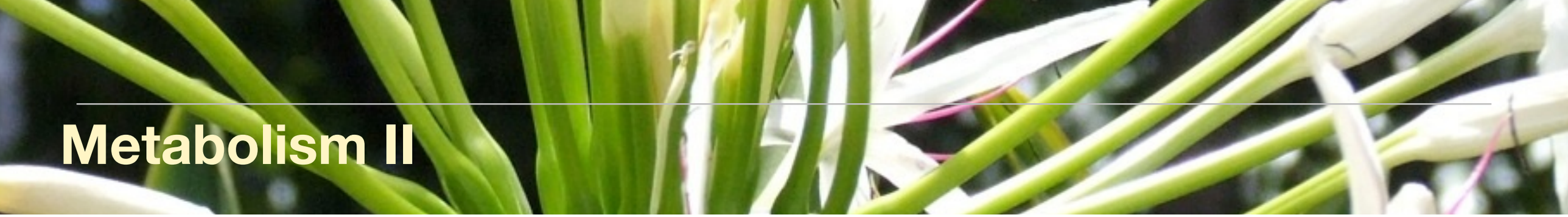
Chapter 7

Metabolism II

In this second section of metabolism, we cover metabolic pathways that do not have a strong emphasis on oxidation/reduction.



Metabolism II

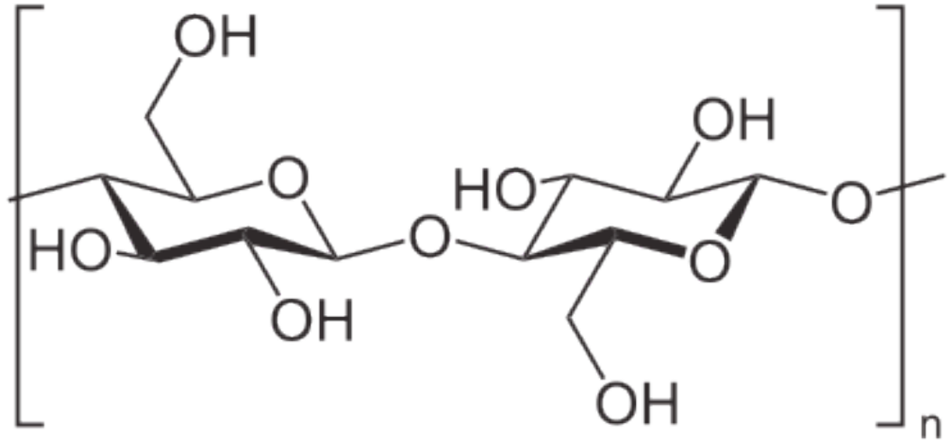
In the last chapter, we focused on metabolic pathways that played important oxidative/reductive roles relative to cellular energy. In this chapter, the pathways that we cover have lesser roles from an energy perspective, but important roles, nonetheless, in catabolism and anabolism of building blocks of proteins and nucleic acids, nitrogen balance, and sugar balance. In a sense, these might be thought of as the "kitchen sink" pathways, but it should be noted that all cellular pathways are important.

Carbohydrate Storage/Breakdown

Carbohydrates are important cellular energy sources. They provide energy quickly through glycolysis and passing of intermediates to pathways, such as the citric acid cycle, amino acid metabolism (indirectly), and the pentose phosphate pathway. It is important, therefore, to understand how these important molecules are made.

Plants are notable in storing glucose for energy in the form of amylose and amylopectin (see HERE) and for structural integrity in the form of cellulose (see HERE). These structures differ in that cellulose contains glucoses solely joined by beta-1,4 bonds, whereas amylose has only alpha1,4 bonds and amylopectin has alpha 1,4 and alpha 1,6 bonds.

Animals store glucose primary in liver and muscle in the form of a compound related to amylopectin known as glycogen. The



The Repeating Structure of Cellulose

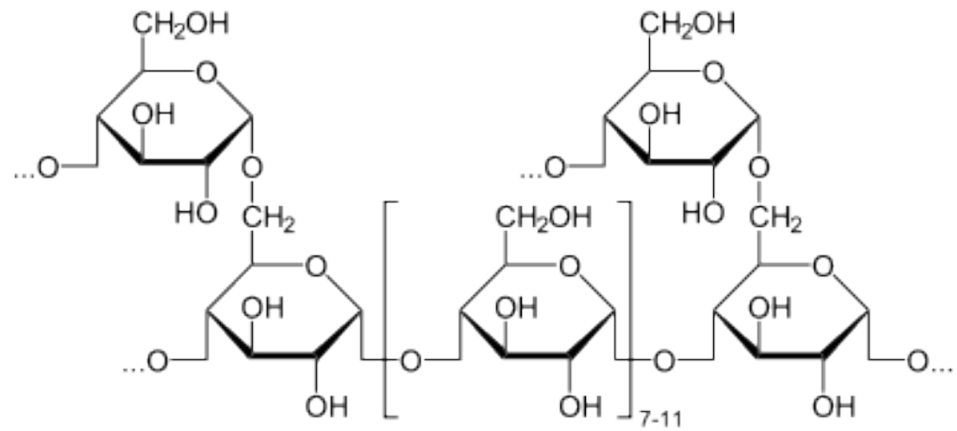
structural differences between glycogen and amylopectin are solely due to the frequency of the alpha 1,6 branches of glucoses. In glycogen they occur about every 10 residues instead of every 30-50, as in amylopectin.

