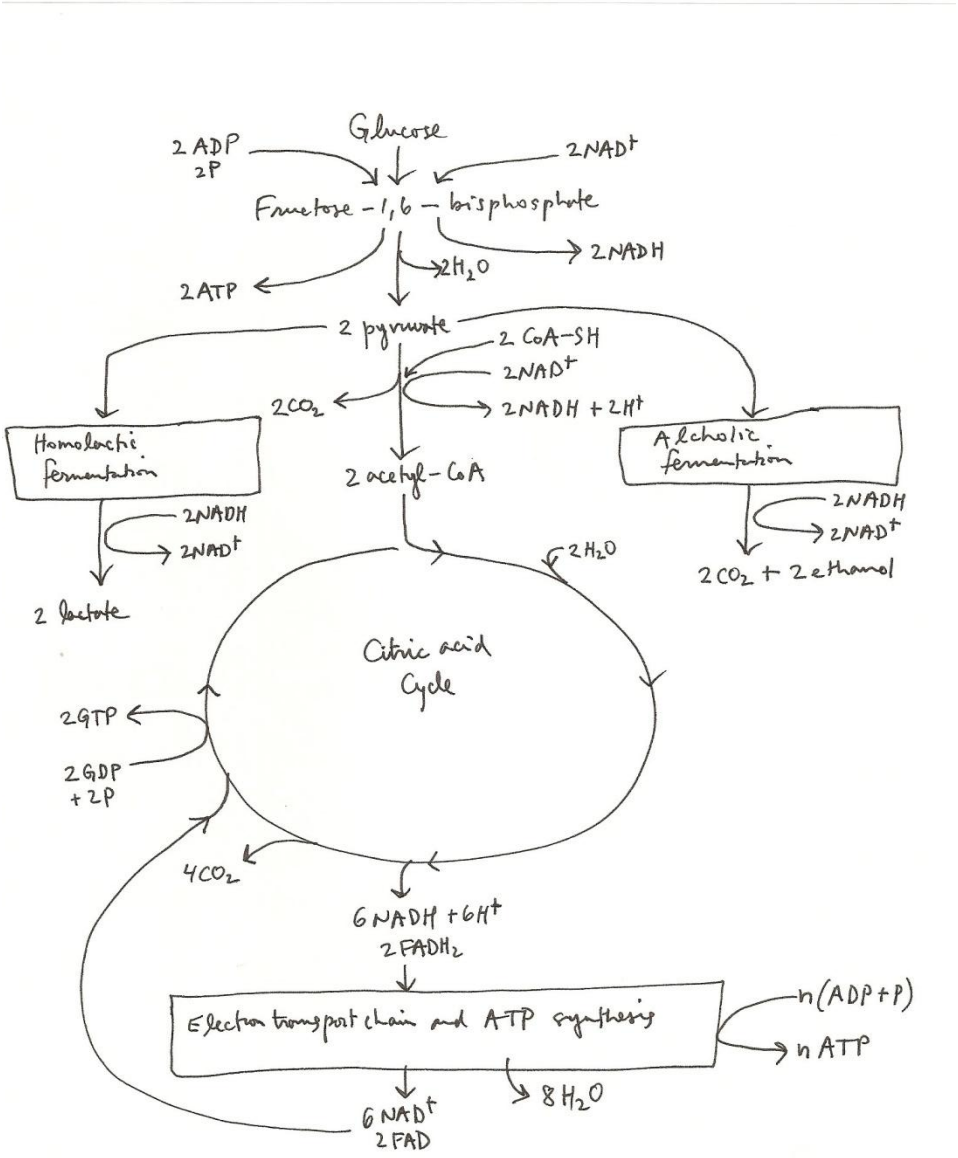
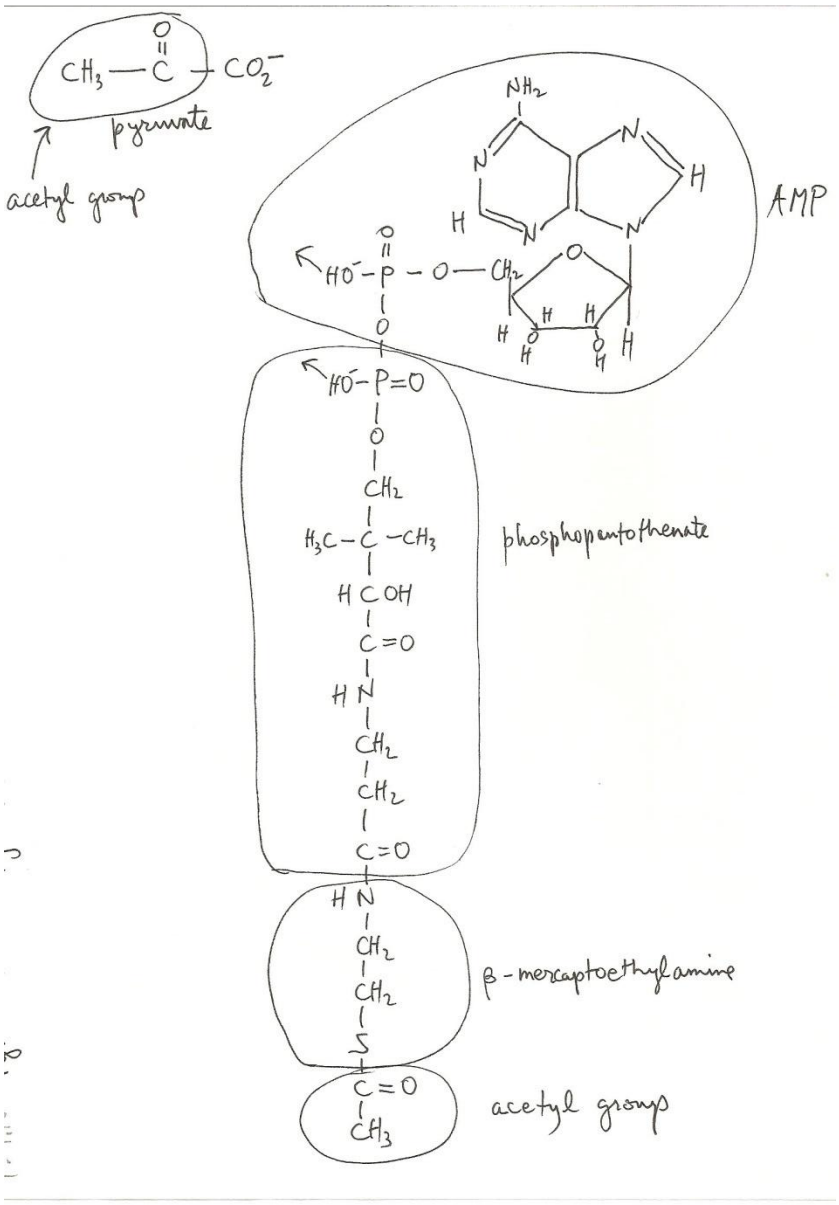
Energy metabolism overview

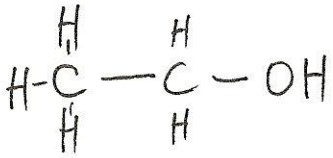
In the figure below, a general outline of energy metabolism is given



There are four distinct segments: glycolysis, conversion of *pyruvate* to *acetyl-CoA*

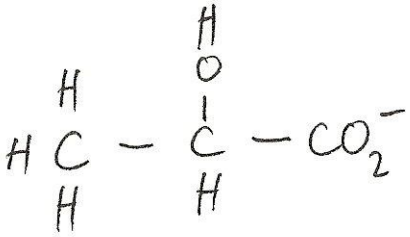


the citric acid cycle, and oxidative phosphorylation driven by the electron transport chain. In some simple organisms, pyruvate is converted to *ethanol*



ethanol

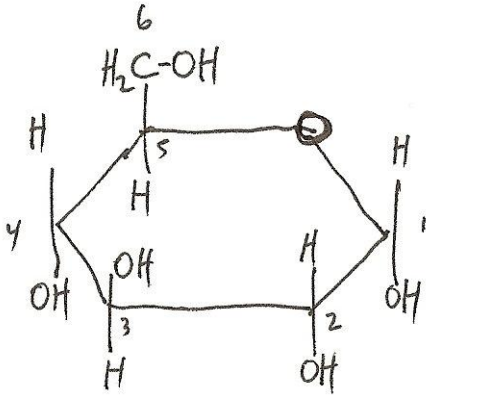
and glycolysis is the only source for generation of ATP. In muscle, this can also be the case but with the conversion of pyruvate to *lactate* instead.



lactate

Remarkably, in muscle, glycolysis is a rapid way to generate ATP compared to the citric acid cycle and electron transport generation of ATP. Thus in situations of heavy need, glycolysis does the job, with the help of ATP regeneration from a *phosphagen*, the molecule *phosphocreatine* that is stored for this purpose. During rest, the entire ATP generation pathway with the citric acid cycle and the electron transport chain is used to generate ATP and to recharge the phosphagen stores. Glycolysis produces a net of 2 ATPs per glucose molecule whereas the electron transport chain accounts for 32 ATPs per glucose. The details of these processes are given below.

