Metabolism 2 :

Anabolic Reactions of the Cell

Using this book: This book is designed to be used in both introductory and advanced cell biology courses. The primary text is generally on the left side of the vertical divider, and printed in black. Details that are usually left to an advanced course are printed in blue and found on the right side of the divider. Finally, additional biomedically relevant information can be found in red print on either side of the divider.

Anabolic Reactions

As pointed out at the beginning of this book, most of the energy for life on this planet originates from the sun. In the last chapter, the discussion was on the breakdown of complex molecules such as sugars and fats that hold great, but difficult to access, potential energy to produce molecules like ATP that can act as more readily accessible sources of cellular energy. This energy is then used to synthesize the more complex biomolecules necessary to build living cells. That synthesis, the formation of sugars, fatty acids, and amino acids, is the focus of this chapter. Although technically the polymerization of nucleic acids and proteins are anabolic processes, they are not included in this chapter and are examined in detail separately.

Photosynthesis

In one way or another, the energy of sugar and fat fuel molecules is derived from photosynthesis - the conversion of solar light energy into chemical bond energy, whether directly in photosynthetic plant cells and certain photosynthetic bacteria, or indirectly by the ingestion of those plants and bacteria. Photosynthesis is a simple idea: atmospheric carbon dioxide molecules are joined with water molecules to form sugars and oxygen:

$$CO_2 + H_2O \xrightarrow{light} CH_2O + O_2$$

The production of usable energy from sunlight and the fixation of atmospheric carbon dioxide are two separate sets of reactions. In plants, photosynthesis takes place only in cells containing chloroplasts. Chloroplasts are organelles with an evolutionary origin suspected to be similar to that of mitochondria, and like mitochondria, chloroplasts generate ATP and use a nicotinamide-based high-energy electron carrier. There are further similarities: they both have highly folded inner membranes, though in chloroplasts, there are three membranes in all, while mitochondria only have two. Finally,

an electron transport chain is embedded in the thylakoid membrane of chloroplasts, functioning very similarly to electron transport in the mitochondria. In addition to the electron transport components and ATP synthase (structurally and functionally almost identical to mitochondrial ATP synthase), the thylakoid membrane is also rich in a set of molecules that are not found in the inner mitochondrial membrane: light-absorbing pigment molecules.

In plants, these pigment molecules fall into two classes: the chlorophylls and the carotenoids (fig. 1) but only the chlorophylls can mediate photosynthesis. Photosynthetic bacteria do not contain chlorophyll, but do have carotenoid pigments that can carry out photosynthesis. Both are hydrophobic hydrocarbons that are held in place within the plane of the membrane by transmembrane proteins. Chlorophylls are easily recognizable by the very large Mg^{++}-containing porphyrin ring, while the carotenoids are long hydrocarbon chains that may or may not have small ring structures on the ends (e.g. β-carotene). While there is variation in the chlorophyll family, they all impart a green color to the leaf. Carotenoids, on the other hand have a much wider range of colors from yellows to reds. Both chlorophylls and carotenoids are able to absorb light energy of a particular wavelength/energy range and enter an unstable excited state. When the molecule returns to its ground state, the energy would be emitted as heat or light in an isolated situation. However within the context of the pigment arrays (antenna complex) in a living cell, most of the energy is shuttled to another pigment molecule of lower energy by resonance transfer. As described below, only one pair of chlorophyll molecules in an antenna complex will actually eject an electron as it drops from an excited state back to ground state. It is the transfer of that high-energy electron that powers photosynthesis.

Photosynthesis can be divided into two mechanisms: the *light reactions*, which use light energy to excite the electrons of certain chlorophylls, and participate in the electron transport chain to generate ATP and NADPH, and the *dark reactions*, which use that ATP and NADPH to fix carbon from CO_2 into organic molecules (carbohydrates). As the name implies, the light reactions require light energy to excite the chlorophyll and begin electron transport. Dark reactions, however, do not require darkness. They are technically light-independent, but in some plants, the dark reactions run better in the light for reasons to be discussed.

The light reactions are intimately tied to the anatomy of the thylakoid membrane; specifically, the arrangement of light-absorbing pigment molecules in antenna complexes, also called light-harvesting complexes (sometimes abbreviated LHC, not to be confused with the Large Hadron Collider). These pigments are held by proteins in ordered three-dimensional groups so that the pigments that absorb the highest energy

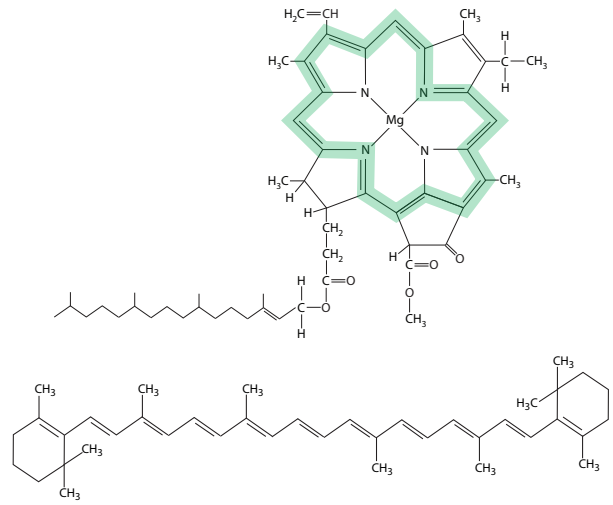


Figure 1. Chlorophyll (top) and β-carotene (bottom)

Chlorophyll molecules are made up of a phytol hydrocarbon tail that anchors the molecule within a membrane, and an electron-carrying porphyrin ring containing a magnesium cation. Note that the phytol tail is not drawn to scale with the porphyrin ring in figure 1. Among different types of chlorophyll, the chemical groups attached to the ring may vary, and this variation is responsible for differences in the absorption spectrum from one type of chlorophyll to the next. For example, chlorophyll a has absorption peaks at approximately 430 and 662 nm, whereas chlorophyll b has peaks at 453 and 642 nm. The difference between the two is small: at C7, there is a $-CH_3$ group on chlorophyll a, but a $-CHO$ group on chlorophyll b. Presently, there are five known chlorophylls: chlorophyll a is found in all photosynthetic organisms, chlorophyll b is only found in plants, chlorophylls c1 and c2 are found in photosynthetic algae, and chlorophyll d is found in cyanobacteria.

Carotenoids have two functions. As noted in the primary text at left, they can participate in energy transfer in toward the reaction center chlorophylls. They are also a protectant molecule, preventing reaction center auto-oxidation. Carotenoids can be highly efficient free radical scavengers due to the conjugation of alternating single-double carbon bond structures.

light are toward the periphery, and the lowest-energy-absorbing chlorophylls are in the center (fig. 2). Sunlight is composed of a broad range of wavelengths, some of which are transiently absorbed by the pigments. After a pigment molecule absorbs a photon, the energy is released and passed on to a pigment tuned to a slightly lower energy level (longer wavelength), and from there to an even lower-energy pigment, and so on until it reaches the reaction center chlorophylls. In this way, energy from a wide range of light wavelengths/energies can all contribute to the ATP and NADPH production by the light reactions. The antenna complex is crucial because it allows the use of a greater portion of the solar light spectrum. And, as a tightly-packed three-dimensional array, photons that pass by one pigment molecule may well hit another one on its way through the array. All of these characteristics combine to increase the efficiency of light use for photosynthesis. The reaction center chlorophylls (P680 for photosystem II, P700 for photosystem I) are the only chlorophylls that actually send excited electrons into the electron transport chain. The other chlorophylls and pigments only act to transfer the energy to the reaction center.