Glycogen provides an additional source of glucose besides that produced *via* gluconeogenesis. Because glycogen contains so many glucoses, it acts like a battery backup for the body, providing a quick source of glucose when needed and providing a place to store excess glucose when glucose concentrations in the blood rise. The branching of glycogen is an important feature of the molecule metabolically as well. Since glycogen is broken down from the "ends" of the molecule, more branches translate to more ends, and more glucose that can be released at once.

Breakdown of glycogen involves 1) release of glucose-1-phosphate (G1P), 2) rearranging the remaining glycogen (as necessary) to permit continued breakdown, and 3) conversion of G1P to G6P for further metabolism. G6P can be 1) broken down in glycolysis, 2) converted to glucose by gluconeogenesis, and 3) oxidized in the pentose phosphate pathway.

Just as in gluconeogenesis, the cell has a separate mechanism for glycogen synthesis that is distinct from glycogen breakdown. As noted previously, this allows the cell to separately control the reactions, avoiding futile cycles, and enabling a process to occur efficiently (synthesis of glycogen) that would not occur if it were simply the reversal of glycogen breakdown.

Synthesis of glycogen starts with G1P, which is converted to an 'activated' intermediate, UDP-glucose. This activated intermediate is what 'adds' the glucose to the growing glycogen

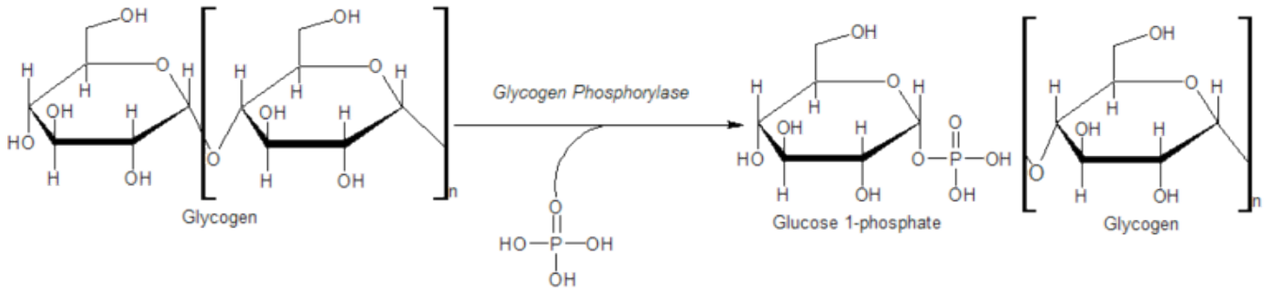


The Repeating Unit of Glycogen

chain in a reaction catalyzed by the enzyme known as glycogen synthase. Once the glucose is added to glycogen, the glycogen molecule may need to have branches inserted in it by the enzyme known as branching enzyme.

Glycogen Breakdown

Glycogen phosphorylase (sometimes simply called phosphorylase) catalyzes breakdown of glycogen into Glucose-1-Phosphate (G1P). The reaction, (see above right) that produces G1P from glycogen is a phosphorolysis, not a hydrolysis reaction. The distinction is that hydrolysis reactions use water to cleave bigger molecules into smaller ones, but phosphorolysis reactions use phosphate instead for the same purpose. Note that the phosphate is just that - it does NOT come from ATP. Since ATP is *not* used to put phosphate on G1P, the reaction saves the cell energy.



Phosphorolysis of Glycogen

Glycogen phosphorylase will only act on non-reducing ends of a glycogen chain that are at least 5 glucoses away from a branch point. A second enzyme, Glycogen Debranching Enzyme (GDE), is therefore needed to convert alpha(1-6) branches to alpha(1-4) branches. GDE acts on glycogen branches that have reached their limit of hydrolysis with glycogen phosphorylase. GDE acts to transfer a trisaccharide from a 1,6 branch onto an adjacent 1,4 branch, leaving a single glucose at the 1,6 branch. Note that the enzyme also catalyzes the hydrolysis of the remaining glucose at the 1,6 branch point. Thus, the breakdown products from glycogen are G1P and glucose (mostly G1P, however). Glucose can, of course, be converted to Glucose-6-Phosphate (G6P) as the first step in glycolysis by either hexokinase or glucokinase. G1P can be converted to G6P by action of an enzyme called phosphoglucomutase. This reaction is readily reversible, allowing G6P and G1P to be interconverted as the concentration of one or the other increases. This is important, because phosphoglucomutase is needed to form G1P for glycogen biosynthesis.