The overall result of glycolysis, a ten step reaction pathway, is the conversion of glucose



glucose $(CH_2O)_6$

α-D – glucopyranose

to pyruvate

$$glu\cos e + 2NAD^+ + 2ADP + 2P \rightarrow$$

$$2\,pyruvate + 2NADH + 2ATP + 2H_2O + 2H^+$$

The standard state Gibbs free energy change at pH 7 is $\Delta G^{0'} = -8.5 kcal/mol$. However, inclusion of the actual physiological concentrations of reactants yields $\Delta G = -18.5 kcal/mol$. In going from glucose to 2 pyruvates, 4 hydrogens and 2 H^+s have been lost. Two hydrogens end up on 2 NADH's, as do 4 electrons leaving 4 H^+s. The remaining 2 H^+s are in the 2 water molecules because the conversion of ADP and P to ATP is a bit more complicated at pH 7. At pH 7 the correct charge states for these phosphate species are ADP^{3-}, P^{2-}, and ATP^{4-}. Thus the generation of of ATP actually is given by

$$ADP^{3-} + P^{2-} \rightarrow ATP^{4-} + HO^-$$

and it takes an H^+ to convert HO^- to H_2O.

The overall result of the conversion of pyruvate to acetyl-CoA, a 6 step pathway occurring on a multienzyme complex, is given by

$$2\,pyruvate + 2CoA - SH + 2NAD^+ \rightarrow$$

$$2acetyl - CoA + 2CO_2 + 2NADH$$

The Gibbs free energy change for this sequence is $\Delta G^{0'} = -16.0 kcal/mol$. Acetyl-CoA carries the two carbon compound acetate to the citric acid cycle where it is completely oxidized.

The overall result of the citric acid cycle may be expressed by

$$2acetyl - CoA + 6NAD^+ + 2FAD + 2GDP + 2P + 4H_2O \rightarrow$$

$$2CoA - SH + 6NADH + 4H^+ + 2FADH_2 + 2GTP + 4CO_2$$

The Gibbs free energy change is $\Delta G^{0'} = -25.6 kcal/mol$.

The overall result of the electron transport chain appears to be

$$10NADH + 6H^+ + 2FADH_2 + 6O_2 \rightarrow 12H_2O + 10NAD^+ + 2FAD$$

with a Gibbs free energy change of $\Delta G^{0'} = -672 kcal/mol$. However, both the citric acid cycle and the pyruvate dehydrogenase enzyme complexes are contained within the mitochondrial matrix and the 8 NADH's they produce are readily available to the electron transport chain complexes located in the inner mitochondrial membrane that borders the matrix. The 2 NADH's produced by glycolysis, however, are initially exterior to the mitochondrial outer membrane. They easily cross the outer membrane but the inner membrane is impermeable to them. The glycerol phosphate shuttle is the mechanism by which the electrons on NADH are communicated to the interior of the inner membrane. NADH itself doesn't actually get inside and the glycerol phosphate shuttle places the electrons on FAD instead, forming $FADH_2$. Thus, from the perspective of the inner surface of the inner mitochondrial membrane where electron transport is initiated, the overall reaction equation should read

$$8NADH + 4H^+ + 4FADH_2 + 6O_2 \rightarrow 12H_2O + 8NAD^+ + 4FAD$$

The NADH's enter the electron transport chain at the top of the chain and they yield enough energy in mitochondria for the synthesis of 3 ATP's. The $FADH_2$'s, however, enter the electron transport chain further down the chain at the CoQ site. This yields enough energy for only 2 ATP's. The net result is 24 ATP's from 8 NADH's, 8 ATP's from 4 $FADH_2$'s, 2 ATP's from glycolysis and 2 GTP's from the citric acid cycle. This is a grand total of 36 high energy phosphate bonds, 34 ATP's and 2 GTP's. Only 2 high energy phosphate bonds arise from glycolysis, the only source in anaerobic organisms and in highly active muscle tissue.

When all of these steps are combined the overall result is

$$glu\cos e + 2ADP + 2P + 2GTP + 2P + 6O_2 \rightarrow$$

$$6CO_2 + 10H_2O + 2ATP + 2GTP + energy$$

The energy is maintained by the membrane potential and can be converted into the synthesis of 32 ATP molecules from 32 ADP's and 32 P's. This balance sheet for the complete oxidation of glucose to CO_2 and H_2O differs from that for the simple burning of glucose in air which would read

$$glu\cos e + 6O_2 \rightarrow 6CO_2 + 6H_2O + heat$$

in the presence of the phosphates and 10 H_2O's. The extra 4 H_2O's represent the *dehydration condensation* of 2 ADP's, 2 GDP's and 4 P's to 2 ATP's and 2 GTP's, i.e. the reversal of these syntheses is *hydrolysis*.

In standard state the Gibbs free energy change for the simple burning of glucose is $\Delta G^0 = 6(-94.3 - 56.7) - (-219.1) kcal/mol = -686.9 kcal/mol$. In the slow *burning* of the energy metabolism pathways 36 high energy phosphate bonds are harvested at a standard state cost of about 7.3 kcal/mol. Thus, 262.8/686.9, or 38% of the available energy is harvested. Compared with, say, man made photovoltaic cells that have efficiencies less than 10%, and the nuclear power plant, the Connecticut Yankee on the Connecticut River that functions like a Carnot cycle engine at 33%, this is very impressive.

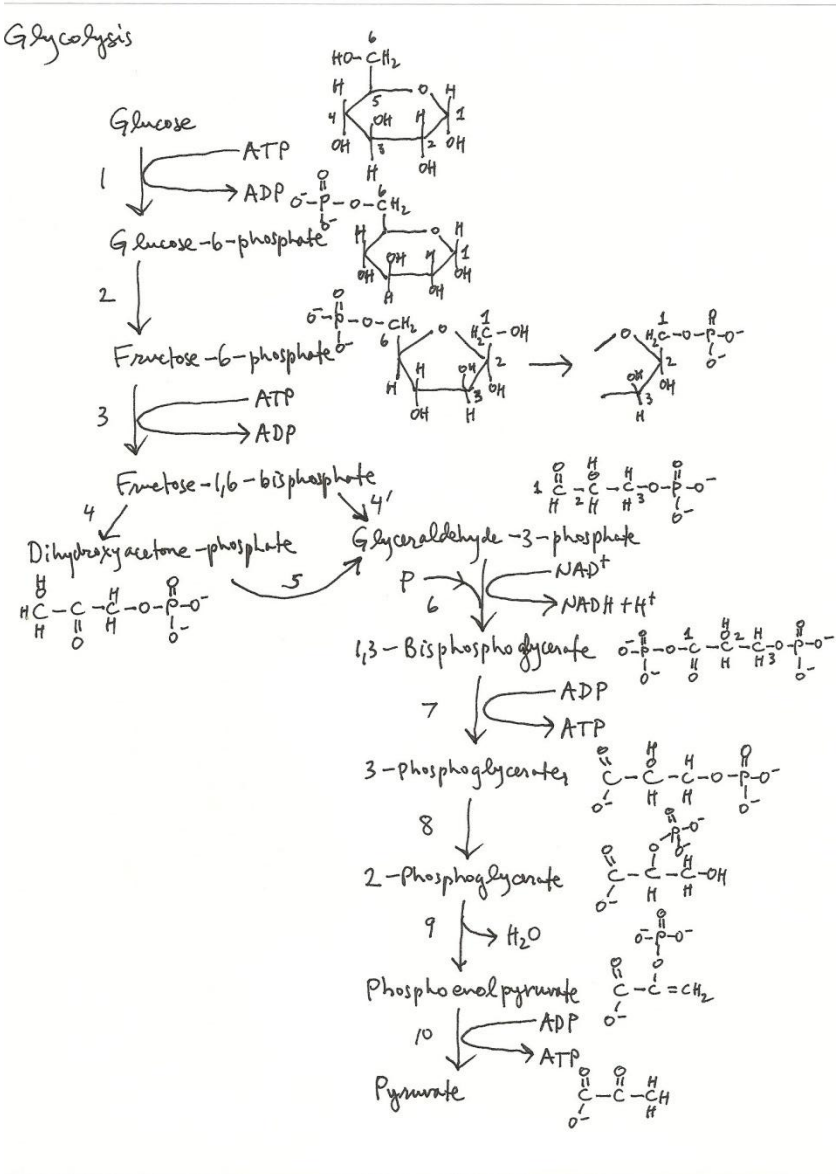
Energy metabolism

Several energy metabolism pathways mentioned earlier are considered in greater detail here. These pathways are central to metabolism generally and they contain mechanisms and processes that are basic to an understanding of nanobiology. The presentation covers glycolysis,

the conversion of pyruvate into acetyl-CoA, the citric acid cycle, the glyoxylate cycle and the Calvin cycle.

