Gluconeogenesis / TCA
11/12/2009

Gluconeogenesis

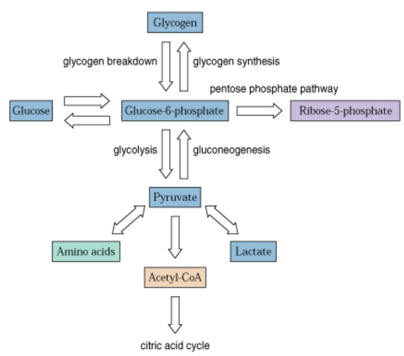
Gluconeogenesis is the process whereby precursors such as lactate, pyruvate, glycerol, and amino acids are converted to glucose.

Fasting requires all the glucose to be synthesized from these non-carbohydrate precursors.

Most precursors must enter the Krebs cycle at some point to be converted to oxaloacetate.

Oxaloacetate is the starting material for gluconeogenesis

Overview of Glucose Metabolism



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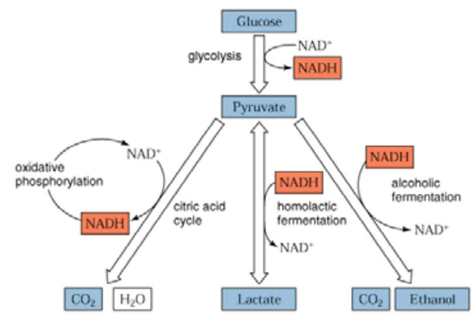
The metabolic fate of pyruvate



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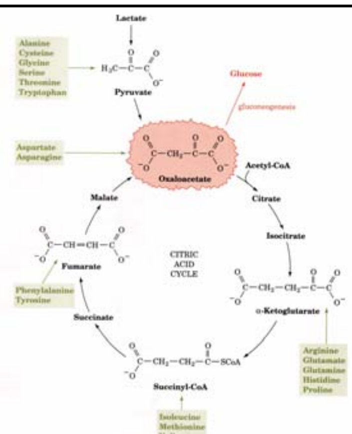


Free energy changes in glycolysis

Reaction	enzyme	ΔG°′	ΔG°
1	Hexokinase	-20.9	-27.2
2	PGI	+2.2	-1.4
3	PFK	-17.2	-25.9
4	Aldolase	+22.8	-5.9
5	TIM	+7.9	+4.4
6+7	GAPDH+PGK	-16.7	-1.1
8	PGM	+4.7	-0.6
9	Enolase	-3.2	-2.4
10	PK	-23.3	-13.9

Gluconeogenesis is not just the reverse of glycolysis

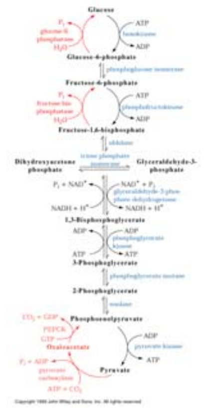
Several steps are different so that control of one pathway does not inactivate the other. However many steps are the same. Three steps are different from glycolysis.

1 Pyruvate to PEP

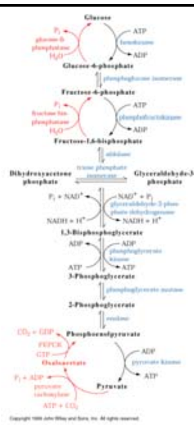
2 Fructose 1,6- bisphosphate to Fructose-6-phosphate

3 Glucose-6-Phosphate to Glucose

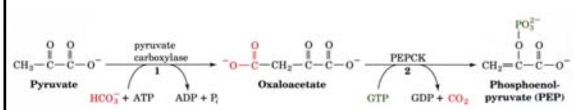
Gluconeogenesis versus Glycolysis



Gluconeogenesis versus Glycolysis



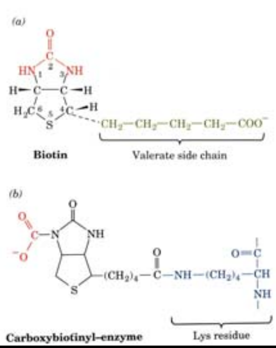
Pyruvate is converted to oxaloacetate before being changed to Phosphoenolpyruvate



1. Pyruvate carboxylase catalyses the ATP-driven formation of oxaloacetate from pyruvate and CO_2

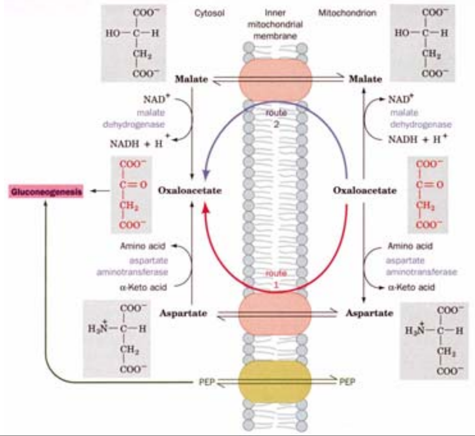
2. PEP carboxykinase (PEPCK) concerts oxaloacetate to PEP that uses GTP as a phosphorylating agent.