See Kevin's YouTube lectures on Glycogen Metabolism HERE, HERE, and HERE

Regulation of Glycogen Metabolism

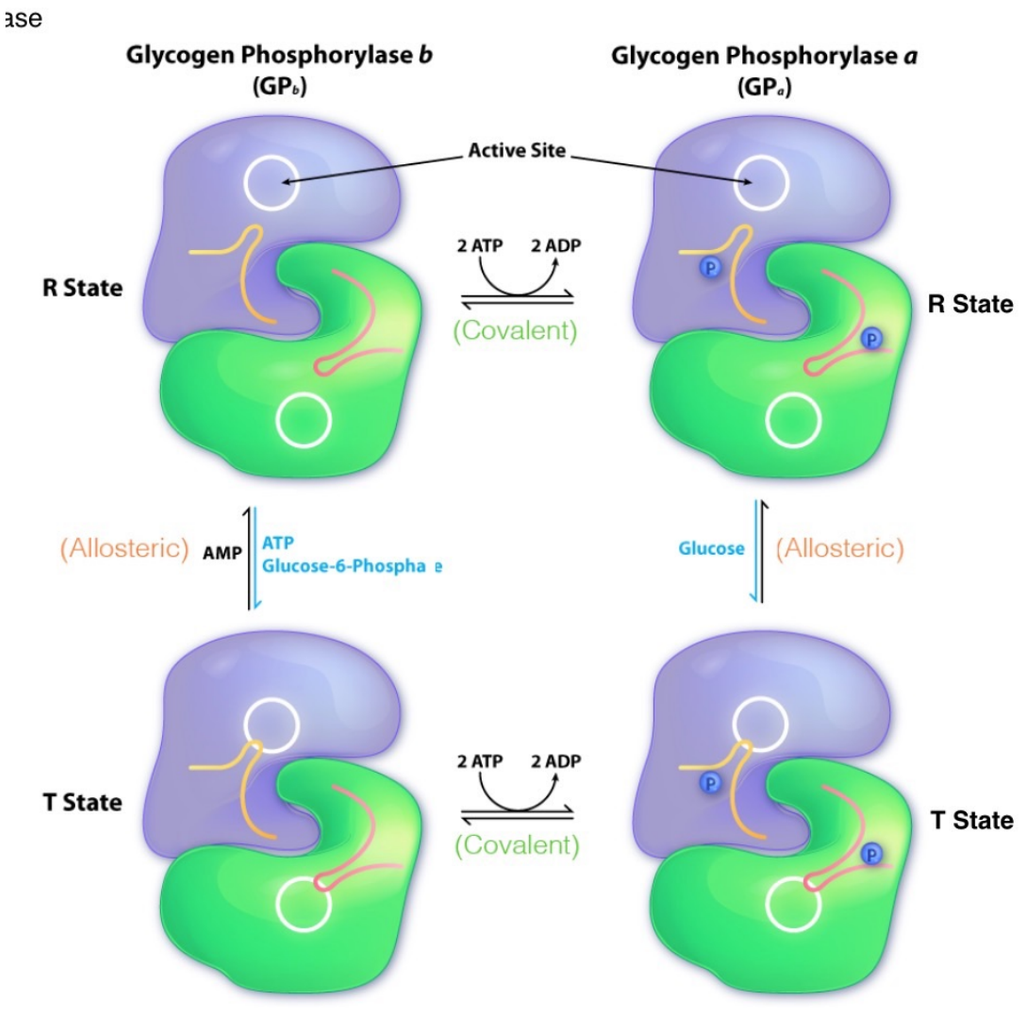
Regulation of glycogen metabolism is complex, occurring both allosterically and *via* hormone-receptor controlled events that result in protein phosphorylation or dephosphorylation. In order to avoid a futile cycle of glycogen synthesis and breakdown

simultaneously, cells have evolved an elaborate set of controls that ensure only one pathway is primarily active at a time.

Regulation of glycogen metabolism is managed by the enzymes glycogen phosphorylase and glycogen synthase. Glycogen phosphorylase is regulated by both allosteric factors (ATP, G6P, AMP, and glucose) and by covalent modification (phosphorylation/ dephosphorylation). Its regulation is consistent with the energy needs of the cell. High energy substrates (ATP, G6P, glucose) allosterically inhibit GP, while low energy substrates (AMP, others) allosterically activate it.

GPa/GPb Allosteric Regulation

Glycogen phosphorylase exists in two different covalent forms – one form with phosphate (called GPa here) and one form lacking phosphate (GPb here). GPb is converted to GPa by phosphorylation by an enzyme known as phosphorylase kinase.



Regulation of Glycogen Phosphorylase

GPa and GPb can each exist in an 'R' state and a 'T' state. For both GPa and GPb, the R state is the more active form of the enzyme. GPa's negative allosteric effector (glucose) is usually not abundant in cells, so GPa does not flip into the T state often. There is no positive allosteric effector of GPa, so when glucose is absent, GPa automatically flips into the R (more active) state.

GPb can convert from the T state to the GPb R state by binding AMP. Unless a cell is low in energy, AMP concentration is low. Thus GPb is not converted to the R state very often. On the other hand, ATP and/or G6P are usually present at high enough concentration in cells that GPb is readily flipped into the T state.