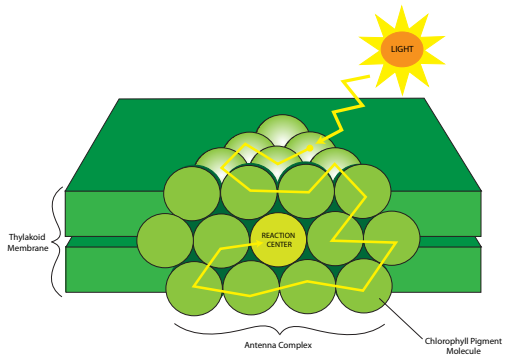


Figure 2. Pigment molecules are arranged in an antenna complex in the thylakoid membrane.

When excited, the reaction center chlorophyll of photosystem II (figure 3) begins the process of electron transport. This chlorophyll is part of a protein complex that also includes a Mn-based oxygen-evolving complex (OEC), pheophytin, and a docking site for plastoquinone. Although the chlorophyll electron is the one excited by the solar energy, the origin of the electrons to keep the chlorophyll replenished comes from the splitting (oxidation) of water to O_2 and 4 H^+.

The question of how a cell could generate the energy needed to split water was long a thorny issue because water is an exceptionally stable molecule. The current model suggests that the energy comes from an extremely strong oxidizer in the form of $P680^+$. After P680 is energized by light, an excited electron has enough energy to break away from the chlorophyll and jumps to pheophytin. Pheophytin becomes $Pheo^-$ temporari-

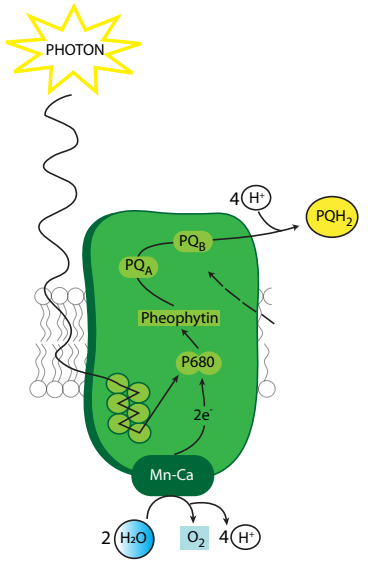


Figure 3. Photosystem II (which feeds electrons into photosystem I).

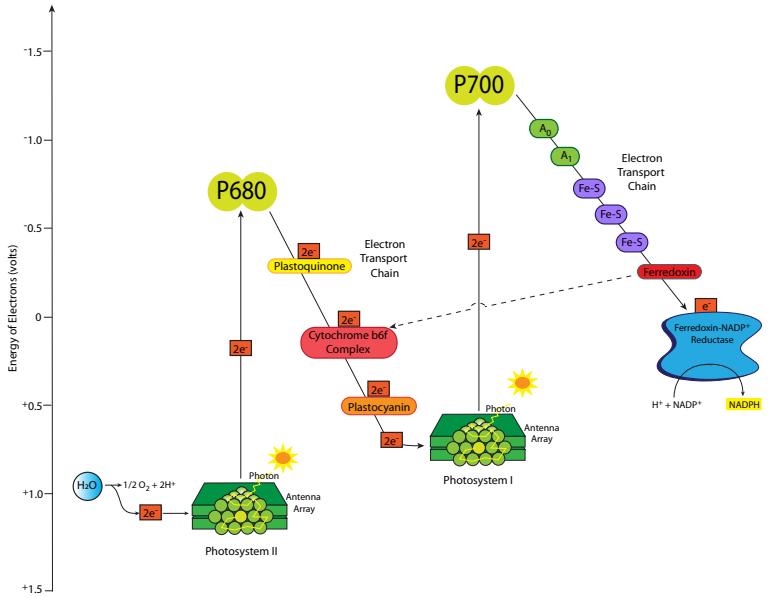


Figure 4. Change in electron energy moving through photosystems II and I. Light energy is needed in both photosystems to boost the electron energy high enough to move to the next electron carrier.

ly, and the charge separation in the complex between $P680^+$ and $Pheo^-$ helps to enhance the oxidative power of $P680^+$. That extraordinarily strong attraction for electrons is what allows the P680 chlorophyll to tear them away from H_2O and split the water. In fact, $P680^+$ is one of the strongest biological oxidizers known. Since four electrons must be taken to fully oxidize two water molecules and generate molecular oxygen, four photoexcitation events are needed. While the exact mechanism is still to be elucidated, it appears that the OEC helps to stabilize the water molecule during this process as well as holding onto each electron as it comes off.

The excited electrons, moving from the OEC to $P680^+$ to pheophytin, next move to the lipid-soluble carrier, plastoquinone. The similarity of the name with the mitochondrial carrier ubiquinone is not a coincidence. They function similarly, and as the plastoquinone takes on the electrons, it also takes on protons from the stromal side of the thylakoid membrane. The PQ moves within the membrane from pheophytin to cytochrome b_6f. As the electrons are transferred to cytochrome b_6f, the protons are then dropped off on the lumenal side of the membrane, increasing their concentration in the chloroplast lumen, and building a proton gradient to power ATP synthase. Cytochrome b_6f passes the electrons on to plastocyanin, an aqueous-phase carrier, which shuttles the electrons to the P700 reaction center chlorophyll of photosystem I. However, after all the transfers, the energy level of the electrons is now fairly low (fig. 4) and unable to power the upcoming reactions. Since it is now on a reaction center chlorophyll, the obvious answer is to re-energize it with a bit of sunlight. This raises the electron energy sufficiently to reduce ferredoxin. Now things get a little complicated.

This part of photosynthesis can take one of two directions, the linear pathway, which generates both NADPH and ATP, and the cyclic pathway which mostly generates ATP. Most of the time, the linear pathway is taken, with the electrons on ferredoxin transferred via ferredoxin-NADPH reductase (FNR) onto NADPH. However, sometimes the cell requires significantly more ATP than NADPH, in which case, the electrons from ferredoxin are transferred back to plastoquinone via ferredoxin-plastoquinone reductase. This acts just as described above, and pumps more protons across the membrane to power the ATP synthase. ATP synthesis goes up and NADPH synthesis goes down.

The ATP and NADPH generated by the chloroplast are almost exclusively used by the chloroplast itself (and not distributed to the rest of the cell) to power the dark reactions, which are energetically expensive. In fact, when the light reactions are not running due to darkness, some plant cells have mechanisms to prevent the dark reactions from using the limited resources of cellular, non-chloroplastic, respiration. The simplest method of such limitation is the pH sensitivity of *rubisco* (ribulose bis-phosphate carboxylase), at least in C3 plants (see below). Rubisco has a very sharp pH optimum

The OEC, or oxygen-evolving complex (also WOC, water oxidizing complex) is a metalloenzyme with a Mn_4O_XCa catalytic cluster, where X is the number of μ-oxo-bridges connecting the metal atoms, with surrounding amino acids, especially crucial tyrosines, also playing a role in the coordination sphere of the active site. The overall complex undergoes a series of 4 oxidation state changes as the P680 chlorophylls are excited by the light energy and transfer electrons, but at present it is not known what the exact oxidation state of any given Mn atom is through this series of state changes. The crucial reaction is the formation of the O-O bond to form O_2. There are two proposed models for this mechanism. One is that the O-O bond is formed when the OEC has reached its fully oxidized state, and an oxygen in a μ-oxo-bridge radical state interacts with a water molecule. The other proposed mechanism is that the O-O bond forms earlier as a complexed peroxide held by the OEC center.

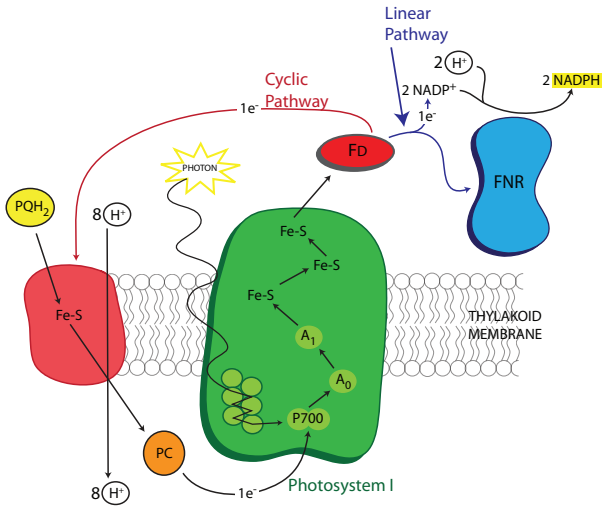


Figure 5. Photosystem I initiates electron movement through two pathways.

at about pH 8.0, so while the light reactions are running and the protons are being pumped, the pH rises to about 8 and rubisco works, but in the dark, the pH drops back to its basal level close to 7.0, inhibiting rubisco activity.

