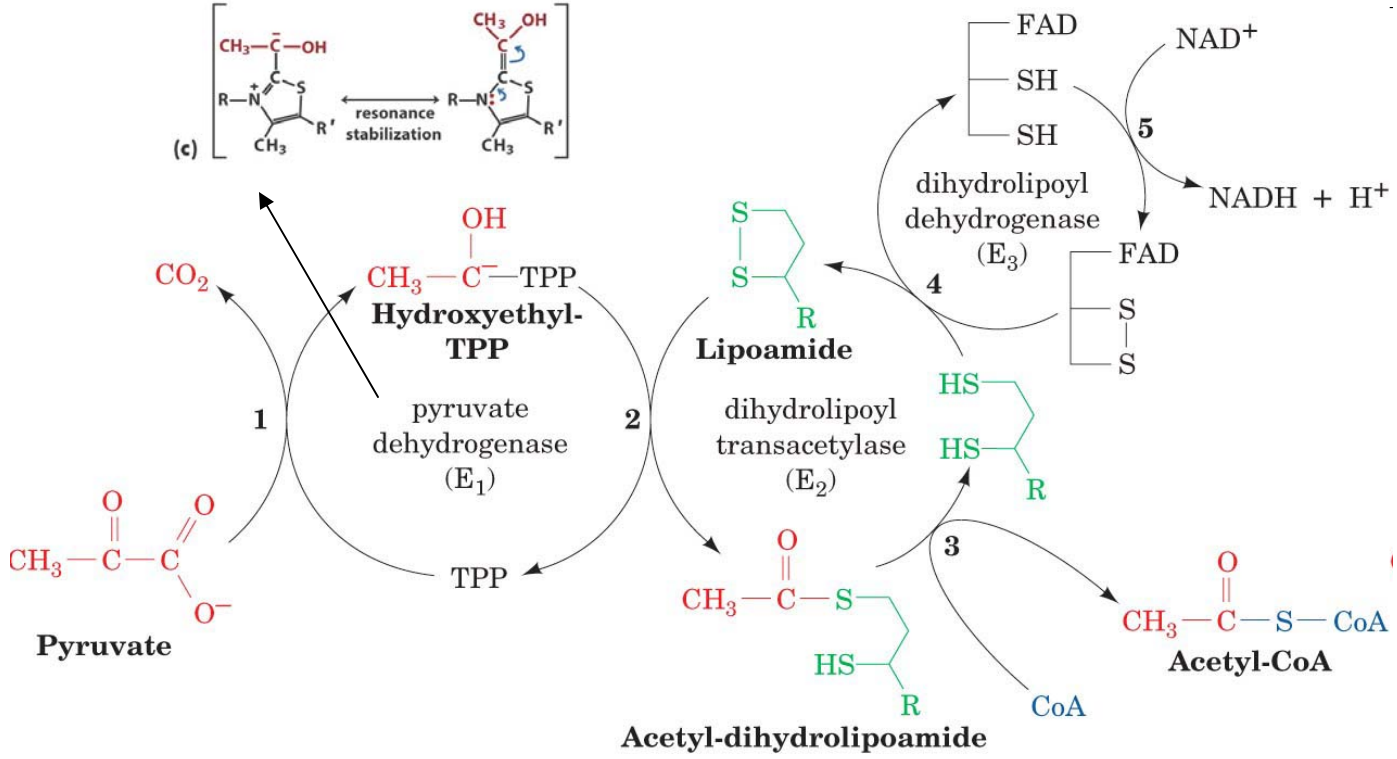
# Pyruvate Dehydrogenase (Multienzyme Complex)