GPa/GPb Covalent Regulation

Because the relative amounts of GPa and GPb largely govern the overall process of glycogen breakdown, it is important to understand the controls on the enzymes that interconvert GPa and GPb. This is accomplished by

the enzyme Phosphorylase Kinase, which transfers phosphates from 2 ATPs to GPb to form GPa. Phosphorylase kinase has two covalent forms – phosphorylated (active) and dephosphorylated (inactive). It is phosphorylated by the enzyme Protein Kinase A (PKA). Another way to activate the enzyme is with calcium. Phosphorylase kinase is dephosphorylated by the same enzyme, phosphoprotein phosphatase, that removes phosphate from GPa.

PKA is activated by cAMP, which is, in turn produced by adenylate cyclase after activation by a G-protein. G-proteins are activated ultimately by binding of ligands to specific 7-TM receptors, also known as G-protein coupled receptors. These are discussed in greater detail in Chapter 8. Common ligands for these receptors include epinephrine (binds beta-adrenergic receptor) and glucagon (binds glucagon receptor). Epinephrine exerts it greatest effects on muscle and glucagon works preferentially on the liver.

Turning Off Glycogen Breakdown

Turning OFF signals is as important, if not more so, than turning them ON. The steps in the glycogen breakdown regulatory pathway can be reversed at several levels. First, the ligand can leave the receptor. Second, the G-proteins have an inherent GTPase activity that serves to turn them off over time. Third, cells have phosphodiesterase (inhibited by caffeine) for breaking down cAMP. Fourth, an enzyme known as phosphoprotein phosphatase can remove phosphates from phosphorylase kinase (inactivating it) AND from GPa, converting it to the much less active GPb.

Glycogen Synthesis

The anabolic pathway contrasting with glycogen breakdown is that of glycogen synthesis. Just as cells reciprocally regulate glycolysis and gluconeogenesis to prevent a futile cycle, so too do cells use reciprocal schemes to regulate glycogen breakdown and synthesis. Let us first consider the steps in glycogen synthesis. 1) Glycogen synthesis from glucose involves phosphorylation to form G6P, and isomerization to form G1P (using phosphoglucomutase common to glycogen breakdown). G1P is reacted with UTP to form UDP-glucose in a reaction catalyzed by UDP-glucose pyrophosphorylase. Glycogen synthase catalyzes synthesis of glycogen by joining carbon #1 of the UDPG-derived glucose onto the carbon #4 of the non-reducing end of a glycogen chain. to form the familiar alpha(1,4) glycogen links. Another product of the reaction is UDP.

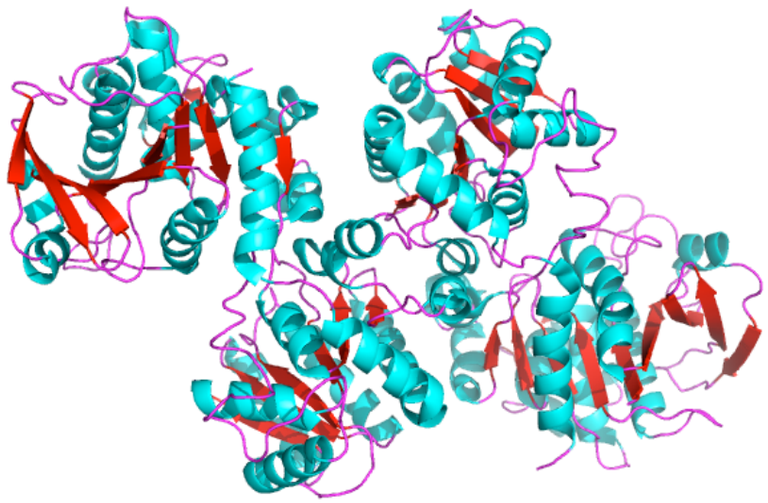
It is also worth noting in passing that glycogen synthase will only add glucose units from UDPG onto a preexisting glycogen chain that has at least four glucose residues. Linkage of the first few glucose units to form the minimal "primer" needed for glycogen synthase recognition is catalyzed by a protein called glycogenin, which attaches to the first glucose and catalyzes linkage of the first eight glucoses by alpha(1,4) bonds. 3) The characteristic alpha(1,6) branches of glycogen are the products of an enzyme

known as Branching Enzyme. Branching Enzyme breaks alpha(1,4) chains and carries the broken chain to the carbon #6 and forms an alpha(1,6) linkage.

Regulation of Glycogen Synthesis

The regulation of glycogen biosynthesis is reciprocal to that of glycogen breakdown. It also has a cascading covalent modification system similar to the glycogen breakdown system described above. In fact, part of the system is identical to glycogen breakdown. Epinephrine or glucagon signaling can stimulate adenylate cyclase to make cAMP, which activates PKA, which activates phosphorylase kinase.

In glycogen breakdown, phosphorylase kinase phosphorylates GPb to the more active form, GPa. In glycogen synthesis, protein kinase A phosphorylates the active form of glycogen synthase (GSa), and converts it into the usually inactive b form (called GSb). Note the conventions for glycogen synthase and glycogen phosphorylase. For both enzymes, the more active forms are called the 'a' forms (GPa and GSa) and the less active forms are called the 'b' forms (GPb and GSb). The *major* difference, however, is that GPa has a phosphate, but GSa does not and GPb has no phosphate, but GSb does. Thus phosphorylation and dephosphorylation have *opposite effects* on the enzymes of glycogen metabolism. This is the hallmark of reciprocal regulation. It is of note that the less active glycogen synthase form, GSb, can be activated by G6P. Recall that G6P had the exactly opposite effect on GPb.