Glycolysis

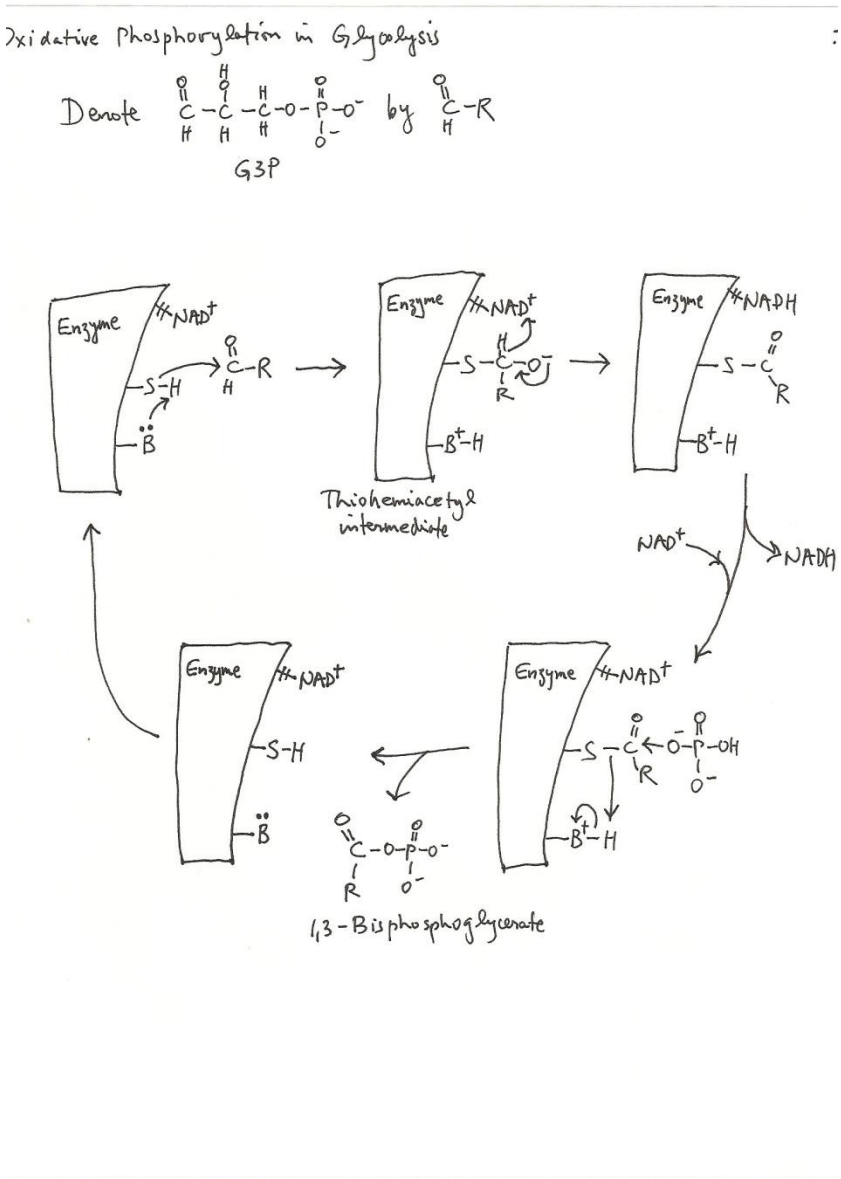
Glycolysis (from the Greek *glycos* for sweet and *lysis* for dissolution) is a ten step sequence of reactions in which glucose is partially oxidized to two molecules of pyruvate along with the generation of two molecules of NADH and a net gain of two molecules of ATP. The pathway is given in the figure.



The first and third steps each require a molecule of ATP for the phosphorylation of glucose and then of fructose-6-phosphate. This activates the pathway and results in the breaking up of the six carbon fructose-1,6-bisphosphate into two three carbon molecules, both of which ultimately end up as glyceraldehyde-3-phosphate. The rest of glycolysis involves the conversion of each

molecule of glyceraldehyde-3-phosphate into pyruvate, along with generation of NADH, two ATP's and one molecule of water. Steps six and seven make up the prototype for *oxidative phosphorylation* in which an oxidation step, the oxidation of glyceraldehyde-3-phosphate by NAD^+, is coupled by a phosphorylated intermediate, 1,3-bisphosphoglycerate, to the phosphorylation of ADP, making ATP. A subsequent phosphorylation generates another molecule of ATP from ADP as phosphoenolpyruvate is converted into pyruvate in step ten. Both phosphorylations involve *chemical coupling* and the the first was taken as the paradigm for oxidative phosphorylation generally for many years. This conception of the mechanism made the discovery and acceptance of the chemiosmotic mechanism of ATP generation much more difficult to achieve.

The oxidative phosphorylation steps in glycolysis, steps six and seven, occur sequentially on an enzyme that catalyzes both the oxidation and the phosphorylation. The NAD^+ participates as a *coenzyme* in this process. This means that a key catalytic step is mediated directly by NAD^+ and not by the enzyme protein itself. The enzyme protein, called the *apoenzyme* (from the Greek *apo* meaning away from), together with the coenzyme, in this case NAD^+, make up the *holoenzyme* (from the Greek *holo* meaning whole). The phosphorylation step, however, is catalyzed by the enzyme through the mediation of a sulfhydryl group, -SH, on an amino acid cysteine residue and the help of an appropriately positioned basic amino acid residue. This is shown in the figure.



This mechanism introduces what will be a recurring theme, the catalytic involvement of sulfur atoms in the formation of *thioesters*. There is reason to believe that this represents an evolutionarily primitive step.

The reaction sequence begins with the holoenzyme containing bound coenzyme NAD^+. The basic amino acid residue takes up the sulfhydryl proton of cysteine as the sulfur reacts with the aldehyde end of glyceraldehyde-3-phosphate by a nucleophilic attack of the aldehyde carbon by sulfur, producing what is called a *thiohemiacetyl* intermediate. The next step is the oxidation of the bound intermediate by NAD^+ producing a thioester. Exchange of NADH for NAD^+ recharges the coenzyme site with oxidized NAD^+. The released NADH is either reoxidized later on when pyruvate is reduced to ethanol or to lactate in anaerobic metabolism, or it is reoxidized by the electron transport chain in aerobic metabolism. An oxygen atom of inorganic phosphate nucleophilically attacks the thioester carbon atom and a *transthiolation* occurs in which 1,3-

bisphosphoglycerate ester is formed. Simultaneously, the reduced basic amino acid residue gives its proton back to the sulfur atom of the apoenzyme's cysteine residue. The holoenzyme is completely regenerated for another cycle, although its NAD^+ is not the same one with which it started. The 1,3-bisphosphoglycerate is energy rich and can phosphorylate ADP making ATP with an overall decrease in Gibbs free energy.

Notice that to initiate the pathway, two ATP's are required and in the second half of the pathway two ATP's are produced for each of two glyceraldehyde-3-phosphates. Thus there is a net gain of two ATP's. However, the four ATP's produced as output can initiated two copies of the pathway, yielding eight ATP's as output. These can prime four copies of the pathway, yielding 16 ATP's and so on. This creates an exponential increase in ATP production provided there are enough copies of the enzymes for the pathway in the cell. The glycolysis enzymes exist inside the cytosol of the cell in a great many multiple copies. They are loosely associated with each other and substrate diffuses from reaction site to reaction site in order to complete the pathway. This is quite primitive compared to the orderly arrangement of enzymes for the electron transport chain in the mitochondrial membrane.