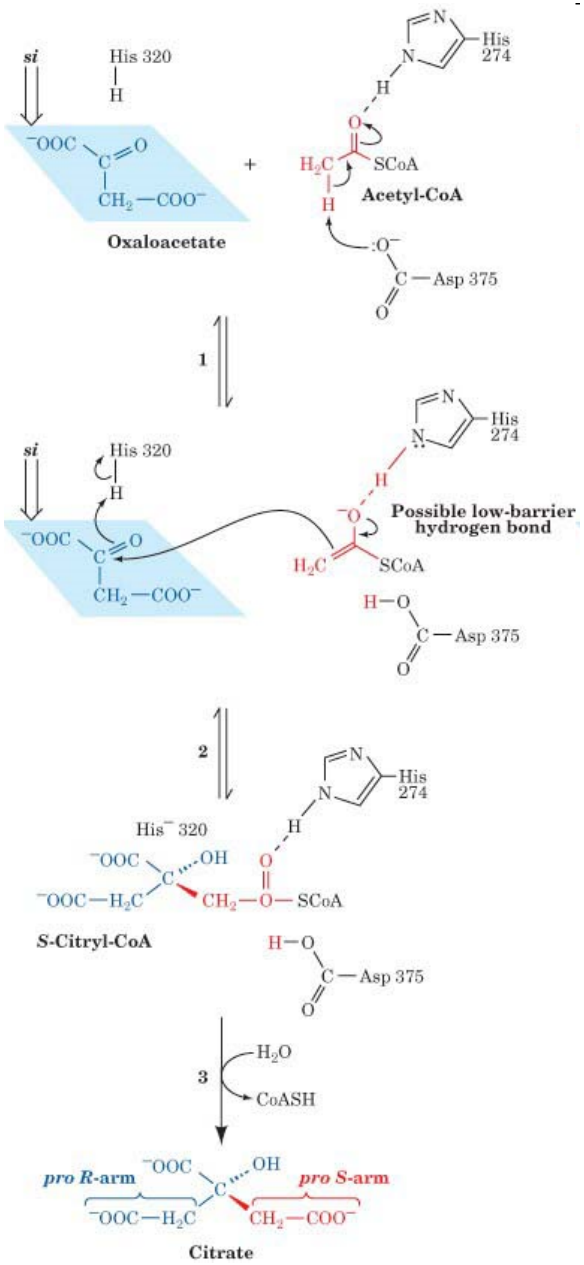




## Advantages of Complex:

- Product release close to next active site
- Coordinated Control



# Step 1: Making Citrate

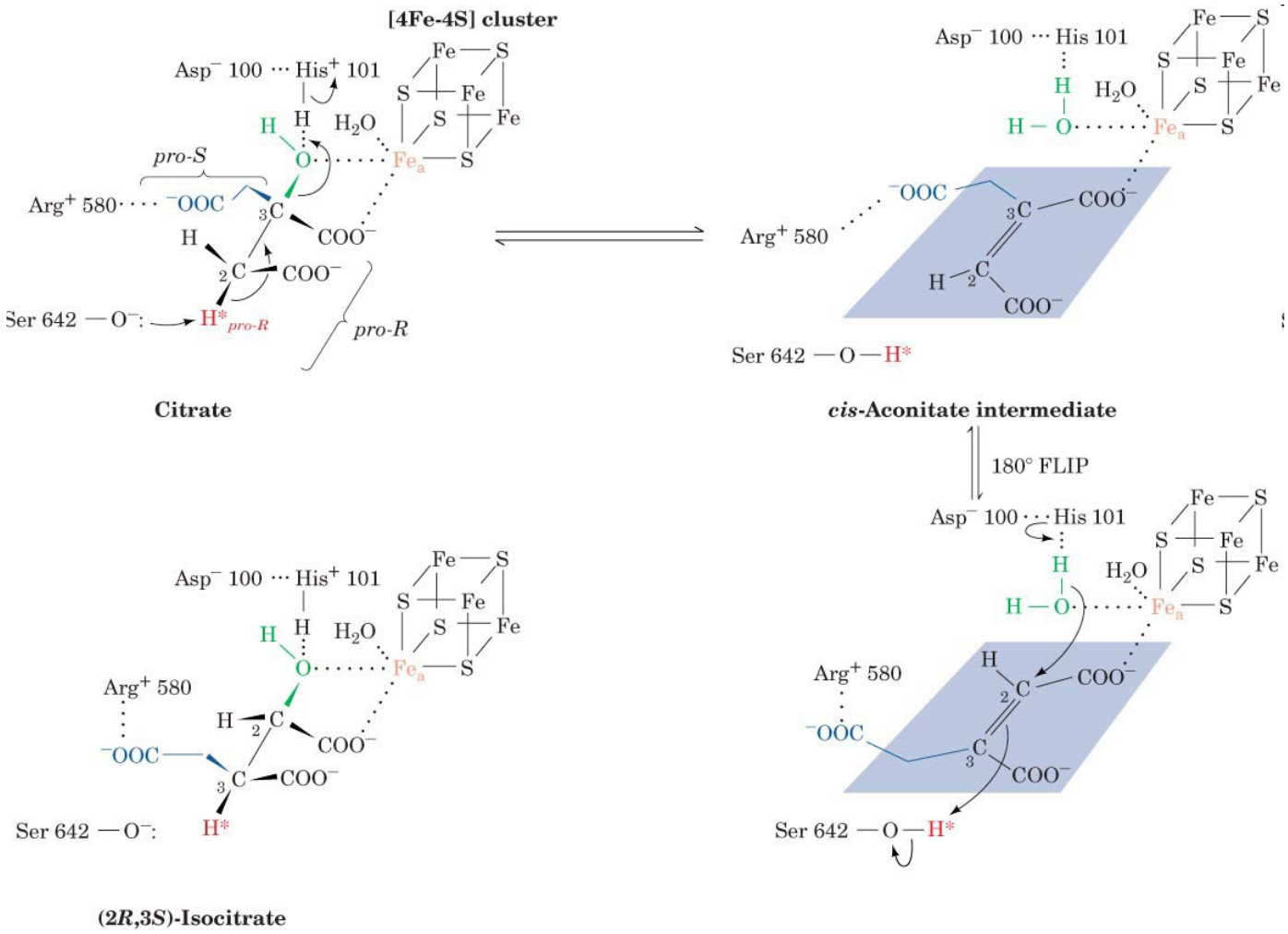



- Citrate is made from Acetyl CoA and Oxaloacetate