The Calvin Cycle

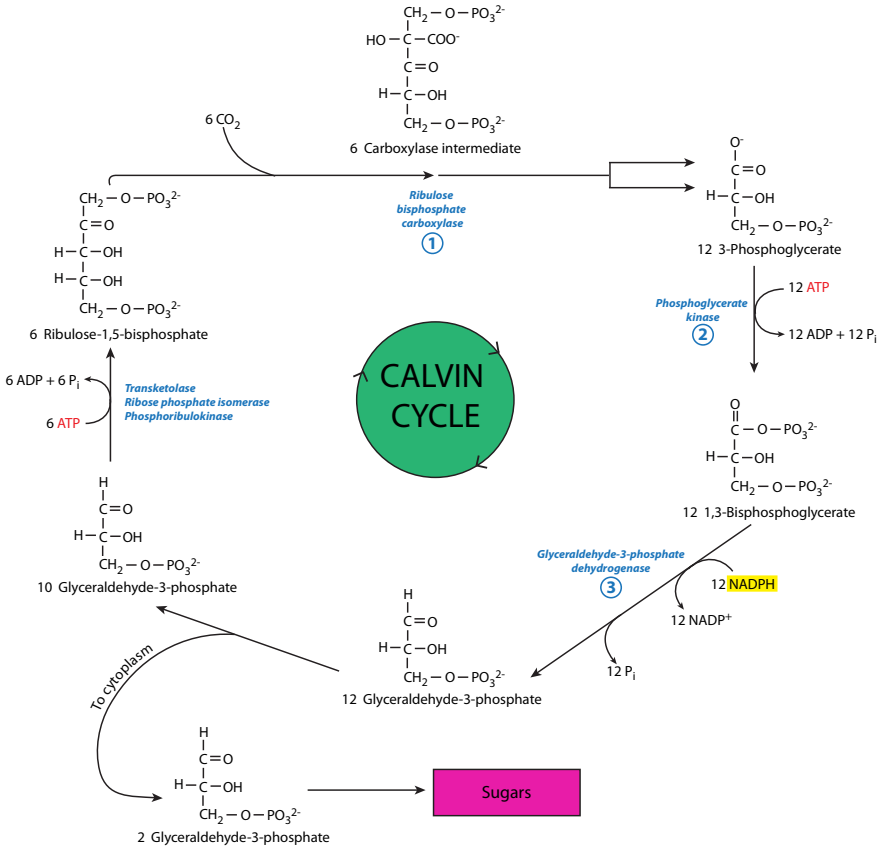


Figure 6. The Calvin cycle fixes atmospheric carbon to ribulose 1,5-bisphosphate to form the organic 3-carbon intermediate 3-phosphoglycerate for the formation of sugars.

The dark (carbon fixation) reactions vary depending on the type of plant. The most common set of carbon fixation reactions is found in C3-type plants, which are so named because the major stable intermediate is the 3-carbon molecule, glyceraldehyde-3-phosphate. These reactions, best known as the Calvin cycle (fig. 6), fix CO_2 onto the pentose, ribulose 1,5-bis-phosphate (RuBP). The production part of the cycle begins with formation of RuBP from glyceraldehyde-3-phosphate. Then, the rate-limiting step occurs:

Ribulose 1,5-bisphosphate and CO_2 are joined together by rubisco. Carboxylases are relatively slow enzymes as a family, and rubisco is one of the slowest. A 6-carbon intermediate is formed but it is unstable, and quickly breaks down to yield two molecules of 3-phosphoglycerate. Some familiar enzymes (from glycolysis, although this is happening in the stroma, not the cytoplasm) now come into play.

Phosphoglycerate kinase phosphorylates 3-PG to 1,3-bisphosphoglycerate. 1,3-BPG is then reduced by glyceraldehyde-3-phosphate dehydrogenase to form glyceraldehyde-3-P. This step requires the energy released from oxidation of NADPH. A small portion ($1/6^{th}$) of the GAP that is made is then exported from the chloroplast and will be used to form more complex carbohydrates. However, the majority is recycled through the recovery phase of the Calvin cycle to regenerate NADP.

As if having a central enzyme that moves at a snail's pace and needing to recycle the majority of its potential product was not bad enough, C3 plants also have to contend with the hijacking of rubisco for a competing, and energy-wasting, set of reactions known as *photorespiration*. Under conditions of low CO_2 and high O_2 in the local atmosphere, oxygen, instead of carbon dioxide, binds to rubisco and forms 3-PG and 2-phosphoglycolate from its reaction with RuBP. As detailed in figure 7, the 2-phosphoglycolate is dephosphorylated to glycolate and transported out of the chloroplast. From there, it undergoes a series of reactions in the peroxisomes and mitochondria to transform it to 3-PG, which can then go in the chloroplast and participate in the Calvin cycle. Unfortunately for the cell, in the course of these reactions, NADH and ATP are used, thus lowering the energy availability inside the cell.

This is a particular problem in hot climates, because the oxygenase activity of rubisco increases more than the carboxylase activity as the temperature increases. This leads to an interesting side effect: in C3 plants, as the temperature rises and CO_2 is outcompeted by O_2 for rubisco binding, the stomata of the leaves need to remain open for longer in order to allow for acquisition of enough CO_2 from the atmosphere. This in turn allows more water vapor from inside the cell to escape, leading to dehydration. C3 plants are thus at a competitive disadvantage in hot dry climates in comparison to plants that do not use rubisco for carbon fixation.

What about plants adapted to such climates? C4 plants, which include some grasses, corn, sugarcane, and weeds, utilize PEP carboxylase (which does not have the annoying photorespiratory capabilities of rubisco and a higher affinity for CO_2) to fix carbon dioxide to PEP, making oxaloacetate. In an interesting twist, the oxaloactetate, after conversion to malate, is decarboxylated to yield CO_2 again, which is fed to rubisco and the calvin cycle. The C4 mechanism, also called the Hatch-Slack pathway, utilizes two

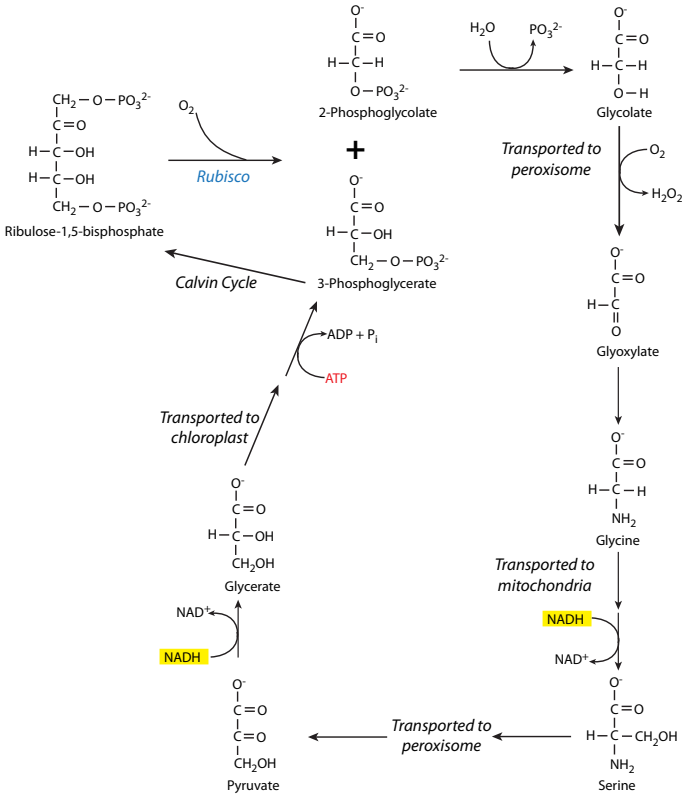


Figure 7. Photorespiration. Rubisco can catalyze the addition of O2 to RuBP, producing 3PG, which can be used by the Calvin cycle, and 2-phosphoglycolate, which is converted to glycolate, transported out of the chloroplast, converted to glycerate over several steps in the peroxisome and mitochondria, and shipped back to the chloroplast. This is an energetically expensive process (note NADH and ATP used).