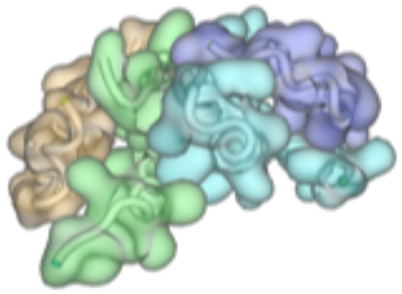


Glycogen Synthase

Glycogen synthase, glycogen phosphorylase (and phosphorylase kinase) can be dephosphorylated by several enzymes called phosphatases. One of these is called Protein Phosphatase and it is activated when insulin binds to a receptor in the cell membrane. It causes PP to be activated, stimulating dephosphorylation,

See Kevin's YouTube lectures on Glycogen Metabolism HERE, HERE, and HERE

Interactive 7.1



The 3D Structure of Insulin

and thus activating glycogen synthesis and inhibiting glycogen breakdown. Again, there is reciprocal regulation of glycogen synthesis and degradation.

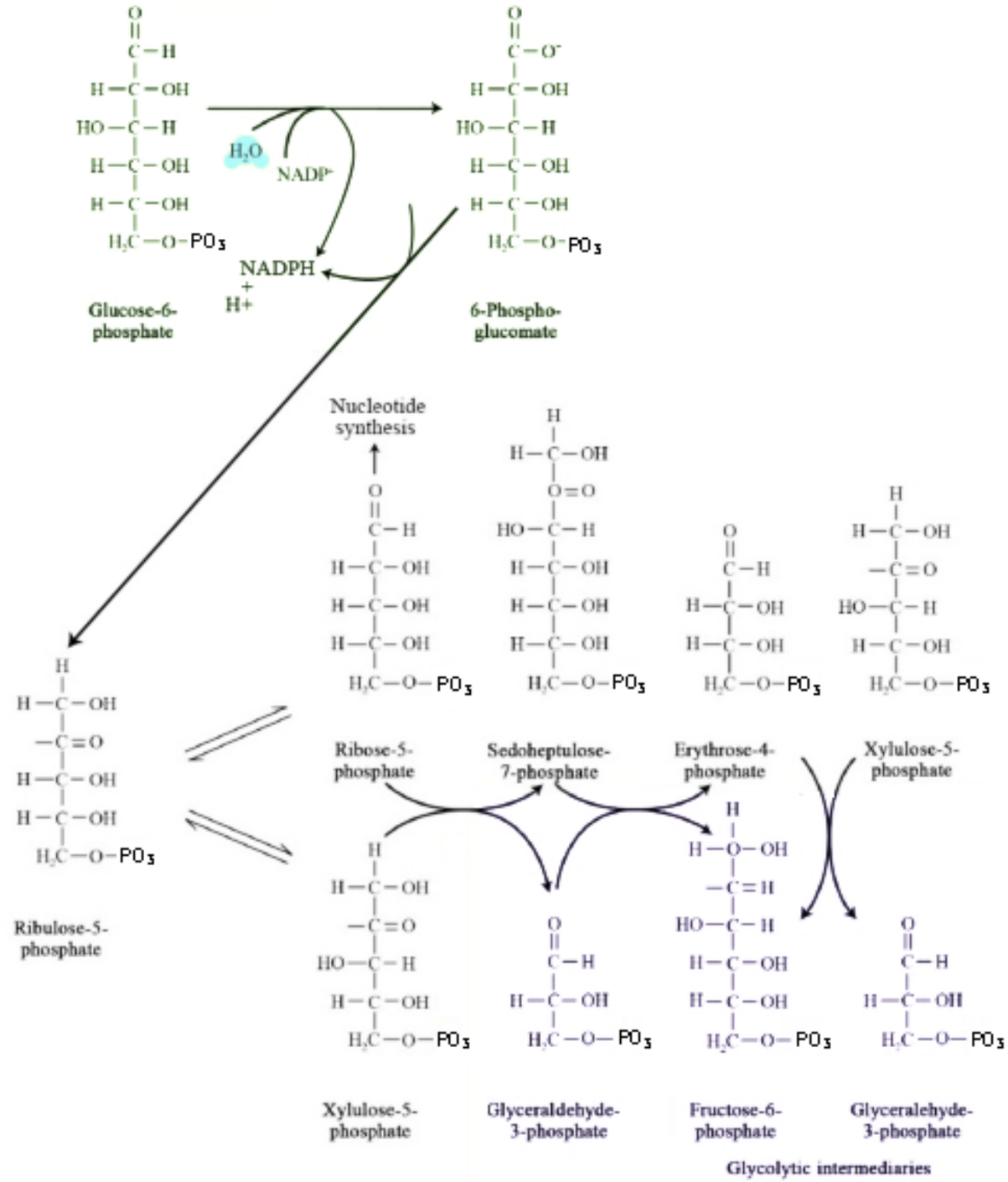
Maintaining Blood Glucose Levels

After a meal, blood glucose levels rise and insulin is released. It simultaneously stimulates uptake of glucose by cells and incorporation of it into glycogen by activation of glycogen synthase and inactivation of glycogen phosphorylase. When blood glucose levels fall, GPa gets activated (stimulating glycogen breakdown to raise blood glucose) and GSb is formed (stopping glycogen synthesis).