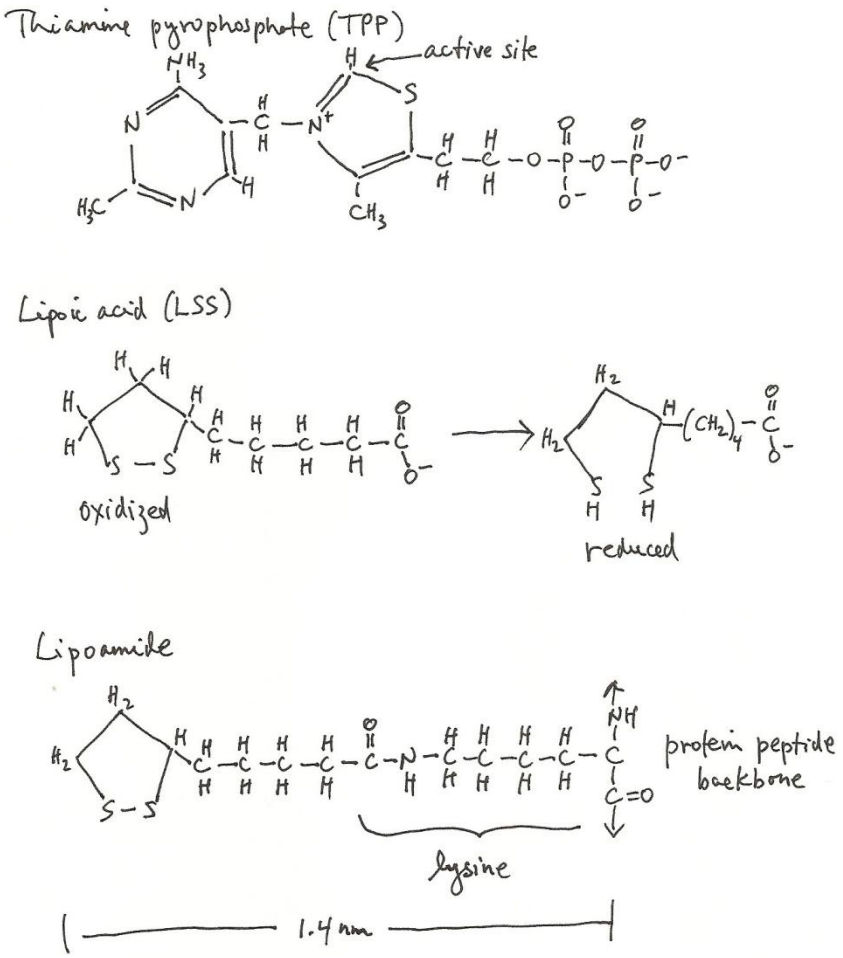
The requirement for priming and the subsequent exponential increase in ATP production can be seen in *in vitro* experiments as an initial induction phase for the process. The enzymes, glucose and ADP and phosphate are incubated together at appropriate temperature, pH and ionic strength. Enough ATP will spontaneously form from ADP and phosphate to prime the reaction although this takes some amount of time. This is seen as a delay in the onset of exponential ATP production.

Conversion of pyruvate to acetyl-CoA

The coupling of glycolysis to the citric acid cycle requires the conversion of pyruvate into acetyl-CoA, a thioester.

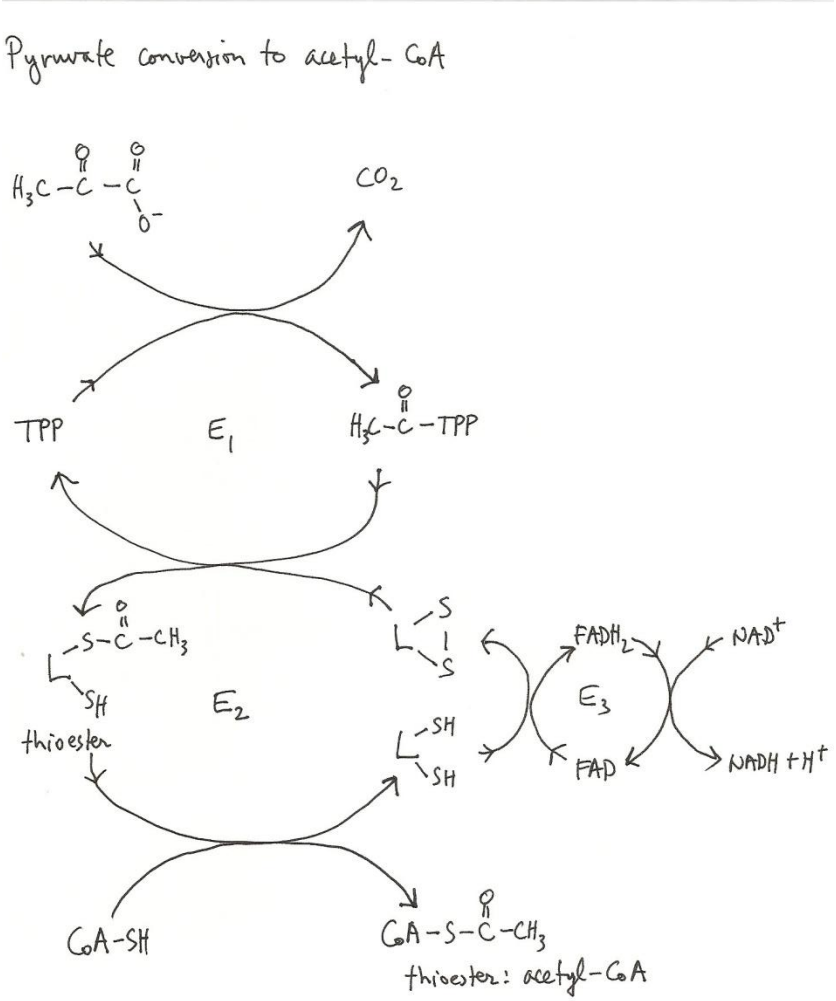
$$pyruvate + CoA - SH + NAD^+ \rightarrow acetyl - CoA + CO_2 + NADH + H^+$$

This is a five step process that takes place on a multienzyme complex that self-assembles from its constituent subunits. It involves five coenzymes, TPP (thiamine pyrophosphate), LSS (lipoic acid), FAD (flavin-adenine dinucleotide), NAD^+ and CoA-SH. Coenzymes TPP and LSS are depicted in the figure.



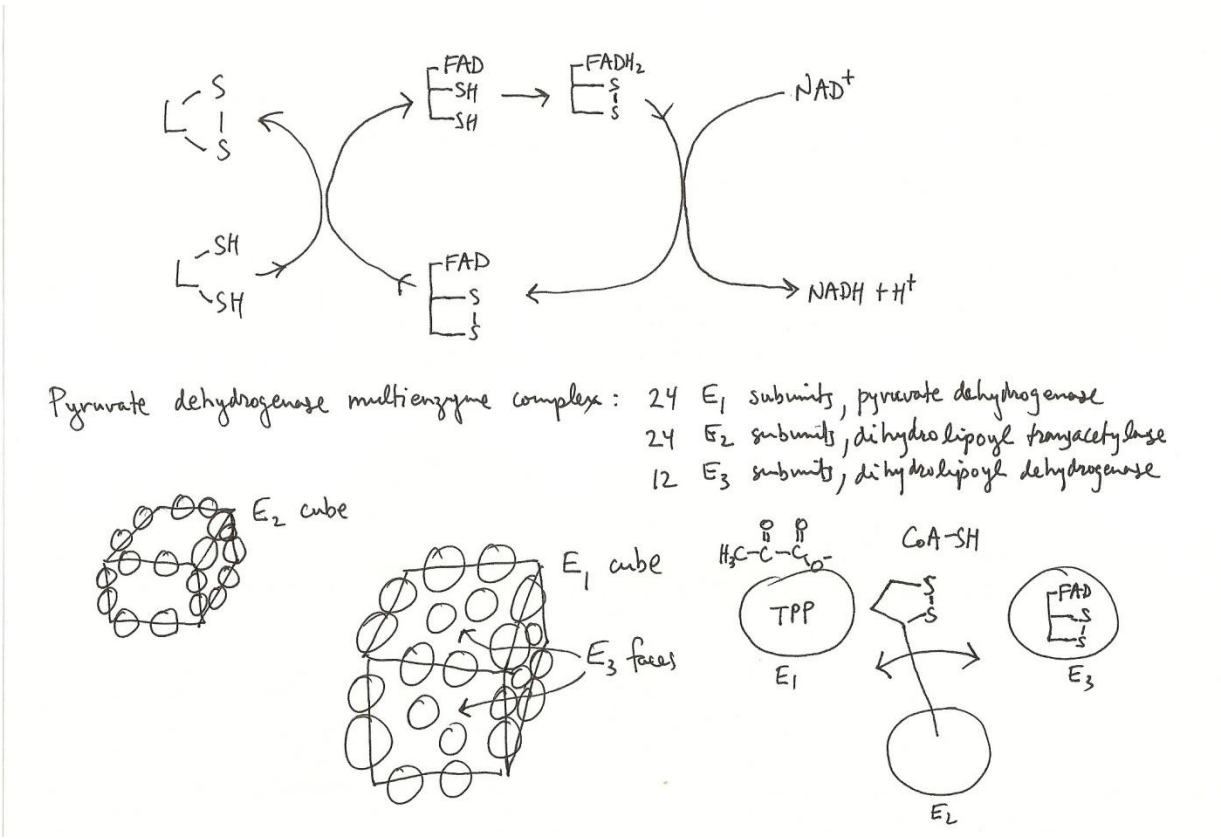
Notice the frequent occurrence of phosphate and ribose in these five coenzymes. These coenzymes had to evolve only after both ribose and phosphate were plentiful constituents of primitive metabolism.