PEP carboxylase actually fixes HCO_3^- to PEP rather than CO_2 directly. The atmospheric CO_2 is converted to the bicarbonate by carbonic anhydrase.

sets of cells, an outer layer (mesophyll) that takes in air and fixes the CO2 to PEP and produces malate, and an inner layer of cells (bundle sheath) that takes the malate, and decarboxylates it for its rubisco enzyme. The two cells are connected via plasmodesmata (see Cell-cell Interactions chapter). Although energetically more expensive than carbon fixation by C3 plants in cooler climates, the C4 pathway overtakes C3 in efficiency as temperatures rise and photorespiration increases.

Desert plants go one step further than C4 plants. Living in environments that are extremely hot and dry during the day, but relatively cool at night, many desert succulents (like cacti) are diurnal, and only open their stomata at night (when temperatures are significantly lower and water evaporates far more slowly) for CO_2 gathering, which is then fixed via the CAM pathway to malate. Then in the daylight hours, CO_2 is released from the malate and used in the Calvin cycle to generate carbohydrates.

Variations of this pathway have been found in which aspartate is transported to the bundle-sheath cells instead of malate.

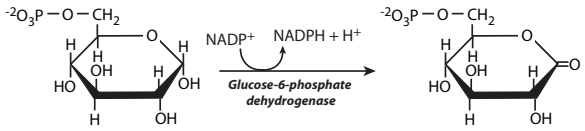
After decarboxylation of the malate by malic enzyme (NAD-dependent in some species, NADP-dependent in others) to release the CO_2 for rubisco, the resulting pyruvate is shuttled back to the mesophyll cell where it is phosphorylated by pyruvate-phosphate dikinase to generate PEP for re-entry into the C4 cycle.

The crassulacean acid metabolism (CAM) pathway is named for a carbon fixation pathway discovered in the Crassulaceae family of succulent plants including pineapples as well as various cactus species. It utilizes a similar biochemical mechanism as the C4 pathway, but occurs within a single photosynthetic cell. The major difference is that the CO_2 is only taken in at night, and it quickly turned into malate, which is stored in vacuoles until daytime. The malate is then released and decarboxylated to provide the RuBP carboxylase (rubisco) with a steady stream of CO_2 for fixation. Because there is such a rush of PEP carboxylase activity at night to fix the atmospheric CO_2 to PEP, there is a high rate of starch breakdown to provide the glucose for glycolytic generation of PEP. Interestingly, as the malate is decarboxylated in the day, its product, pyruvate, can then be used to re-synthesize glucose (see gluconeogenesis below) and then starch.

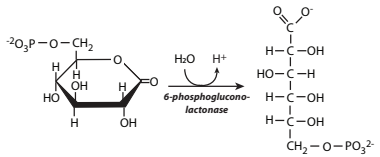
Pentose Phosphate Pathway

NADPH is found not only in plants, but in animal cells as well. Although our first discussion of NADPH was in the context of photosynthesis, it is also a general reducing agent in any cell. It is also crucial to note that though introductory texts often consider NAD^+/NADH and NADP/NADPH similarly as high energy electron carriers, and although they are structurally differentiated only by a phosphate group (on the 2'-OH of adenosine), they are *not* interchangeable in the metabolic pathways of a cell. NADP/NADPH is used in reductive metabolic pathways, whereas NAD+/NADH is used in oxidative pathways. With such an important role in biosynthesis, it is no surprise that its production is part of a major metabolic pathway, the pentose phosphate pathway (figure 7), also called the phosphogluconate pathway, and the hexose monophosphate shunt.

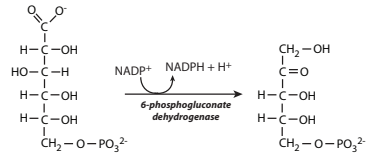
In step 1 of this pathway, glucose-6-phosphate and NADP+ are bound to glucose-6-phosphate dehydrogenase, which transfers a hydride ion from glucose-6-phosphate to NADP+ to form 6-phosphoglucono-δ-lactone and NADPH.



In step 2, the 6-phosphoglucono-δ-lactone is hydrolyzed to 6-phosphogluconate using 6-phosphogluconolactonase. This reaction actually proceeds fairly quickly even without the enzyme.



In step 3, the 6-phosphogluconate is decarboxylated by 6-phosphogluconate dehydrogenase, in the process producing more NADPH, as well as the five-carbon sugar, ribulose-5-phosphate. This metabolite is used by the cell as the basis for nucleotide synbthesis. This concludes the NADPH-producing portion of the pentose phosphate pathway.



However, it is useful, in the context of this chapter, to also consider the fate the Ru5P, which is converted to ribose-5-P by ribulose-5-P isomerase or it is converted to xylulose-5-phosphate using ribulose-5-P epimerase. The ribose-5-phosphate is used in nucleotide synthesis, so plays an important role in not only nucleic acid production, but general metabolism (e.g. for ATP).

Ribulose-5-phosphate and NADPH are the most significant products of this pathway. As mentioned earlier, NADPH is important as a general reducing agent. The mechanism for this involves glutathione and glutathione reductase. Glutathione is the primary scavenger of reactive oxygen species such as oxides and peroxides, and the key regulator of cellular oxidative stress. The reduced form of the glutathione tripeptide (Glu-Cys-Gly) dimerizes with another glutathione via disulfide bond as they donate electrons to oxidizers, and is regenerated by glutathione reductase. NADPH is a necessary cofactor for glutathione reductase activity, providing the electrons to reduce the G-S-S-G dimer.

A metabolic disorder known as G6PD deficiency manifests itself in erythrocytes, or red blood cells. NADPH in this case, is needed not for biosynthetic pathways, but to regenerate reduced glutathione (GSH). GSH is needed to detoxify peroxides through the action of glutathione peroxidase. Since the NADPH is in short supply (due to defect in G6PD), peroxides build up and cause damage to the membrane lipids. Extensive damage can lead to premature cell death by autolysis.