Pentose Phosphate Pathway

The Pentose Phosphate Pathway (PPP) is one that many students are confused by. Perhaps the reason for this is that it doesn't really have a single direction in which it proceeds, as will be apparent below.

Portions of the PPP are similar to the Calvin Cycle of plants, also known as the dark reactions of photosynthesis. We discuss these reactions separately in the next section. The primary functions of the PPP are to produce NADPH (for use in anabolic reductions), **ribose-5-phosphate** (for making nucleotides), and **erythrose-4-phosphate** (for making aromatic amino



Pentose Phosphate Pathway

acids). Three molecular intermediates of glycolysis can funnel into PPP (or be used as usual in glycolysis). They include G6P, fructose-6-phosphate (in two places), and glyceraldehyde-3-phosphate (also in two places).

A starting point for the pathway (though there are other entry points) is the oxidative phase. It includes two reactions generating NADPH. In the first of these, oxidation of glucose-6-phosphate (catalyzed by **glucose-6-phosphate dehydrogenase**), produces NADPH and 6-phosphogluconolactone. 6-phosphogluconolactone spontaneously gains water and loses a proton to become **6-phosphogluconate**. Oxidation of this produces **ribulose-5-phosphate** and another NADPH and releases CO_2. The remaining steps of the pathway are known as the non-oxidative phase and involve interconversion of sugar phosphates.

I need erythrose phosphate
And don't know what to do
My cells are full of G-6-P
Plus NADP too

But I just hit upon a plan
As simple as can be
I'll run reactions through the path
That's known as PPP

In just two oxidations
There's ribulose-5P
Which morphs to other pentoses
Each one attached to P

The next step it is simple
Deserving of some praise
The pentose carbons mix and match
Thanks to transketolase

Glyceraldehyde's a product
Sedoheptulose is too
Each with a trailing phosphate
But we are not quite through

Now three plus seven is the same
As adding six and four
By swapping carbons back and forth
There's erythrose-P and more

At last I've got the thing I need
From carbons trading places
I'm happy that my cells are full
Of these transaldolases

For example, ribulose-5-phosphate is converted to ribose-5-phosphate (R5P) by the enzyme **ribulose-5-phosphate isomerase**. Alternatively, ribulose-5-phosphate can be converted to **xylulose-5-phosphate** (Xu5P). R5P and Xu5P (10 carbons total) can be combined and rearranged by **transketolase** to produce intermediates with 3 and 7 carbons (glyceraldehyde-3-phosphate and **sedoheptulose-7-phosphate**, respectively). These last two molecules can, in turn be rearranged by **transaldolase** into 6 and 4 carbon sugars (fructose-6-phosphate and erythrose-4-phosphate, respectively). Further, the erythrose-4-phosphate can swap parts with Xu5P to create glyceraldehyde-3-phosphate and fructose-6-phosphate.