- Oxaoloacetate feeds in from the 'end' of the cycle

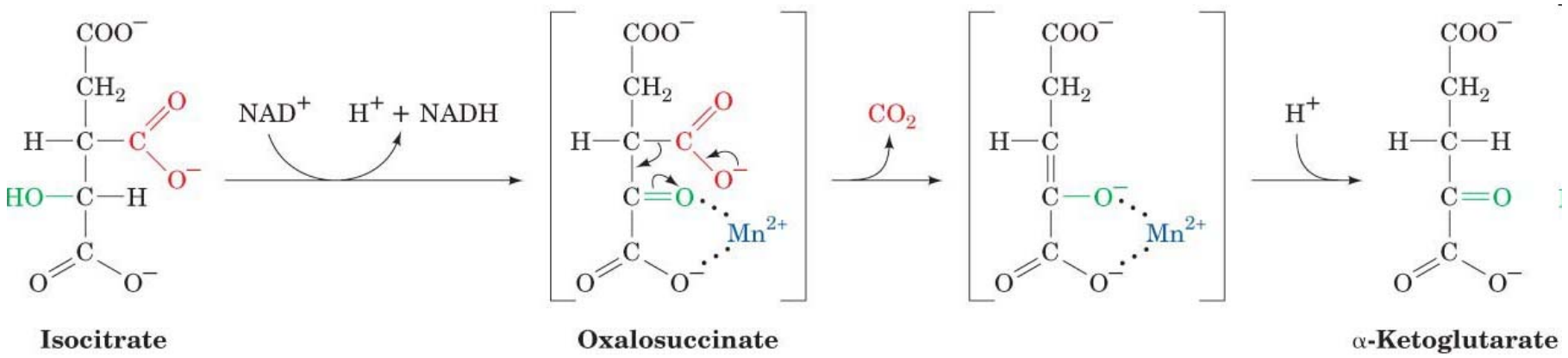
# Step 2: Making iso-Citrate

- This sets sets up reduction of $NAD^+$
- Uses a *catalytic* Iron / Sulphur cluster (4Fe-4S)