The multienzyme complex on which this process takes place contains multiple copies of three enzyme subunits labeled E_1, E_2 and E_3. These subunits self-assemble into a cubic structure. The core is made up of 24 E_2 subunits, two for each of the twelve edges of the cube. Assembled on this core are 24 E_1 subunits, again two per edge. In addition, 12 E_3 subunits adorn each of the cubic faces, two per face. A schematic of the reaction sequence is given in the figure.



The next figure shows the involvement of FAD in greater detail indicating that additional sulfhydryl groups are part of the FAD/$FADH_2$ redox cycle. This part of the pathway is remarkably similar to the mechanism of *glutathione reductase*. In fact, the structure of E_3 from bacteria is incredibly similar to that of human glutathione reductase.

The carboxyl group of lipoic acid is connected to the enzyme complex through an amino acid lysine residue of the E_2 subunit. The ε-amino group of the lysine and the carboxyl group of the lipoic acid form a peptide bond. This combination creates a freely rotating arm 1.4 nm long. The transfer of acetate from thiamine pyrophosphate to coenzyme CoA is mediated by attachment of acetate to the sulfhydryl group of the rotating lipoic acid. This results in a reduction of the lipoate sulfur atoms. Their re-oxidation is catalyzed by the FAD's on the E_3 subunits



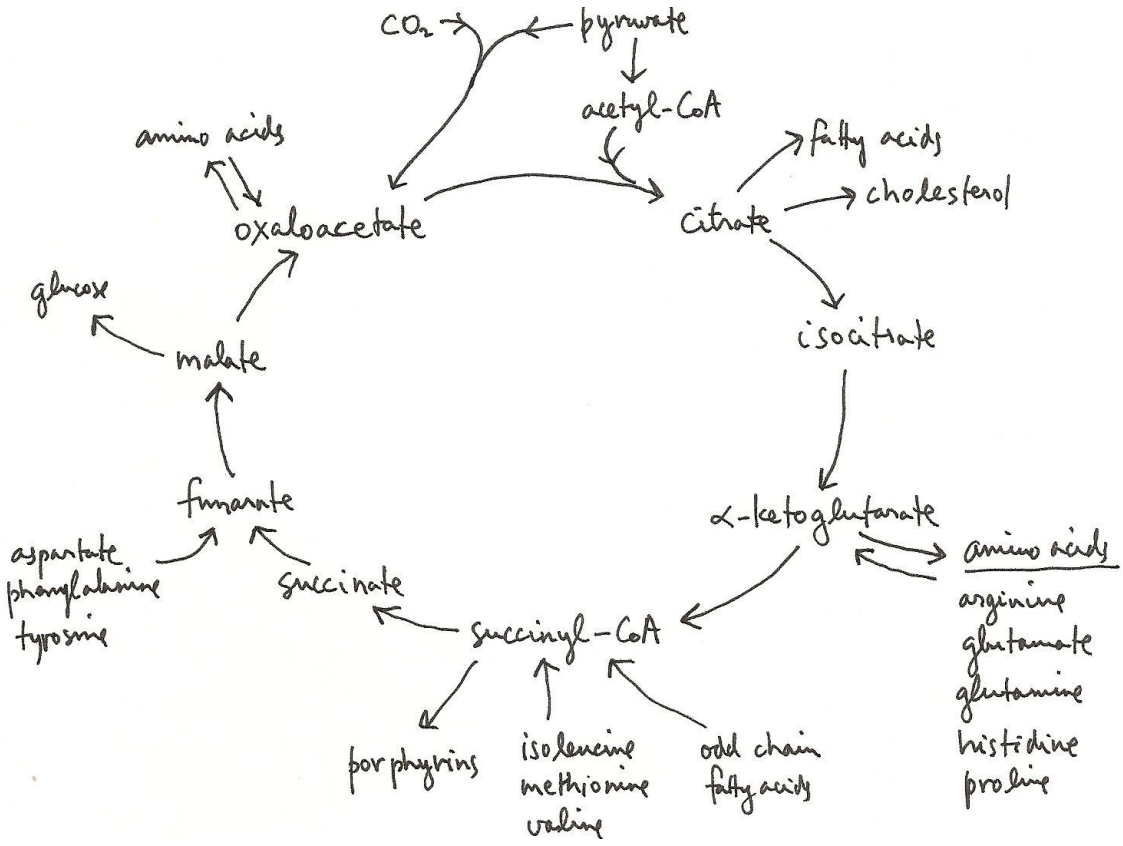
A very similar structure exists for the multienzyme complex *α-ketoglutarate dehydrogenase* that occurs in the citric acid cycle and produces *succinyl-CoA*.

$$\alpha - ketoglutarate + CoA - SH + NAD^+ \rightarrow succinyl - CoA + CO_2 + NADH + H^+$$

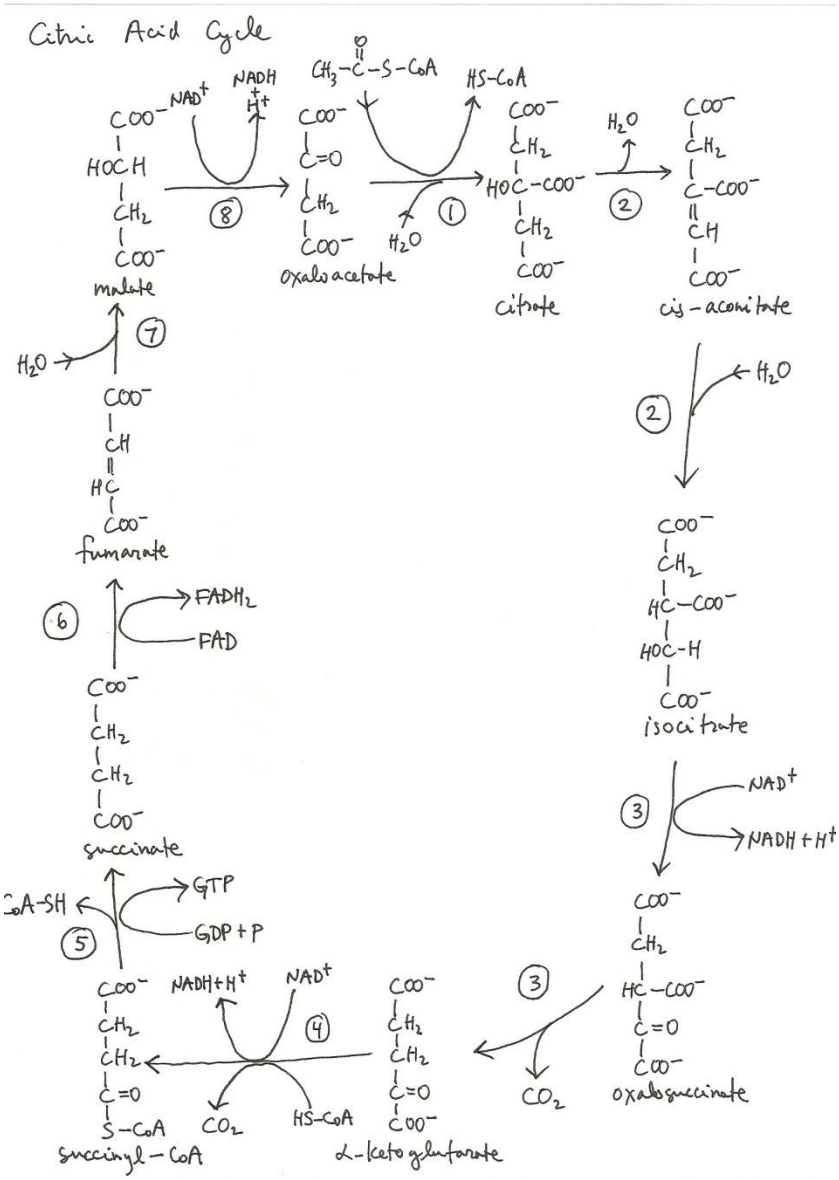
The same five coenzymes are involved and the lipoyllysine rotating arm serves the same purpose. While the E_1 and E_2 subunits are different in α-ketoglutarate dehydrogenase, the E_3 subunit is the same as in pyruvate dehydrogenase. The degradation of the amino acids isoleucine, leucine and valine involves a similar complex. This exhibits the conservative nature of molecular and cellular evolution in that a mechanism is used for several different purposes once it has evolved for a single purpose.