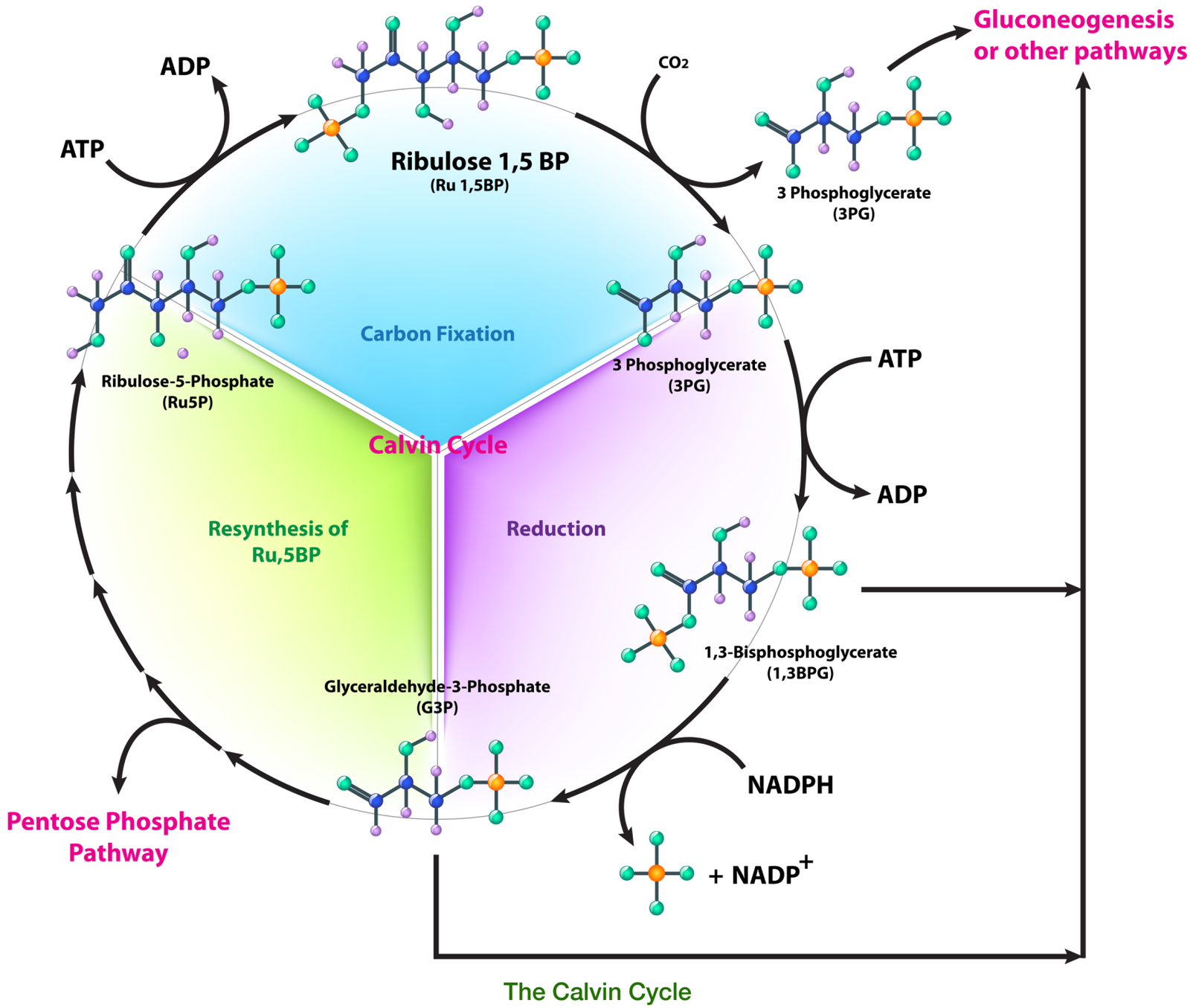
It is important to recognize that the

PPP pathway is not a “top-down” pathway, with all the intermediates derived from a starting G6P. All of the reactions are reversible, so that, for example, fructose-6-phosphate and glyceraldehyde-3-phosphate from glycolysis can reverse the last reaction of the previous paragraph to provide a means of synthesizing ribose-5-phosphate non-oxidatively. The pathway also provides a mechanism to cells for metabolizing sugars, such as Xu5P and ribulose-5-phosphate. In the bottom line of the pathway, the direction the pathway goes and the intermediates it produces are determined by the needs of, and intermediates available to, the cell.

As noted above, the pathway connects in three places with glycolysis. In non-plant cells, the PPP pathway occurs in the cytoplasm (along with glycolysis), so considerable “intermingling” of intermediates can and does occur. Erythrose-4-phosphate is an important precursor of aromatic amino acids and ribose-5-phosphate is an essential precursor for making nucleotides.

Calvin Cycle

The Calvin Cycle occurs exclusively in photosynthetic organisms and is the part of photosynthesis referred to as the “Dark Cycle.”



The Calvin Cycle

It is in this part of the process that carbon dioxide is taken from the atmosphere and ultimately built into glucose (or other sugars). Though reduction of carbon dioxide to glucose ultimately requires electrons from twelve molecules of NADPH (and 18 ATPs), it is a bit confusing because one reduction occurs 12 times (1,3 BPG to G3P) to achieve the reduction necessary to make one glucose.

One of the reasons students find the pathway a bit confusing is because the carbon dioxides are absorbed one at a time into six different molecules of **ribulose-1,5-bisphosphate** (Ru1,5BP). At no point are the six carbons ever together in the same molecule to make a single glucose. Instead, six molecules of Ru1,5BP (30 carbons) gain six more carbons via carbon dioxide and then split into 12 molecules of 3-phosphoglycerate (36 carbons). The gain of six carbons

Photosynthesis is Divine

To the tune of "*Scarborough Fair*"

Photosynthesis is divine
Fixing carbon using sunshine
It's thanks to plants that we've got a prayer
They pull CO_2 from the air

Reaping energy from the sun
It's efficient second to none
You grab the photons almost at will
Protoporphyrin chlorophyll

Light reactions of System II
Split up water, making O_2
Electrons pass through schemes labeled 'Z'
Pumping protons gradiently

ATP's made due to a shift
Of the protons spinning quite swift
An enzyme turbine, cellular maze
You know as A-T-P synthase

Carbon's fixed onto a substrate
Ribulose-1,5-bisphosphate
Rubisco acts in-e-fficient-ly
Splitting it into 3PGs