# Step 3: Making α-Ketoglutarate

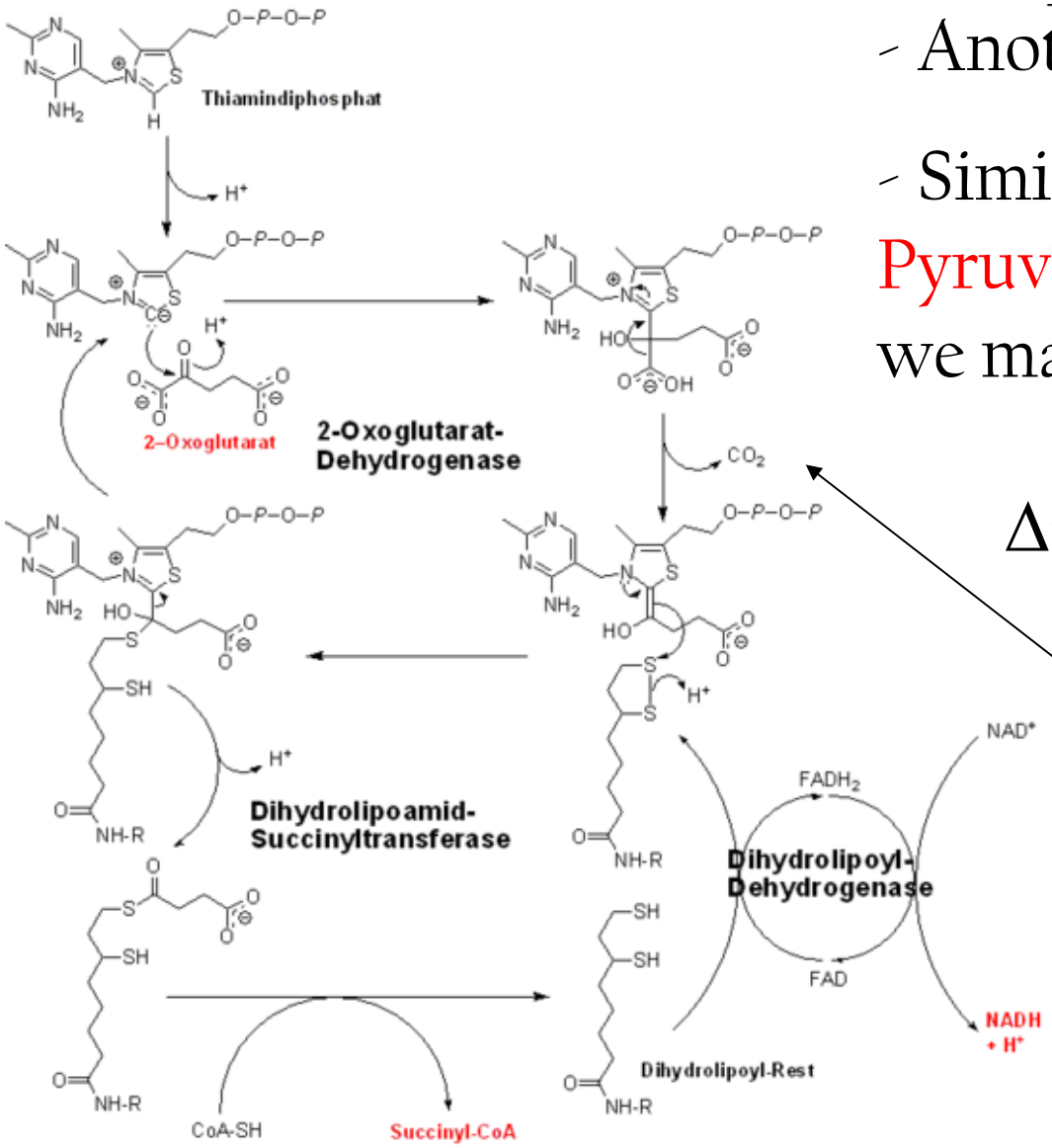
- Uses $NAD^+$ to oxidize isocitrate




- Detailed mechanism unknown

- Our second oxidative decarboxylation!

# Step 4: Making Succinyl CoA

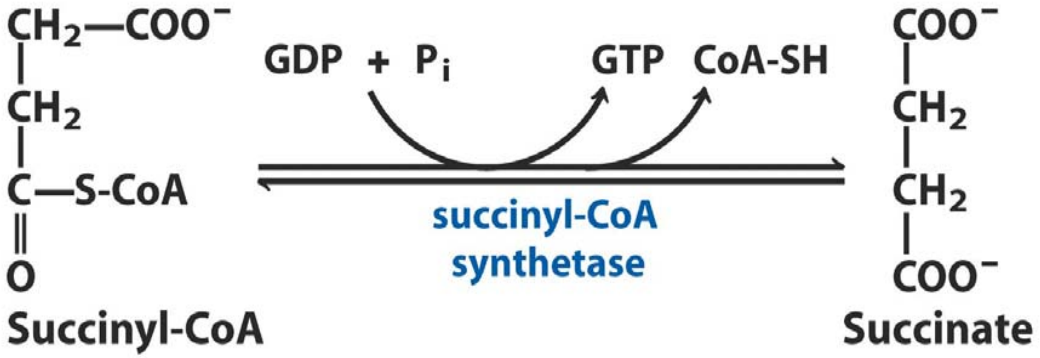



- Another enzyme complex
- Similar mechanism to Pyruvate Dehydrogenase when we made Acetyl CoA

$\Delta G'^{o} = -33.5$ kJ/mol!