Citric acid cycle

The citric acid cycle is a major hub for metabolism



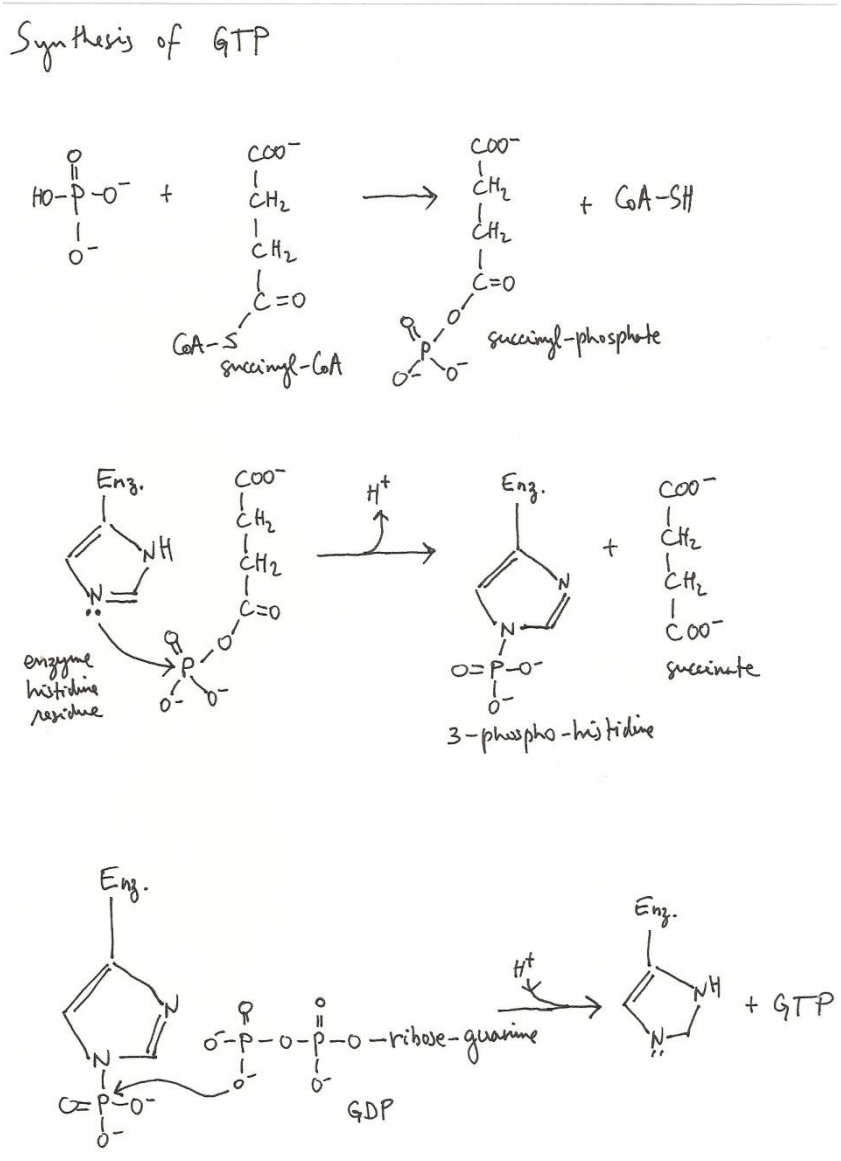
Fatty acid and amino acid metabolism are directly connected to citric acid intermediates. The cycle generate reduced coenzymes NADH and $FADH_2$ that ultimately drive the electron transport chain and the production of chemiosmotic membrane energy. Thus, the citric acid cycle is a major power generator as well. The cycle involves eight enzymes, all located in an organized way inside the mitochondrial matrix



The enzymes are: (1) *citrate synthase* catalyzing the hydrolytic release of CoA-SH and incorporation of acetate into *oxaloacetate* to form *citrate*; (2) *aconitase*, an FeS containing protein [4Fe-4S] catalyzing the elimination of a molecule of water from citrate to form *cis-aconitate*, an enzyme bound intermediate, and then catalyzing the rehydration of cis-aconitate with the same eliminated water molecule to form *isocitrate*; (3) *isocitrate dehydrogenase* catalyzing the oxidation of isocitrate by NAD^+ to form enzyme bound intermediate, *oxalosuccinate*, which is decarboxylated to α-ketoglutarate; (4) *α-ketoglutarate dehydrogenase* catalyzing the oxidative (by NAD^+) decarboxylation (elimination of CO_2) and incorporation of CoA-SH to form *succinyl-CoA*; (5) *succinyl-CoA synthase* catalyzing the formation of *succinate* along with the elimination of CoA-SH and the phosphorylation of GDP to GTP; (6) *succinate dehydrogenase* catalyzing the oxidation of succinate by FAD to form *fumarate*; (7) *fumarase* catalyzing the hydration of fumarate to form *malate*; and (8) *malate dehydrogenase* catalyzing

the oxidation of malate by NAD^+ to form oxaloacetate which completes the cycle. The similarity of α-ketoglutarate dehydrogenase to pyruvate dehydrogenase was already mentioned in the last chapter. Unlike their role in the elctron transport chain where FeS proteins are associated with redox processes, in aconitase the role is different. In this case, one special iron atom in the cluster is centrally involved in the coordination of the water molecule that is first eliminated from citrate and then used to rehydrate cis-aconitate. The formation of GTP is another example to be added to the two examples from glycolysis of phosphorylation of a nucleotide diphosphate to make a triphosphate by chemical coupling.

The mechanism of GTP formation in the citric acid cycle is of interest because of the involvement of the amino acid *histidine*. This mechanism is shown in the figure.



Inorganic phosphate nucleophilically attacks the ester carbon of succinyl-CoA, releasing CoA-SH and forming *succinyl-phosphate*, a high energy phosphate intermediate. Histidine, an amino acid constituent of the enzyme in turn nucleophilically attacks the phosporus atom of succinyl-phosphate to form the enzyme bound *3-phospho-histidine* and free succinate. Finally, a terminal oxygen of GDP nucleophilically attacks the phosphorus atom of 3-phospho-histidine to form GTP and a normal histidine residue. Histidine plays a key role in the mechanisms of enzymes (1), (2) and (7) as well and in (7) the FAD is covalently bound to a histidine residue of the enzyme.

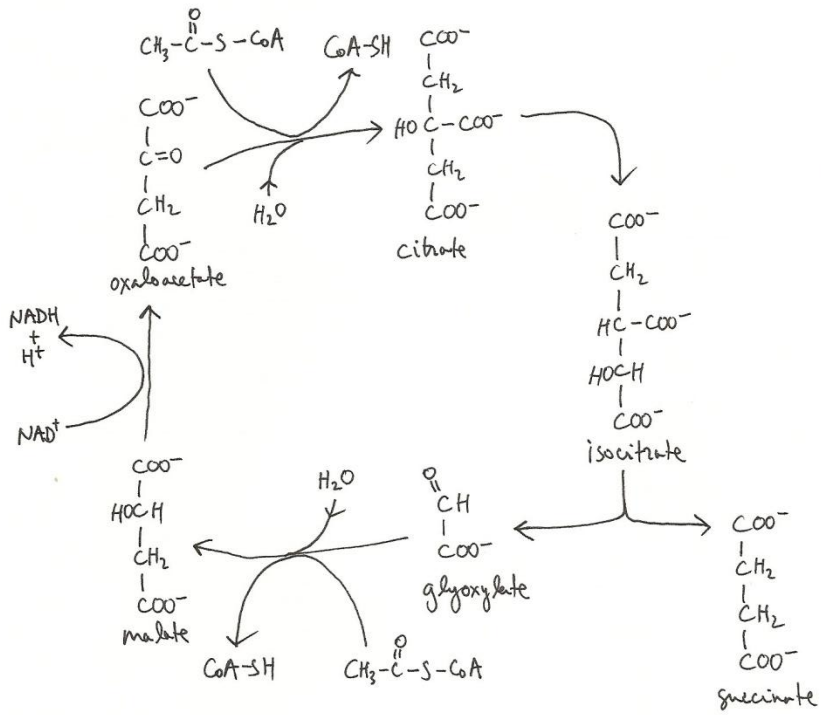
For the citric acid cycle, acetate is oxidized to 2 CO_2's, 3 ($NADH+H^+$) and 1 $FADH_2$, along with 1 GTP. The electron transport chain can generate 3 ATP's from 1 NADH and 2 ATP's from 1 $FADH_2$. Thus, one passage through the citric acid cycle can generate 3 x 3 + 2 + 1 = 12 high energy phosphate bonds. For glucose, 2 acetates are produced, as well as a net of 2 ATP's during glycolysis. Thus 2 x 12 + 2 = 26 high energy phosphate bonds can be made. As pointed out in the previous chapter, glycolysis also generates 2 NADH's per glucose and the pyruvate dehydrogenase reaction does too. At a possible 3 ATP's per NADH, it appears that 12 more ATP's are possible. However, those NADH's from glycolysis have the get their electrons to the electron transport chain and this is done through the intermediacy of $FADH_2$ which can only generate 2 ATP's per $FADH_2$. As described in the last chapter, the $NADH_2$'s from pyruvate dehydrogenase don't have this problem. Thus, there are only 10 extra ATP's possible for a grans total of 26 + 10 = 36 high energy phosphate bonds.