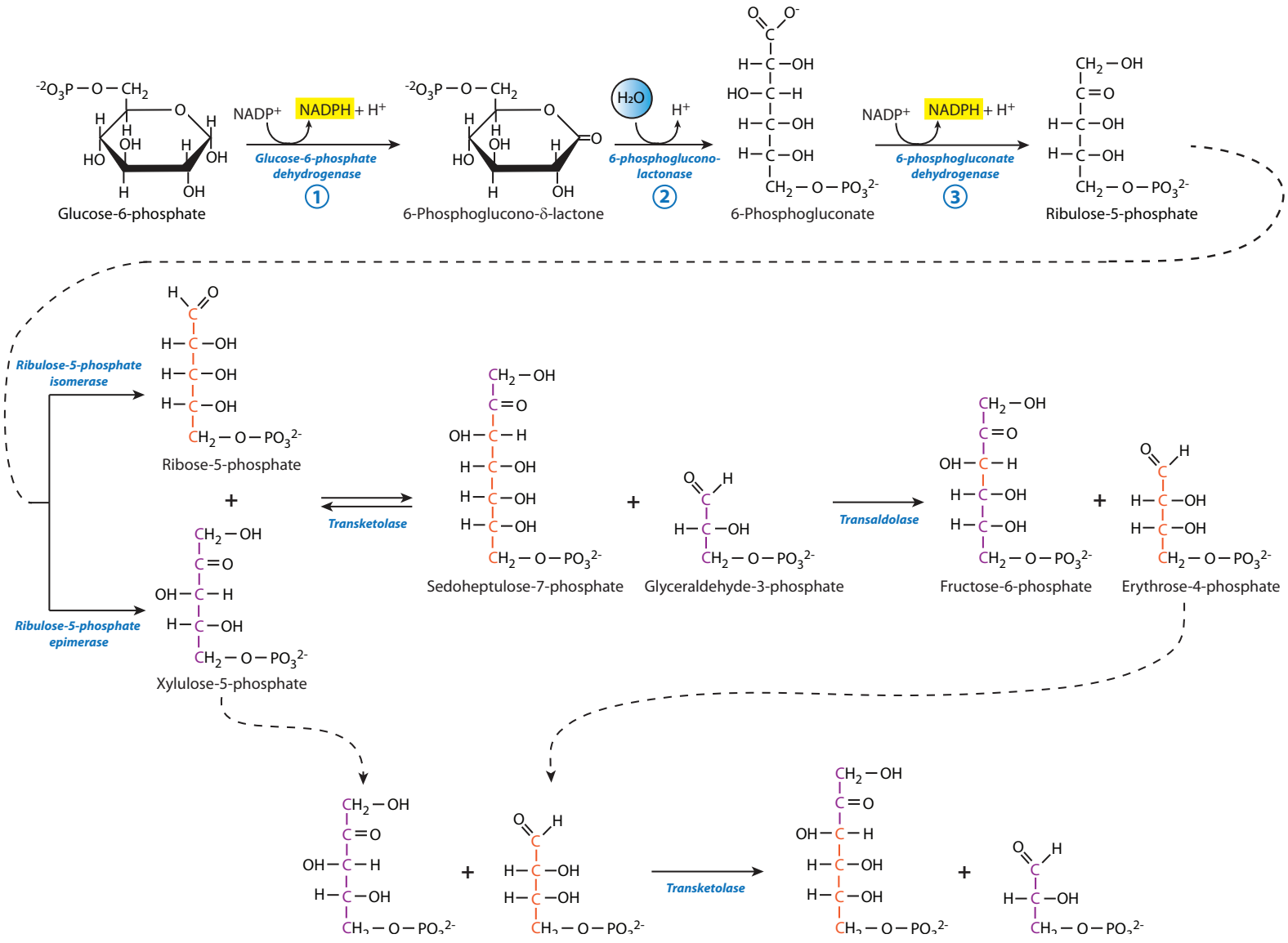


Figure 7. The pentose phosphate pathway. The first three reactions generate the energy carrier NADPH in the process of converting glucose-6-phosphate to ribulose-5-phosphate. The Ru5P is important as a precursor to nucleotide synthesis, as well as for production of other sugars and important metabolic intermediates, such as fructose-6-phosphate and glyceraldehyde-3-phosphate. Transketolase then transfers the terminal two carbons of ribulose-5-P to xylulose-5-P, making sedoheptulose-7-phosphate and G3P. Transaldolase comes up next. It transfers a 3-carbon unit from sedoheptulose-7-P to the G3P, forming erythrose-4-phosphate and fructose-6-phosphate.Transketolase is used again at this point, transferring a 2-carbon unit from xylulose-5-phosphate - to erythrose-4-phosphate and generating more G3P and fructose-6-P.

Gluconeogenesis

Having considered the initial anabolic reaction of life - carbon fixation by photosynthesis, we now turn our attention to utilizing the smaller metabolites to generate glucose and other sugars and carbohydrates. Glucose is the most important fuel for most organisms, and the only fuel for some cell types, such as brain neurons. Potential building blocks of glucose include many of the products and intermediates of glycolysis and the TCA cycle, as well as most amino acids. The key reaction is conversion of any of these compounds into oxaloactetate before using them to make glucose. In animals, the amino acids leucine and isoleucine, as well as any fatty acids, cannot be used to build glucose because they convert first to acetyl-CoA, and animals have no pathway for acetyl-CoA to oxaloacetate conversion. Plants, on the other hand, can push acetyl-CoA to oxaloacetate through the glyoxylate cycle, which will be discussed shortly.

The process of gluconeogenesis is in many ways the simple opposite of glycolysis, so it is not surprising that some of the enzymes used in glycolysis are the same as those used for gluconeogenesis. However, there are a few exceptions. These arose (and have probably evolved) for two major reasons - (1) the thermodynamics of the reaction are prohibitive, and (2) the need for independent control of the catabolic and anabolic processes. Since there is this parallel, we will explore gluconeogenesis first by starting with one of the major products of glycolysis, pyruvate. Pyruvate can be converted to oxaloacetate by pyruvate carboxylase, in a reaction requiring ATP hydrolysis. The oaxaloacetate is then converted to phosphoenolpyruvate (PEP) by PEP carboxykinase, which also uses nucleotide triphosphate hydrolysis for energy, though this time it is GTP.

As shown in the summary/comparison (figure 8), from the formation of PEP to the formation of fructose-1,6-bisphosphate the enzymes used in gluconeogenesis are exactly the same enzymes used in glycolysis. This works because the free energy change in those reactions is relatively small. However, in the dephosphorylation of fructose-1,6-bisphosphate to to fructose-6-phosphate, and subsequently in the dephosphorylation of glucose-6-phosphate to glucose, there is a large free energy change that works against the gluconeogenic reactions. Thus, the enzymes that drive these reactions are different from the enzymes that drive the reverse reactions in glycolysis (i.e. hexokinase, phosphofructokinase). These two hydrolytic reactions are catalyzed by *fructose bisphosphatase* and *glucose-6-phosphatase*, respectively. Full reversal of glycolysis in animals is limited, however, to liver and kidney, since they are the only tissues that express glucose-6-phosphatase. Other tissues use different mechanisms for generating glucose (e.g. glycogenolysis).

Interestingly, PEP carboxykinase (PEPCK) is unregulated at the protein level. There are no known activators or inhibitors of its activity. The only regulation of PEPCK appears to be at the level of transcription: glucagon can stimulate it (as can gluocorticoids and thyroid hormone), while insulin can inhibit it. The other gluconeogenic enzymes, though, do have direct activators and inhibitors. They are allosteric modulators, binding away from, but influencing the shape and efficacy of the substrate binding site. In examining the regulation of these enzymes, one important regulator stands out because it is not a metabolite of either glycolysis or gluconeogenesis. Fructose-2,6-bisphosphate (F2,6P) is an activator of phosphofructokinase, and an inhibitor of fructose bis-phosphatase. F2,6P levels are controlled by fructose-bisphosphatase-2 and phosphofructokinase-2, which are themselves controlled by levels of fructose-6-phosphate, as well as through a hormone-driven signaling cascade shown in the figure on the next page.