Pyruvate carboxylase requires biotin as a cofactor



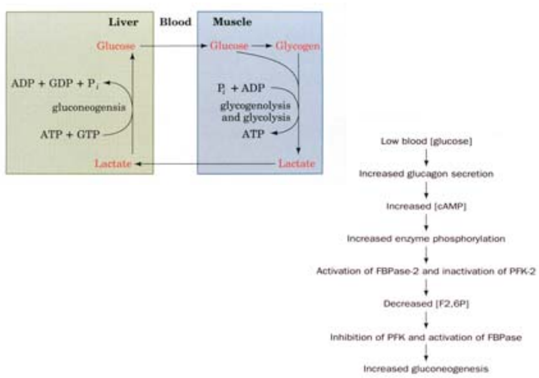
Acetyl-CoA regulates pyruvate carboxylase

Increases in oxaloacetate concentrations increase the activity of the Krebs cycle and acetyl-CoA is a allosteric activator of the carboxylase. However when ATP and NADH concentrations are high and the Krebs cycle is inhibited, oxaloacetate goes to glucose.



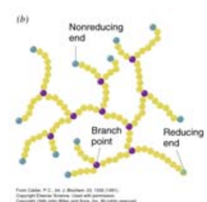
Regulators of gluconeogenic enzyme activity

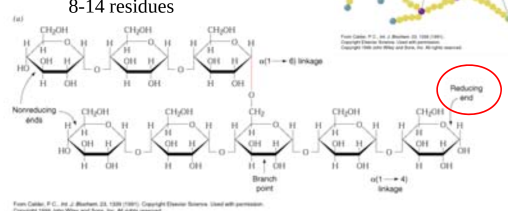
Enzyme	Allosteric Inhibitors	Allosteric Activators	Enzyme Phosphorylation	Protein Synthesis
PFK	ATP, citrate	AMP, F2-6P		
FBPase	AMP, F2-6P			
PK	Alanine	F1-6P	Inactivates	
Pyr. Carb.		AcetylCoA		
PEPCK				Glucogon
PFK-2	Citrate	AMP, F6P, Pi	Inactivates	
FBPase-2	F6P	Glycerol-3-P	Activates	



Glycogen Storage

- Glycogen is a D-glucose polymer
- α(1→4) linkages
- α(1→6) linked branches every
  8-14 residues





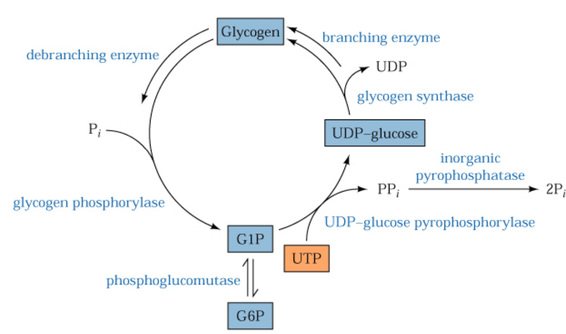
Glycogen Breakdown or Glycogenolysis

- Three steps
  - Glycogen phosphorylase

    Glycogen + Pi <-> glycogen + G1P
    (n residues) (n-1 residues)

  - Glycogen debranching
  - Phosphoglucomutase

Glycogen Syntheisis



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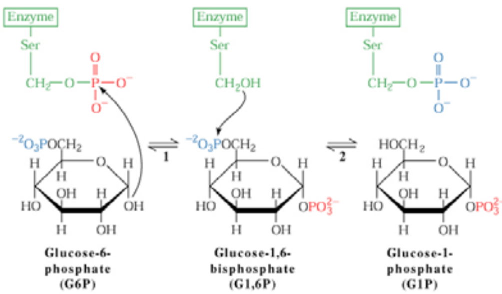
Phosphoglucomutase



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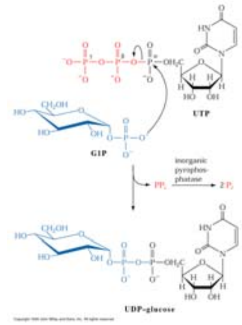
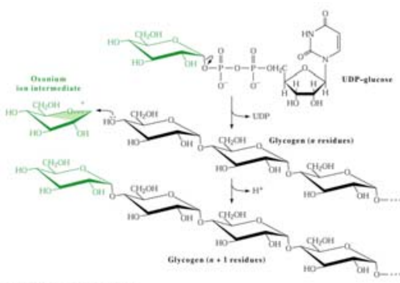
UDP-glucose Pyrophorylase