Glyoxylate cycle

In bacteria and plants there exists the glyoxylate cycle which is a shortened version of the citric acid cycle that generates the four carbon compound succinate.

$$2acetyl-CoA+NAD^{+}+2H_2O \rightarrow succinate+2CoA-SH+NADH+H^{+}$$

This cycle is given in the figure.



In plants, this cycle involves the organelle called the *glyoxysome*. The reaction cycle is divided between the glyoxysome and the plant mitochondria. The net result is

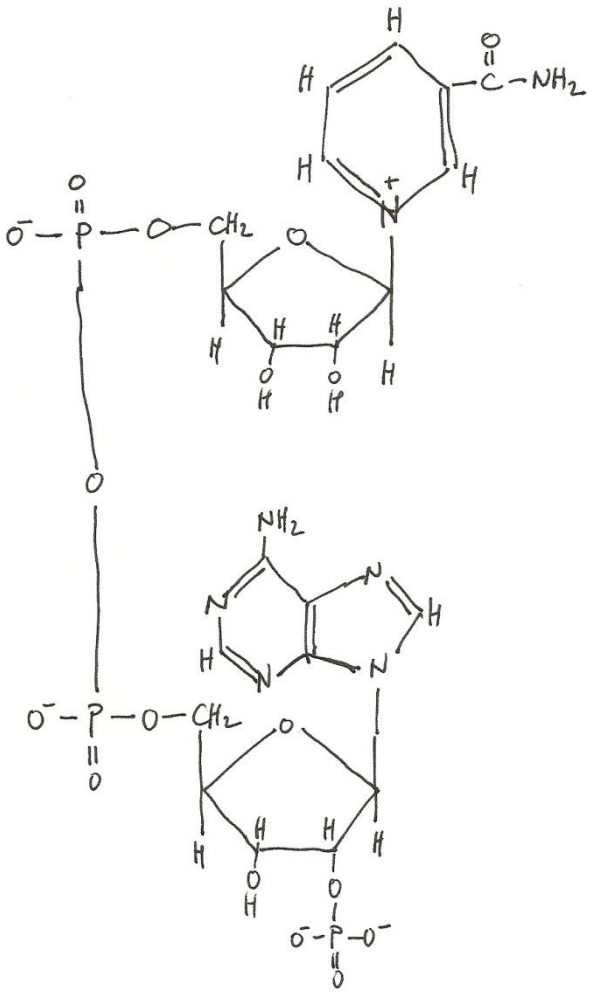
$$2acetyl-CoA+NAD^{+}+2H_2O \rightarrow oxaloacetate+NADH+H^{+}$$

What happens is that a portion of the citric acid cycle occurring in the mitochondrial matrix is coupled to a portion of the total glyoxylate cycle occurring in the glyoxysome that resides in the cellular cytosol. Inside the mitochondrial matrix, oxaloacetate from the citric acid cycle is aminated by the enzyme *aspartate aminotransferase* using the amino acid *glutamate* as amino group donor producing *aspartate*, another amino acid, and α-ketogluarate, the deamination product of glutamate. Asparate and α-ketoglutarate can be transported out of the mitochondria into the cytosol and then into the glyoxysome. Inside the glyoxysome, α-ketoglutarate and aspartate react to produce oxaloacetate and glutamate, the reverse of the initial mitochondrial

reaction. This is also catalyzed by aspartate aminotransferase, but inside the glyoxysome. The glutamate can be transported back into the mitochondrial matrix for another round. Inside the glyoxysome the steps of the cycle leading from oxaloacetate to malate, with the production of succinate, take place. The succinate is transported back into the mitochondrial matrix where it is converted into oxaloacetate by a portion of the citric acid cycle. The malate is transported out of the glyoxysome into the cellular cytosol where it is converted into oxaloacetate by malate dehydrogenase. Here, the oxaloacetate is available for the synthesis of glucose, a process called *gluconeogenesis*. This means that the acetate of acetyl-CoA is ultimately converted into glucose that may fuel the germination of plant seeds. The acetyl-CoA is produced by the oxidation of fatty acids, in particular *triacylglycerols*, that are the latent fuel for the seeds.

Calvin cycle

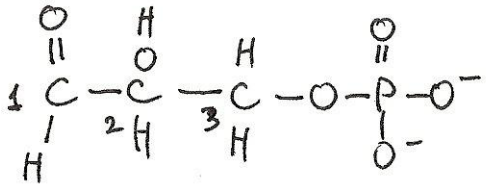
The pathways of glycolysis, the citric acid cycle and electron transport are designed to *catabolize* glucose. They oxidize glucose completely to CO_2 and water and capture much of the energy released by this process as high energy phosphate bonds, principally in ATP, and as energized membrane potential. The glyoxylate cycle, however, is aimed at *anabolism*, i.e. the generation of glucose from the smaller acetate precursors. In plants, an even grander anabolic process occurs in which water and CO_2 are combined to make glucose and other sugars. This process begins by harnessing energy from a light driven electron transport chain that partially transduces the light energy into the energy of ATP and the energy of reducing potential in a variant of NADH, NADPH (*nicotinamide adenine dinucleotide phosphate*)



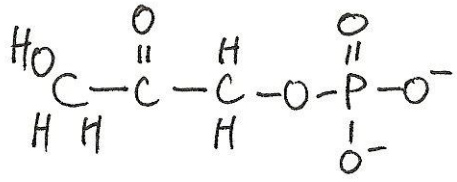
The Calvin cycle utilizes this energy to condense water and CO_2 into sugar molecules.

The Calvin cycle interconverts twelve different *carbohydrate* species ranging from compounds with three carbons to those with seven. The twelve species names, abbreviations and chemical structures are:

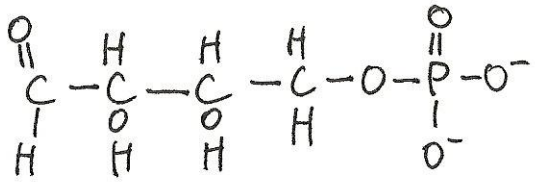
glyceraldehyde-3-phosphate (GAP)



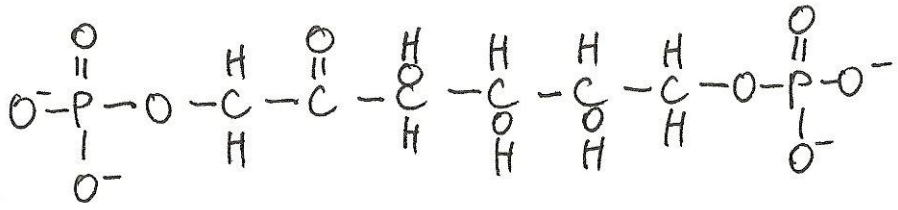
dihydroxyacetone-phosphate (DHAP)



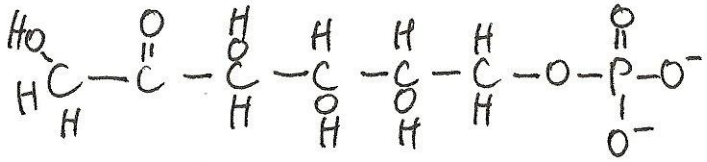
erythrose-4-phosphate (E4P)



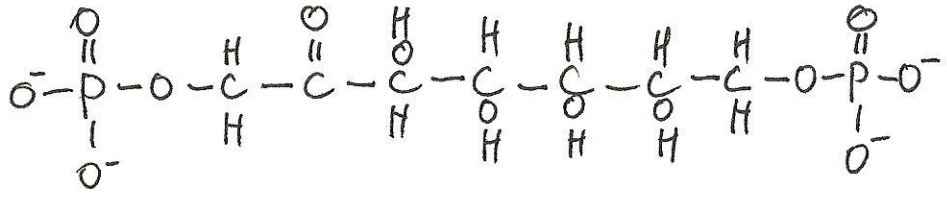
fructose-1,6-bisphosphate (FBP)



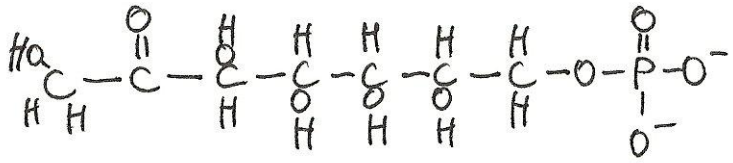
fructose-6-phosphate (F6P)