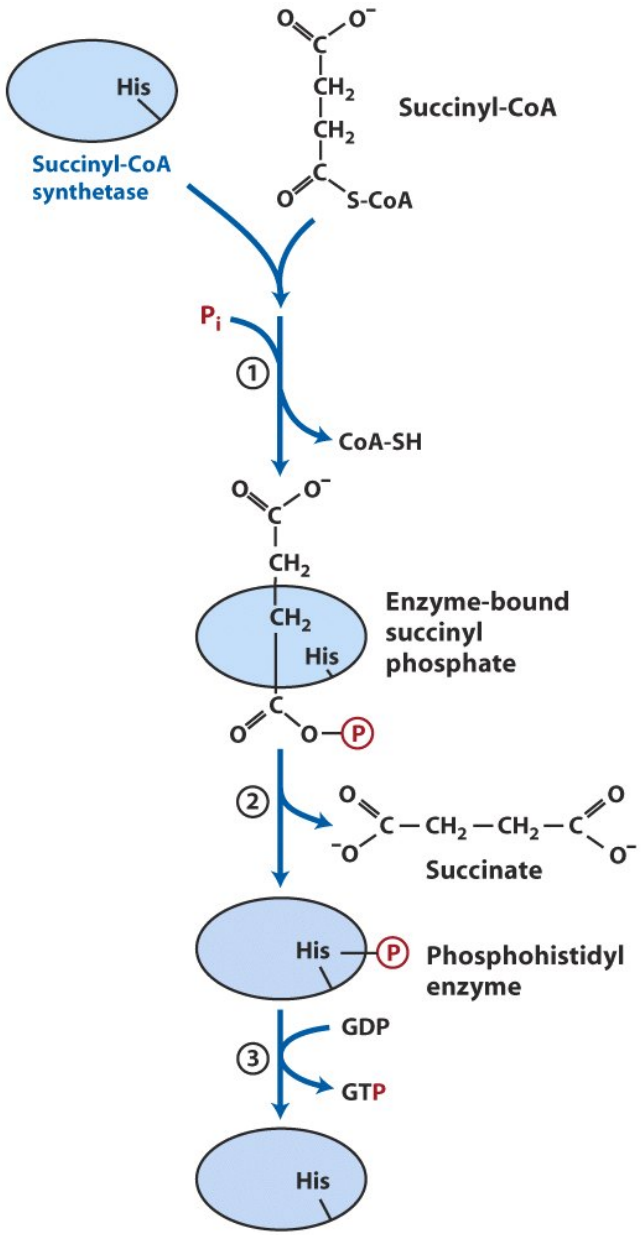
- Our third oxidative decarboxylation

# Step 5: Releasing Succinate



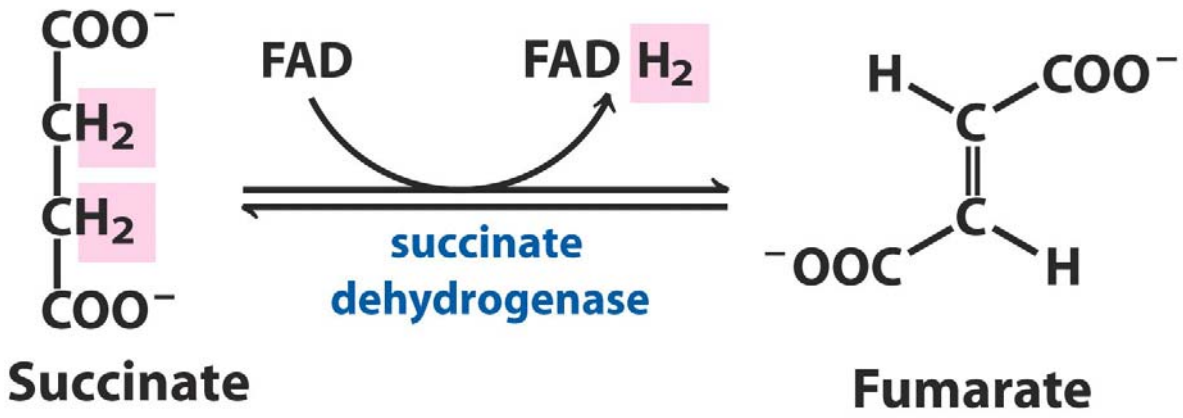

$\Delta G'^{\circ} = -2.9$ kJ/mol

- Transfer of high energy thioester to high energy phosphoester
- Mammals make GTP, plants make ATP
- GTP can be converted to ATP by nucleotide diphosphate kinase



# Step 6: Oxidation of Succinate to Fumarate

- We've done about all the oxidative decarboxylation we can handle. Time to start moving back towards oxaloacetate