Glycogen Synthase



The Citric acid cycle

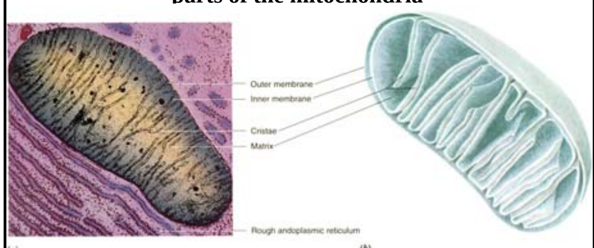
It is called the Krebs cycle or the tricarboxylic and is the "hub" of the metabolic system. It accounts for the majority of carbohydrate, fatty acid and amino acid oxidation. It also accounts for a majority of the generation of these compounds and others as well.

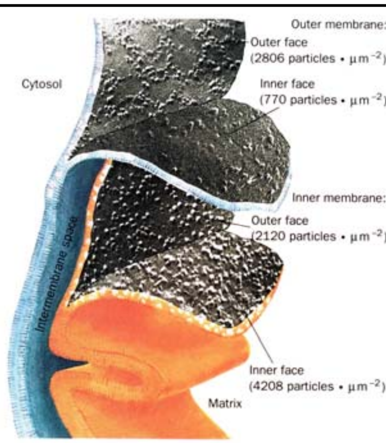
Amphibolic - acts both catabolically and anabolically

$$3NAD^+ + FAD + GDP + Pi + acetyl\text{-}CoA \longrightarrow 3NADH + FADH_2 + GTP + CoA + 2CO_2$$

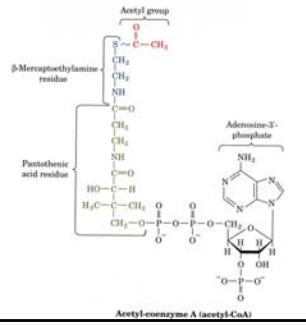
The citric acid cycle enzymes are found in the matrix of the mitochondria

Substrates have to flow across the outer and inner parts of the mitochondria





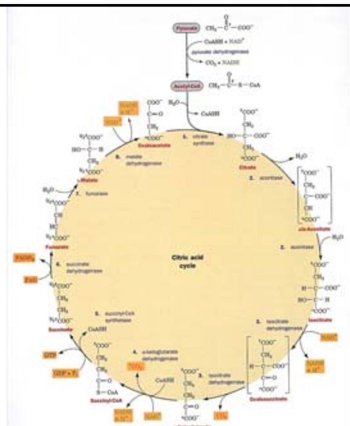
Nathan Kaplan and Fritz Lipmann discovered Coenzyme A and Ochoa and Lynen showed that acetyl-CoA was intermediate from pyruvate to citrate.



CoA acts as a carrier of acetyl groups

Acetyl-CoA is a "high energy" compound: The ΔG°' for the hydrolysis of its thioester is -31.5 kJ• mol^{-1} making it greater than the hydrolysis of ATP

Pyruvate dehydrogenase converts pyruvate to acetyl-CoA and CO_2

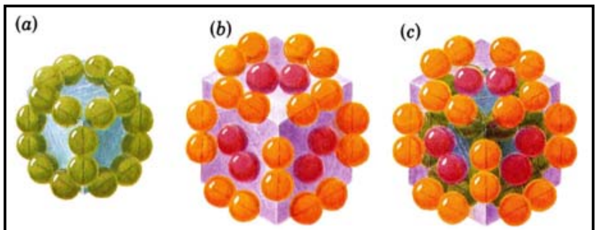


Pyruvate dehydrogenase

A multienzyme complexes are groups of non covalently associated enzymes that catalyze two or more sequential steps in a metabolic pathway.

Molecular weight of 4,600,000 Da

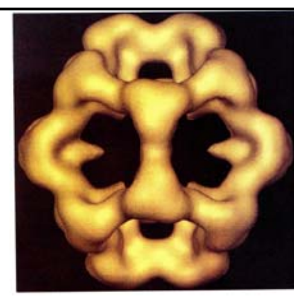
		E. coli	yeast
Pyruvate dehydrogenase --	**E1**	**24**	**60**
dihydrolipoyl transacetylase --	**E2**	**24**	**60**
dihydrolipoyl dehydrogenase--	**E3**	**12**	**12**



24 E2 subunits

24 E1 orange
12 E3 Red

a and b together



EM based image of the core E2 from yeast pyruvate dh

60 subunits associated as 20 cone-shaped trimers that are verticies of a dodecahedron

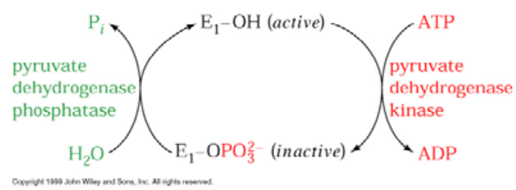
Why such a complex set of enzymes?