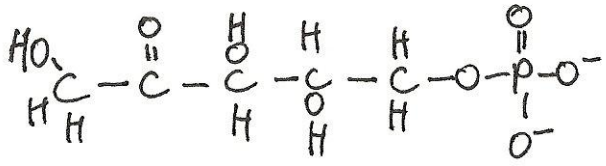
sedoheptulose-1,7-bisphosphate (SBP)



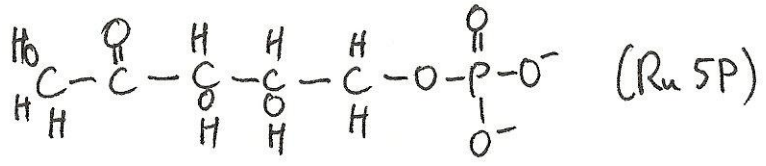
sedoheptulose-7-phosphate S7P)

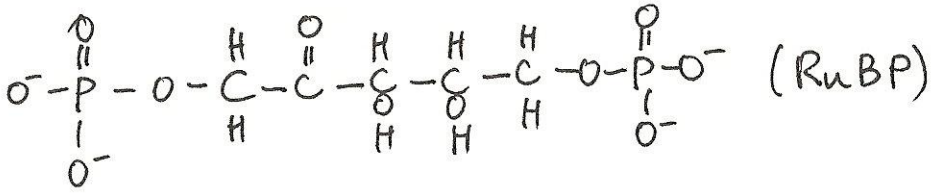


xylulose-5-phosphate (Xu5P)

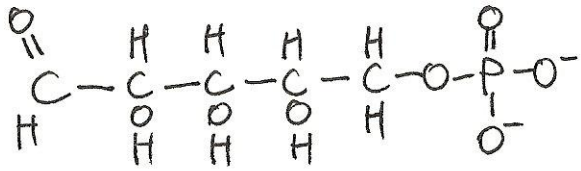


ribulose-5-phosphate (Ru5P)

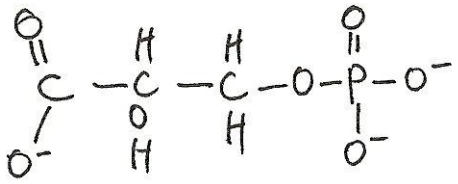




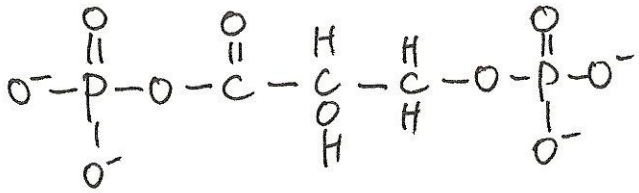
ribose-5-phosphate (R5P)



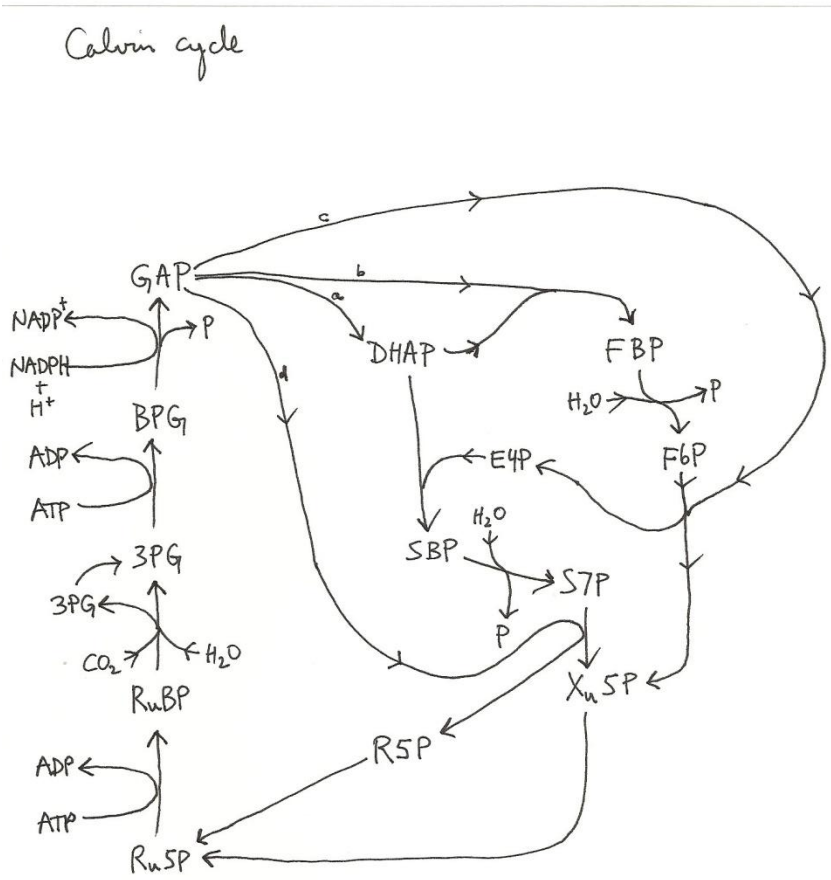
3-phosphoglycerate (3PG)



1,3-bisphosphoglycerate (BPG)



Using these abbreviations the reaction pathway is represented as

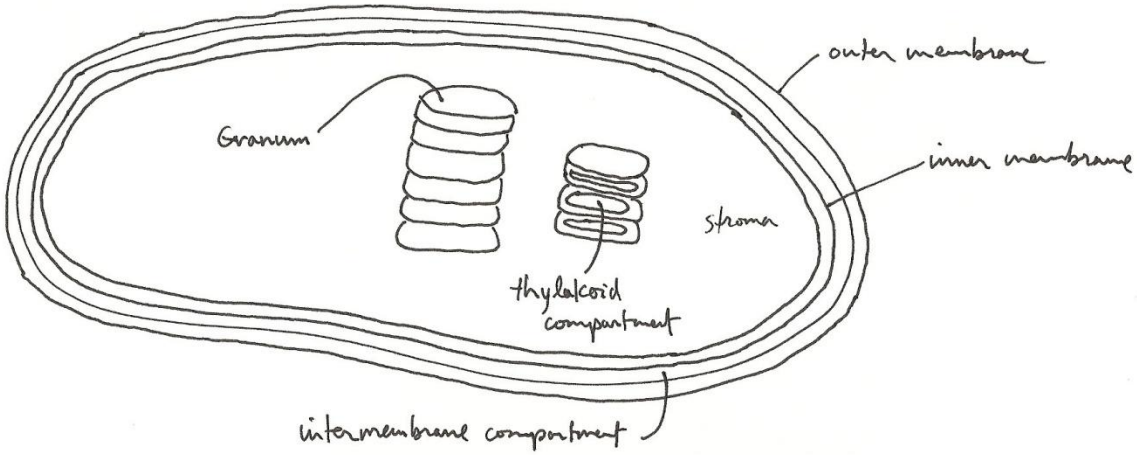


The key step is the incorporation of a molecule of CO_2 and a molecule of H_2O into Ru5P to make rwo molecules of 3PG. Thus, a five carbon sugar-phosphate adds one more carbon from CO_2 to make two three carbon molecules of 3PG.

Imagine initiating the pathway with 5 molecules of GAP (15 carbon atoms). If two go by path a and one each take paths b, c and d, then the result is three Ru5P's (15 carbon atoms), one by way of R5P and two by way of Xu5P. The ATP and NADPH activation steps convert these three Ru5P's into six GAP's. This is a multiplicative factor of 6/5. Recall that in glycolysis the ATP generation factor was 4/2 and caused exponential growth of the ATP population. Here, 6/5 will do the same thing, albeit at a slower rate of exponentiation. If instead, this pathway is initiated with just three GAP's (9 carbon atoms) and one each take paths a, b and c, then one molecule of Xu5P (5 carbon atoms) and one molecule of E4P (4 carbon atoms) are produced. The Xu5P converts to Ru5P which can be activated to make two GAP's (6 carbon atoms). One

of these takes path a to combine with E4P to make S7P. The remaining GAP takes path d and combines with S7P to ultimately make two Ru5P's. These are then activated to produce 4 GAP's (12 carbon atoms). Save one of these GAP's and convert the remaining three into four as was just done and the result is five GAP's, enough to prime exponential production.

The primary product of the Calvin cycle is GAP. By means of other pathways, GAP can be converted into glucose-1-phosphate as well as many other products. The primary light reactions of photosynthesis occur inside chloroplasts in the *thylakoid* (from the Greek *thylakos* meaning sac or pouch) membranes



much like the electron transport chains in mitochondria. The Calvin cycle occurs in the *stroma* of the chloroplast. The stroma is the analogue of the mitochondrial matrix, where the citric acid cycle takes place. GAP must be exported to the chloroplast exterior in order to be utilized for other syntheses.