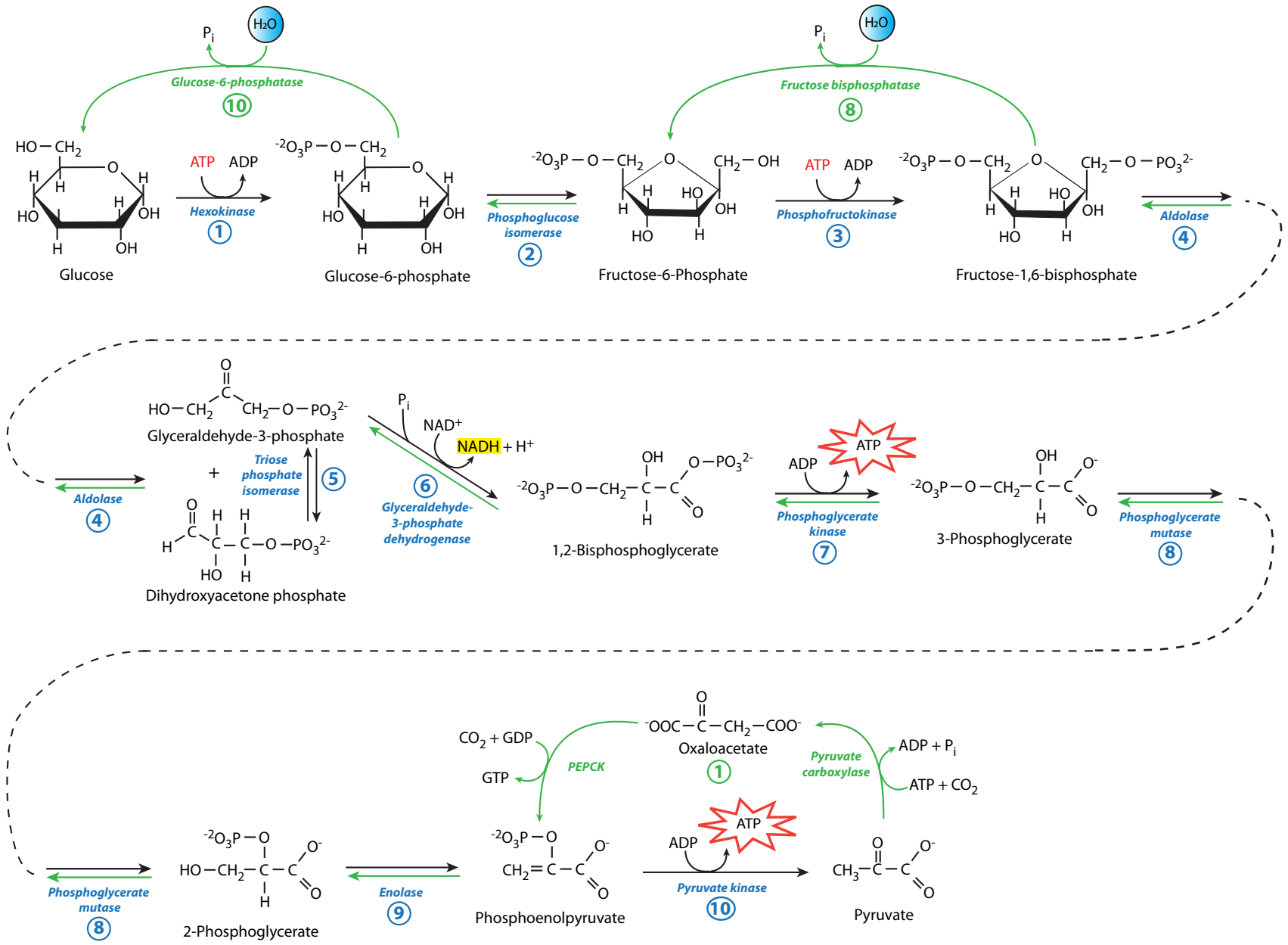


Figure 8. Gluconeogenesis (shown in green arrows) shares some, but not all enzymes with the reverse process, glycolysis (black arrows).

The glyoxylate cycle provides a mechanism for plants to convert acetyl-CoA into oxaloacetate, and therefore contribute to gluconeogenesis. This allows them to convert fatty acids and the hydrophobic amino acids leucine and isoleucine into glucose when necessary. The ability to do this comes from a plant-specific organelle called the glyoxysome, as well as some mitochondrial enzymes. The glyoxysomal part of the cycle consists of five steps, of which the first three contribute to the conversion, while the last two steps regenerate the glyoxysomal oxaloacetate (figure 9).

Once the macromolecules have been broken down to acetyl-CoA, they enter the glyoxysome and combine with oxaloacetate to make citrate. This is catalyzed by citrate synthase just as in the mitochondrial TCA cycle. The next reaction also uses a familiar enzyme: aconitase catalyzes the conversion of citrate to isocitrate. However, the aconitase is a cytosolic enzyme, so the citrate is transported out of the glyoxysome and then the isocitrate transported back in.

At this point, the glyoxysomal-specific enzyme, isocitrate lyase, hydrolyzes isocitrate to yield succinate and glyoxylate. The succinate is transported to the mitochondrion, where TCA cycle enzymes convert it to fumarate and then malate, which is transported out to the cytosol. In the cytosol, the malate is converted to oxaloactetate through malate dehydrogenase, and gluconeogenesis can proceed.

The glyoxylate is acted upon by another glyoxysomal enzyme, malate synthase, which adds it to acetyl-CoA to form malate.

The final step of the glyoxysomal portion of the glyoxylate cycle is oxidation of the malate to oxaloacetate by glyoxysomal malate dehydrogenase.

So, to summarize, the pool of oxaloacetate within the glyoxysome is used and regenerated within the glyoxysome. Acetyl-CoA is converted to succinate within the glyoxysome, but then goes to the mitochondrion for conversion to malate, and finally the cytosol for conversion to a separate pool of oxaloacetate that is then used in gluconeogenesis.

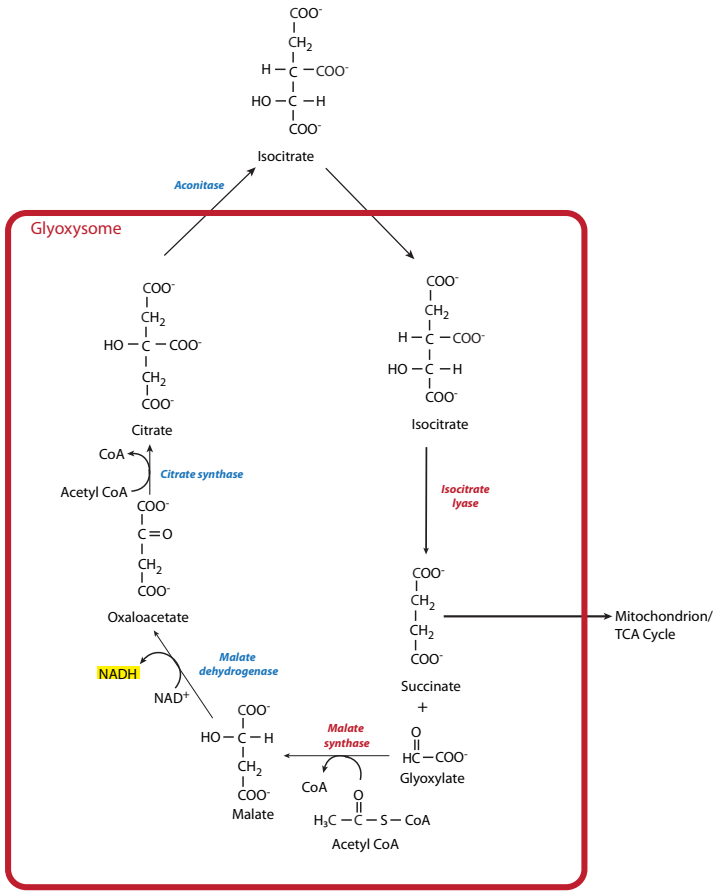


Figure 9. The glyoxylate cycle.

Glycogen synthesis

Although glucose is the primary fuel for cells, it is not an efficient molecule for long-term storage in complex (i.e. greater than single-celled) organisms. Therefore, in both plants and animals, the glucose molecules are linked together to form polysaccharides known as glucans. In animals, the glucan formed is glycogen, which consists of glucose molecules linked by α(1->4) glycosidic bonds, and branching α(1->6) bonds approximately between 8 to 14 residues apart. The average size of a glycogen unit is a cytoplasmic granule containing over 100000 glucose molecules. The addition of a glucose-1-phosphate to another (or to a glycogen chain) is energetically unfavorable, so it must be coupled with a sufficiently exergonic reaction to proceed.

Glycogen synthesis begins with UDP-glucose phosphorylase, which combines the nucleotide uridine triphosphate (UTP) with glucose-1-phosphate to release pyrophosphate (PPi) and form UDP-glucose.