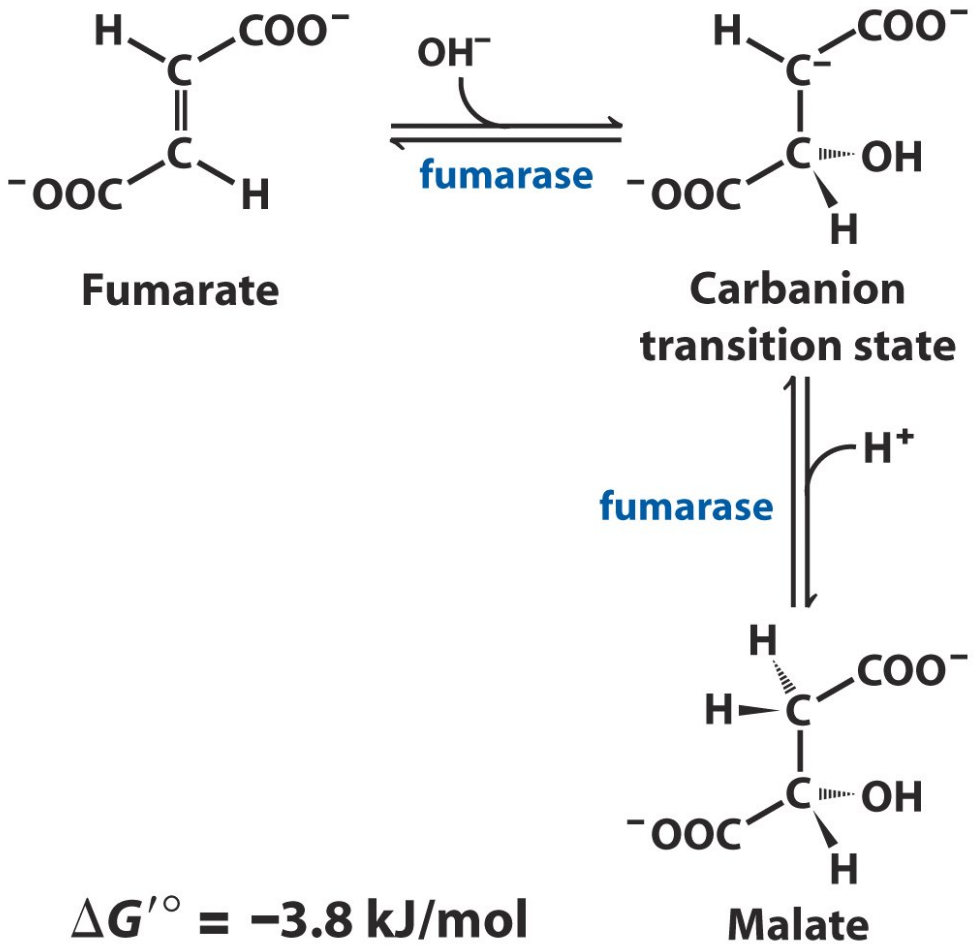

$\Delta G'^{\circ} = 0$ kJ/mol

- This step requires a strong oxidant

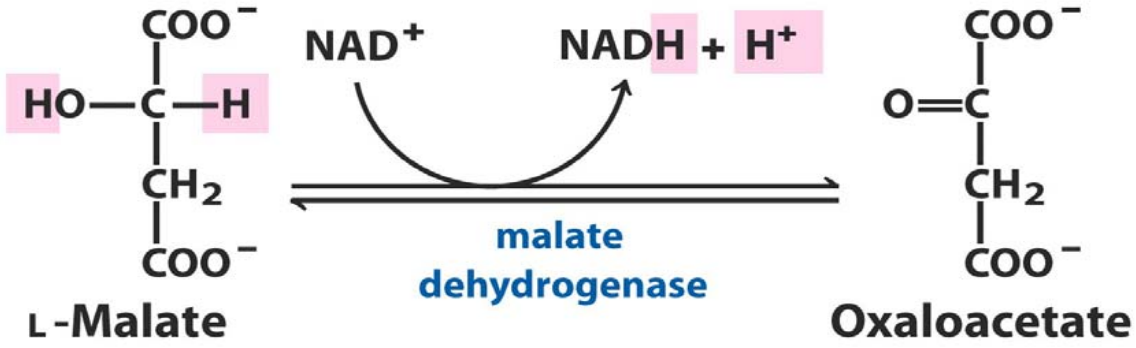
- This reaction is run in reverse for oxidative phosphorylation; FAD is covalently linked to the enzyme

# Step 7: Hydration of Fumarate to Malate

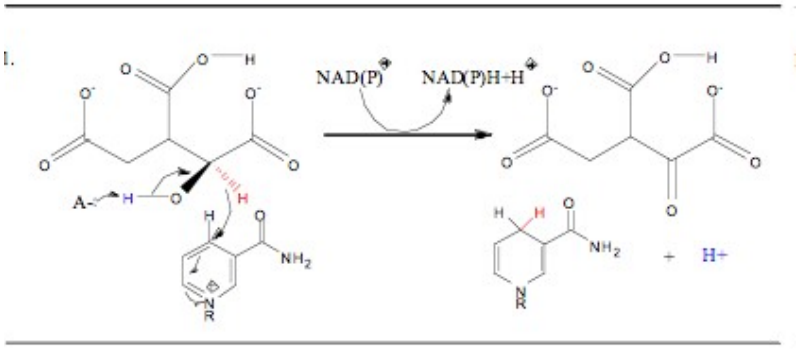
- Detailed mechanism unknown



# Step 8: Oxidation of Malate to Oxaloacetate




$\Delta G'^{\circ}$ = 29.7 kJ/mol




- Detailed mechanism unknown, though it must involve a hydride transfer to $NAD^+$ in a manner similar to the other dehydrogenases.