1 Enzymatic reactions rates are limited by diffusion, with shorter distance between subunits a enzyme can almost direct the substrate from one subunit (catalytic site) to another.

2. Channeling metabolic intermediates between successive enzymes minimizes side reactions

3. The reactions of a multienzyme complex can be coordinately controlled

Covalent modification of eukaryotic pyruvate dehydrogenase



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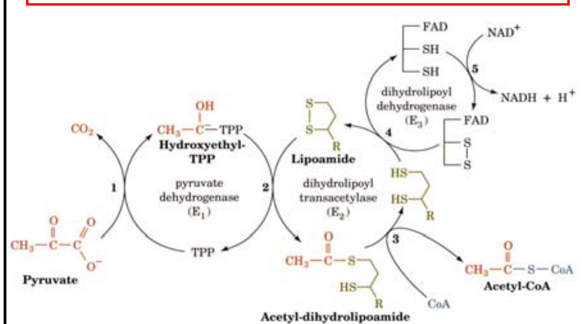
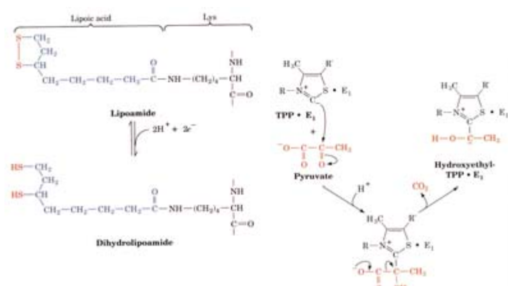
The five reactions of the pyruvate dehydrogenase multi enzyme complex



The enzyme requires five coenzymes and five reactions

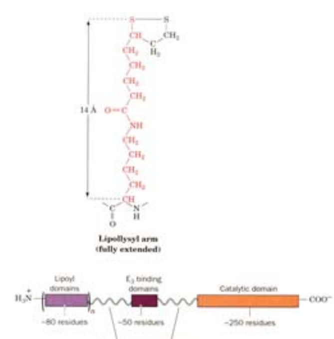
Pyruvate + CoA + NAD^+ ⟶ acetyl-CoA + CO_2 + NADH



The Coenzymes and prosthetic groups of pyruvate dehydrogenase

Cofactor	Location	Function
Thiamine pyrophosphate	Bound to E1	Decarboxylates pyruvate
Lipoic acid	Covalently linked to a Lys on E2 (lipoamide)	Accepts hydroxyethyl carbanion from TPP
CoenzymeA	Substrate for E2	Accepts acetyl group from lipoamide
FAD (flavin)	Bound to E3	reduced by lipoamide
NAD^+	Substrate for E3	reduced by FADH2

Domain structure of dihydrolipoyl transacetylase E2



Pyruvate dehydrogenase

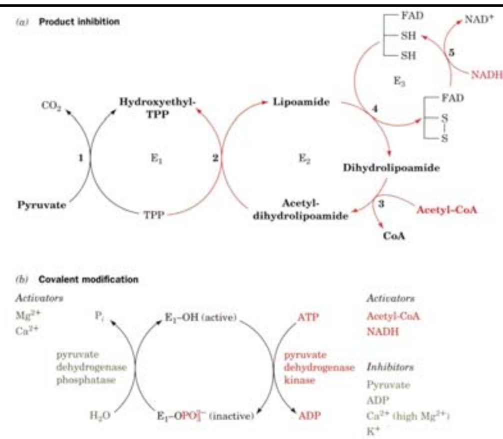
1. Pyruvate dh decarboxylates pyruvate using a TPP cofactor forming hydroxyethyl-TPP.

2 The hydroxyethyl group is transferred to the oxidized lipoamide on E2 to form Acetyl dihydrolipoamide-E2

3 E2 catalyzes the transfer of the acetyl groups to CoA yielding acetyl-CoA and reduced dihydrolipoamide-E2

4 Dihydrolipoyl dh E3 reoxidizes dihydrolipoamide-E2 and itself becomes reduced as $FADH_2$ is formed

5 Reduced E3 is reoxidized by NAD^+ to form FAD and NADH The enzymes SH groups are reoxidized by the FAD and the electrons are transferred to NADH



Next Lecture
Tuesday 11/17/09
Citric Acid Cycle