The phosphoanhydride exchange reaction catalyzed by UDP-glucose phosphorylase is minimally exergonic. However, the pyrophosphate released is quickly hydrolyzed by inorganic pyrophosphatase, a ubiquitous cytosolic enzyme, in a highly exergonic reaction. This pyrophosphate hydrolysis is a mechanism utilized in many biosynthetic pathways to provide energy for otherwise endergonic reactions.

In the next step, glycogen synthase attaches the UDP-glucose to the pre-existing glycogen chain with an α(1->4) linkage. It cannot join two individual glucoses together, only add to a pre-existing chain. This means that there must be some workaround for the frst two glucoses: glycogenin is an enzyme that catalyzes the addition of UDP-glucose to itself, and can do so for up to seven UDP-glucose molecules, thus forming a short primer for glycogen synthase to work with. Furthermore, glycogen synthase can only add glucoses with an α(1->4) link. For branching to occur, a branching enzyme (specifically, amylo-(1,4->1,6)-transglycosylase is needed. This enzyme can transfer terminal chain segments to the 6-carbon hydroxyl of any glucose in a glycogen chain. However, the branches can only be added if there are at least 4 glucose residues between them, and if the originating chain was at least 11 residues in length.

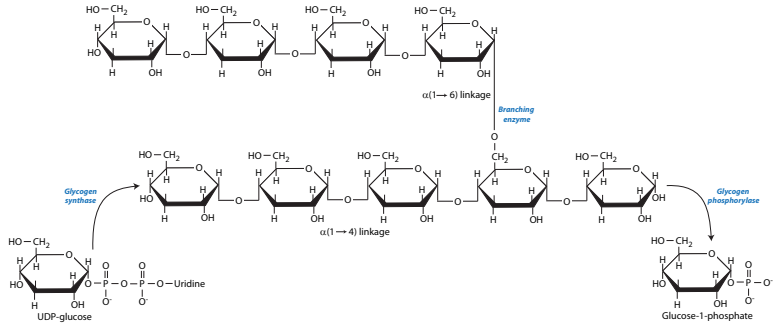


Figure 10. Glycogen synthesis

Oligosaccharide synthesis

Like glycogen synthesis, oligosaccharide synthesis also requires the initial step of coupling the sugar with a nucleotide. In mammals, a major disaccharide is lactose, which is the linkage of a galactose and a glucose, and the formation is catalyzed by lactose

synthase. However, before the lactose synthase is able to act, the galactose must first be in the form of a UDP-galactose. Similarly, in plants, the major disaccharide is sucrose, formed by the linkage of UDP-glucose and fructose-6-phosphate. This results in sucrose-6-phosphate, which is then readily dephosphorylated to sucrose. These kinds of mechanisms are also used in the glycosylation of proteins and lipids, which will be discussed primarily in the protein processing and trafficking chapter.

The major hexose species besides glucose are fructose, mannose, and galactose. Interconversion between these hexoses can occur via intermediates, as demonstrated in glycolysis (glucose-6-P to fructose-6-P). Mannose-6-P can be converted to fructose-6-P by phosphomannose isomerase. Galactose can be converted similarly, to galactose-1-P and then to glucose-1-P. The galactose to glucose conversion can also take place by epimerization of UDP-Glucose to UDP-galactose via intermediate redox using NAD^+/NADH.

Mutation of galactose-1-phosphate uridylyltransferase or mutations of other enzymes in this pathway (uridylyl transferase mutations are most common and usually most severe) can lead to galactosemia, a human genetic disease whose symptoms begin in infancy and may include mental retardation, liver damage, jaundice, vomiting, and lethargy. The cause of these symptoms is generally a buildup of galactose-1-phosphate, especially in the liver and nervous tissue. Fortunately, with early diagnosis, the symptoms can be prevented by avoiding milk products (lactose).

Fatty acid synthesis

This anabolic process is accomplished using a different set of enzymes than the catabolism of fatty acids discussed earlier. Fatty acid synthesis (fig. 11) starts with the formation of palmitic acid (C16) from acetyl-CoA and malonyl-CoA (which is itself a 3-carbon molecule formed from acetyl-CoA). Another difference between the catabolic and anabolic reactions for fatty acids is the location: whereas we saw that catabolism occurs largely in the mitochondria, fatty acid synthesis is run from a single large cytoplasmic enzyme complex. The fatty acid synthase system is comprised of seven enzymes linked together with an acyl carrier protein (ACP). As mentioned, this complex is found in the cytoplasm, so its substrates must be as well. The acetyl-CoA in the cytoplasm is primarily derived from the mitochondrial acetyl-CoA via a citrate-malate shuttle that couples deacetylation in the mitochondrion with acetylation in the cytosol.

The acetyl-CoA and malonyl-CoA are linked to the synthase and ACP, then there is a sequence of acetyl group transfers that runs a total of seven times to form palmitoyl-ACP, from which the palmitic acid is finally released. Palmitic acid is the precursor for variety of long-chain fatty acids such as stearic acid, palmitoleic acid, and oleic acid. Generally, there is either an elongation or sometimes a desaturation step. However, desaturation is a tricky process for vertebrates. The desaturation at C9 to form oleic acid from stearic acid can occur; however, other desaturations such as desaturation at C-12 to generate linoleic acid are not possible in vertebrates. Interestingly, they can be carried out in plant species. Furthermore, even though linoleic acid cannot be synthesized by vertebrates, it is nevertheless needed by vertebrates, which build arachidonic

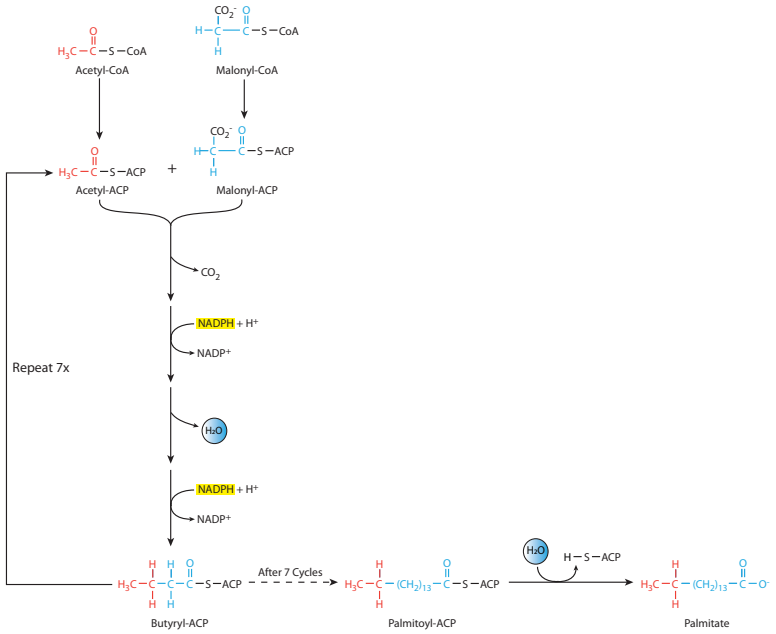


Figure 11. Fatty Acid Synthesis

acid, prostaglandins, and other molecules from it. Linoleic acid is therefore considered an *essential* fatty acid, since it must be ingested by the animal.

These fatty acids are then used to form the triacylglycerols that form the bulk of the energy storage molecules in most animals. Triacylglycerols are synthesized by the reaction of fatty acyl-CoA chains with glycerol-3-phosphate. Two rounds of this reaction yields diacylglygerol-3-phosphate (phosphatidic acid). After the action of phosphatidate phosphatase, the phosphatidic acid is converted to 1,2-diacylglycerol. This reacts with fatty acyl-CoA to form the final triacyglycerol.

Each of the fatty acyl chain additions generates an ester bond, which requires a significant energy input: that energy comes from a linked ATP hydrolysis reaction for each chain addition.