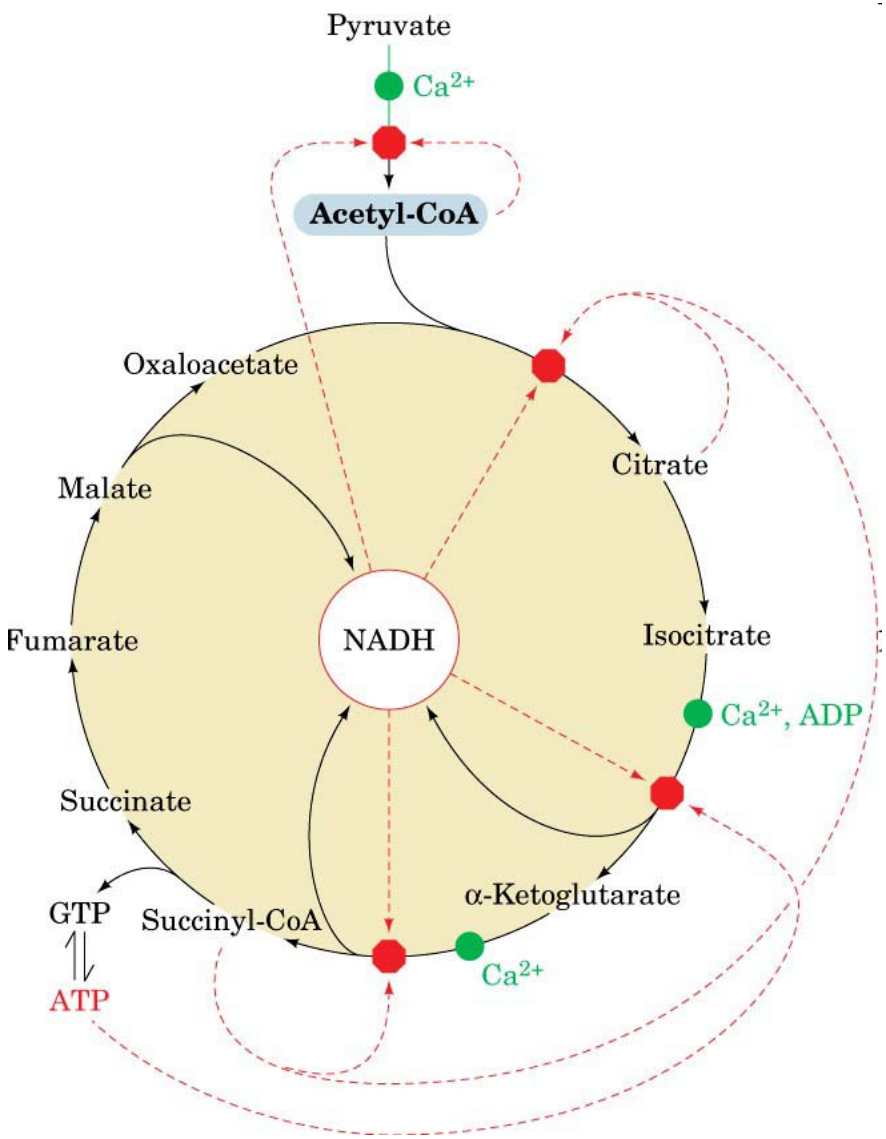
- This reaction is highly endergonic

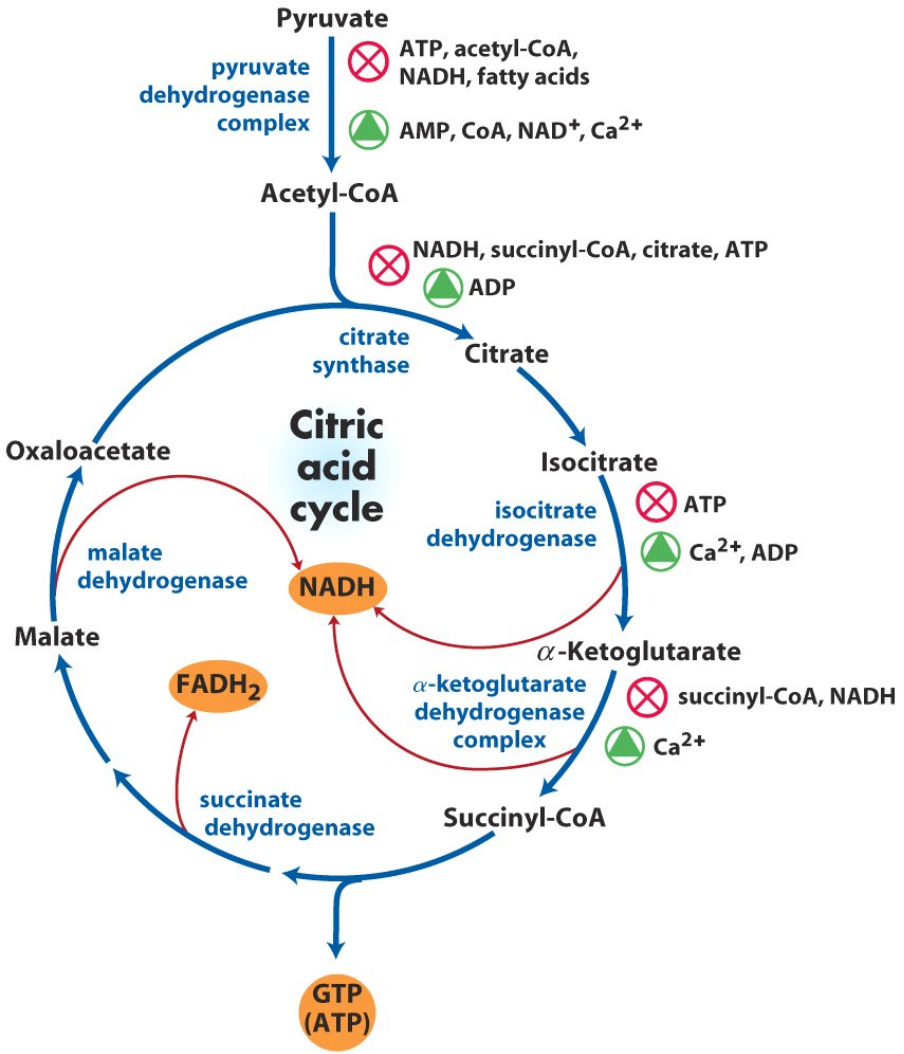
# Energetics of the Citric Acid cycle

| Reaction | ΔG°' | est. ΔG heart, liver | $K_{eq}$ |
|---|---|---|---|
| 1 (citrate synthase) | -31.4 | -53.9 | $3.2 \times 10^{5}$ |
| 2 (aconitase) | +6.7 | +0.8 | 0.0067 |
| 3 (isocitrate dehydrogenase) | -8.4 | -17.5 | 29.7 |
| 4 (αketoglutarate dehydrogenase) | -30 | -43.9 | $1.8 \times 10^{5}$ |
| 5 (succinyl CoA synthetase) | -3.3 | ~0 | 3.8 |
| 6 (succinate dehydrogenase) | +0.4 | ~0 | 0.85 |
| 7 (fumarase) | -3.8 | ~0 | 4.6 |
| 8 (malate dehydrogenase) | +29.7 | ~0 | $6.2 \times 10^{-6}$ |

- Note how many ΔG in vivo are close to zero. This should give an idea of the concentrations of intermediates

# Regulation of Citric Acid cycle

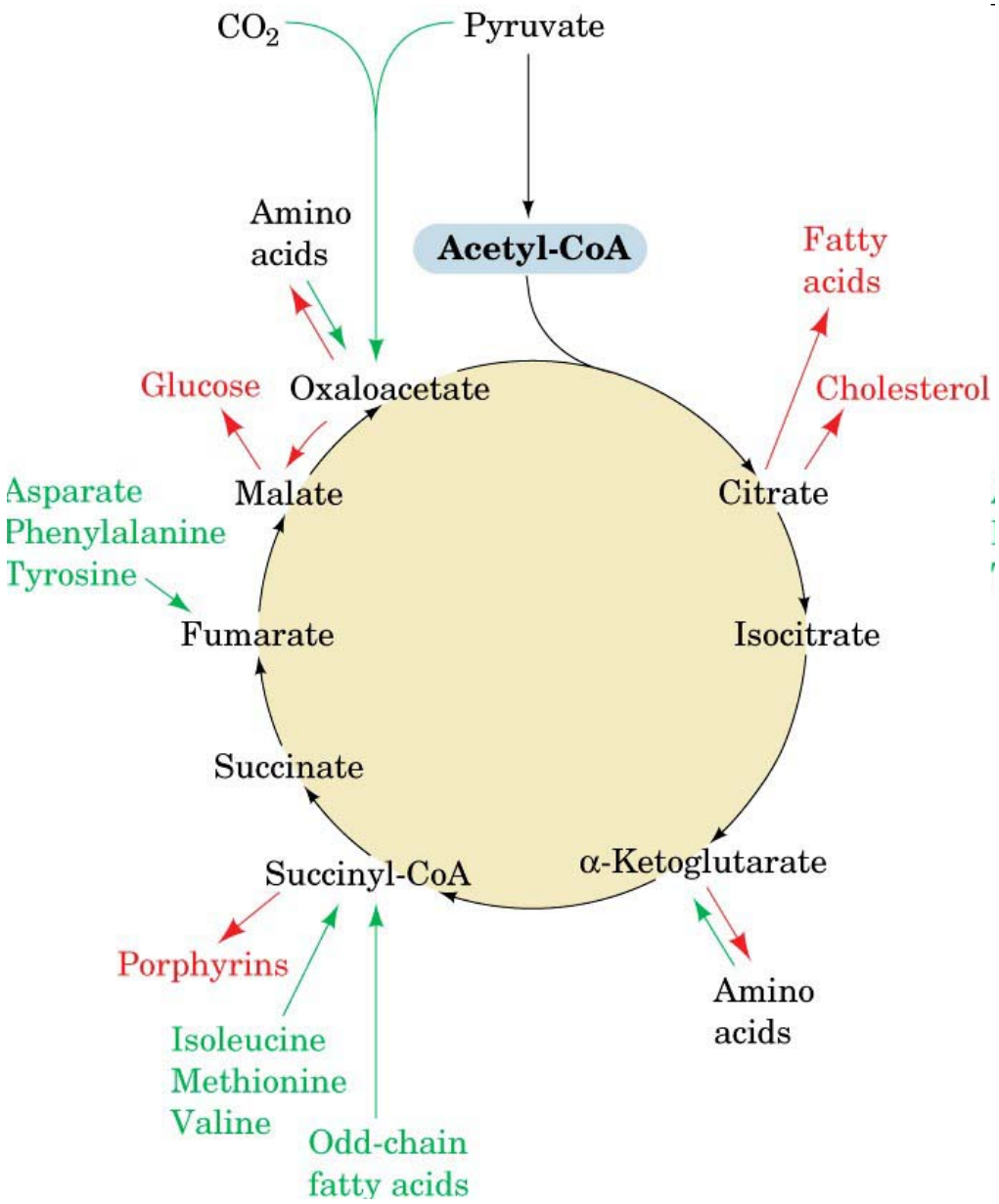


# Anapleroticity

- The citric acid cycle is both catabolic and anabolic

- Intermediates can feed in and out to keep their concentrations constant



# Linearized Michaelis-Menten Kinetics

# Linearized Michaelis-Menten Kinetics