Amino acid synthesis

In humans, only half of the standard amino acids (Glu, Gln, Pro, Asp, Asn, Ala, Gly, Ser, Tyr, Cys) can be synthesized (fig. 12 and 13), and are thus classified the nonessential amino acids. Within this group, the first three, glutamate, glutamine, and proline, have a shared anabolic pathway. It begins with glutamate dehydrogenase, which adds ammonia to α-ketoglutarate in the presence of NADPH to form glutamate. This is a key reaction for all amino acid synthesis: glutamate is a nitrogen (amino group) donor for the production of all the other amino acids.

Glutamine synthetase catalyzes the formation of glutamine from glutamate and ammonia. This is an important biochemical reaction for a completely different reason: it is the primary route for ammonia detoxification.

Proline is synthesized from glutamate in a two-step process that begins with the reduction of glutamate to a semialdehyde form that spontaneously cyclizes to D-pyrroline-5-carboxylate. This is reduced by pyrroline carboxylate reductase to proline.

Alanine and Aspartate are the products of glutamate-based transamination of pyruvate and oxaloacetate, respectively.

Asparagine is synthesized through one of two known pathways. In bacteria, an asparagine synthetase combines aspartate and ammonia. However, in mammals, the aspartate gets its amino group from glutamine.

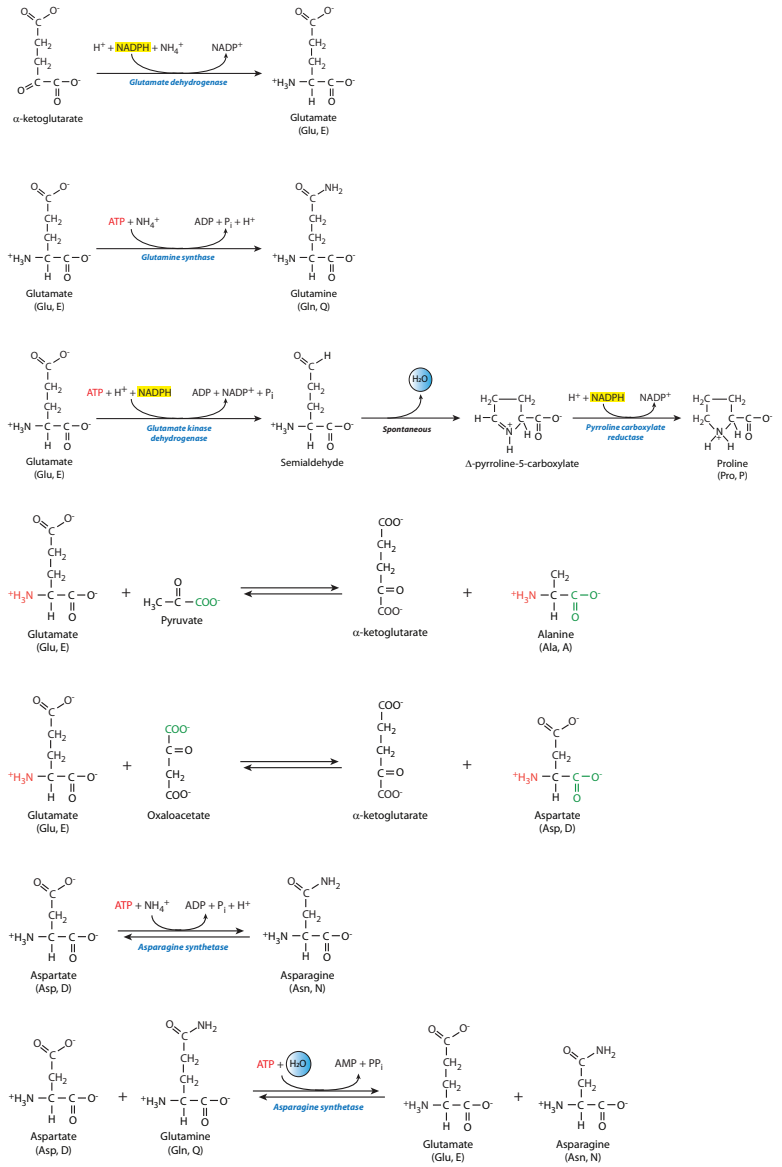


Figure 12. Synthetic reaction for amino acids: Glutamate, Glutamine, Proline, Alanine, Aspartate, Asparagine.

The synthesis of serine begins with the metabolic intermediate 3-phosphoglycerate (glycolysis). Phosphoglycerate dehydrogenase oxidizes it to 3-phosphohydroxypyruvate. An amino group is donated by glutamate in a reaction catalyzed by phosphoserine transaminase, forming 3-phosphoserine, and finally the phosphate is removed by phosphoserine phosphatase to produce serine.

Serine is the immediate precursor to glycine, which is formed by serine hydroxymethyltransferase. This enzyme requires the coenzyme tetrahydrofolate (THF), which is a derivative of vitamin B9 (folic acid).

Serine is also a precursor for cysteine, although the synthesis of cysteine actually begins with the essential amino acid methionine. Methionine is converted to S-adenosylmethionine by methionine adenosyltransferase. This is then converted to S-adenosylhomocysteine by a member of the SAM-dependent methylase family. The sugar is removed by adenosylhomocysteinase, and the resultant homocysteine is connected by cystathionine synthase to the serine molecule to form cystathionine. Finally, cystathionine-γ-lyase catalyzes the production of cysteine.

Tyrosine is another amino acid that depends on an essential amino acid as a precursor. In this case, phenylalanine oxygenase reduces phenylalanine to produce the tyrosine.

In general, the synthesis of essential amino acids, usually in microorganisms, is much more complex than for the nonessential amino acids and is best left to a full-fledged biochemistry course.

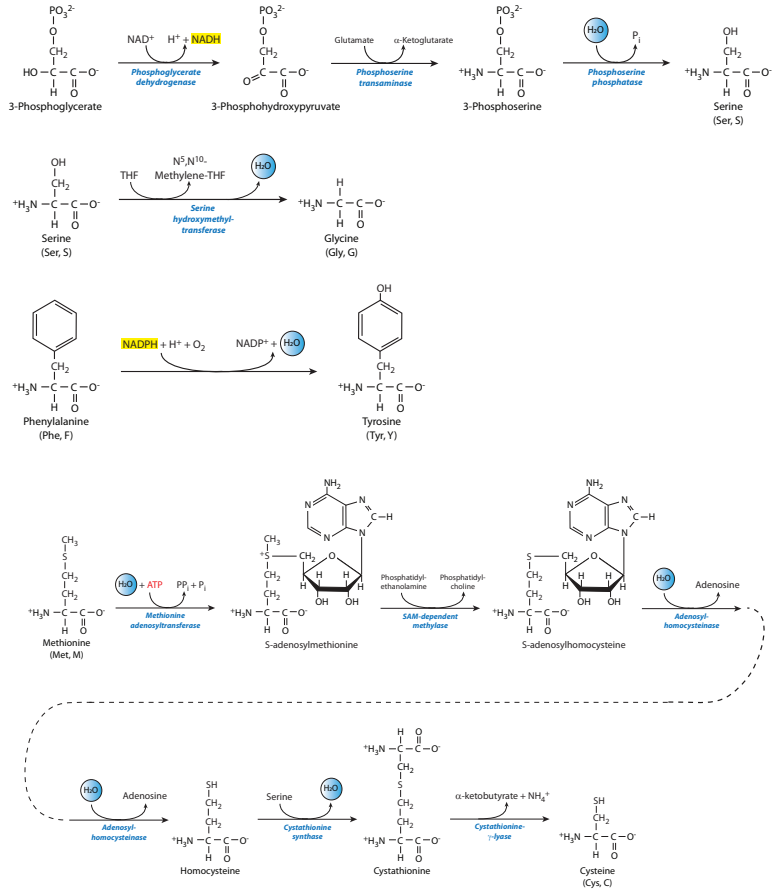


Figure 13. Synthetic reactions for amino acids: Serine, Glycine, Tyrosine, and Cysteine.