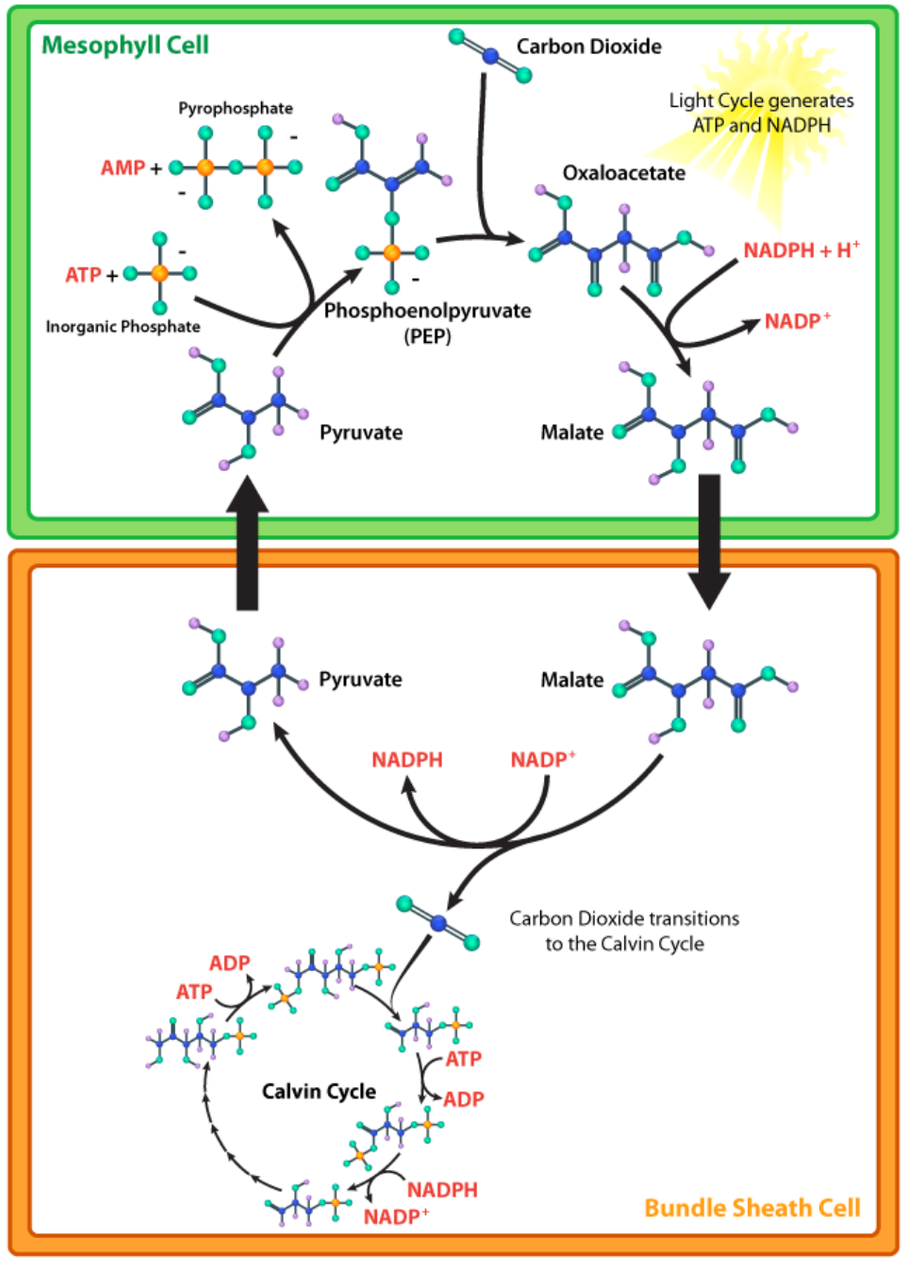
If the enzyme grabs an O_2
It makes glycolate, it is true
The Calvin Cycle works in a wheel
Giving plants a sugary meal

So photosynthesis is divine
'Cause it happens all of the time
From dawn to dusk and times in between
Solar panels truly are green

Recording by David Simmons
Lyrics by Kevin Ahern

allows two three carbon molecules to be produced in excess for each turn of the cycle. These two molecules molecules are then converted into glucose using the enzymes of gluconeogenesis.

Like the citric acid cycle, the Calvin Cycle doesn't really have a starting or ending point, but can we think of the first reaction as the fixation of carbon dioxide to Ru1,5BP. This reaction is catalyzed by the enzyme known as ribulose-1,5bisphosphate carboxylase (RUBISCO). The resulting six carbon intermediate is unstable and each Ru1,5BP is rapidly converted to 3-phosphoglycerate. As noted, if one starts with 6 molecules of Ru1,5BP and makes 12 molecules of 3-PG, the extra 6 carbons that are a part of the cycle can be shunted off as two three-carbon molecules of glyceraldehyde-3-phosphate (GA3P) to gluconeogenesis, leaving behind 10 molecules of GA3P to be reconverted into 6 molecules of Ru1,5BP. That part of the pathway requires multiple steps, but only utilizes two enzymes unique to plants - **sedoheptulose-1,7bisphosphatase** and **phosphoribulokinase**.



C4 Plant Cycle

RUBISCO is the third enzyme of the pathway that is unique to plants. All of the other enzymes of the pathway are common to plants and animals and include some found in the pentose phosphate pathway and gluconeogenesis.

C_4 Plants

The Calvin Cycle is the means by which plants assimilate carbon dioxide from the atmosphere, ultimately into glucose. Plants use two general strategies for doing so. The first is employed by plants called C_3 plants (most plants) and it simply involves the pathway described above. Another class of plants, called C_4 plants employ a novel strategy for concentrating the

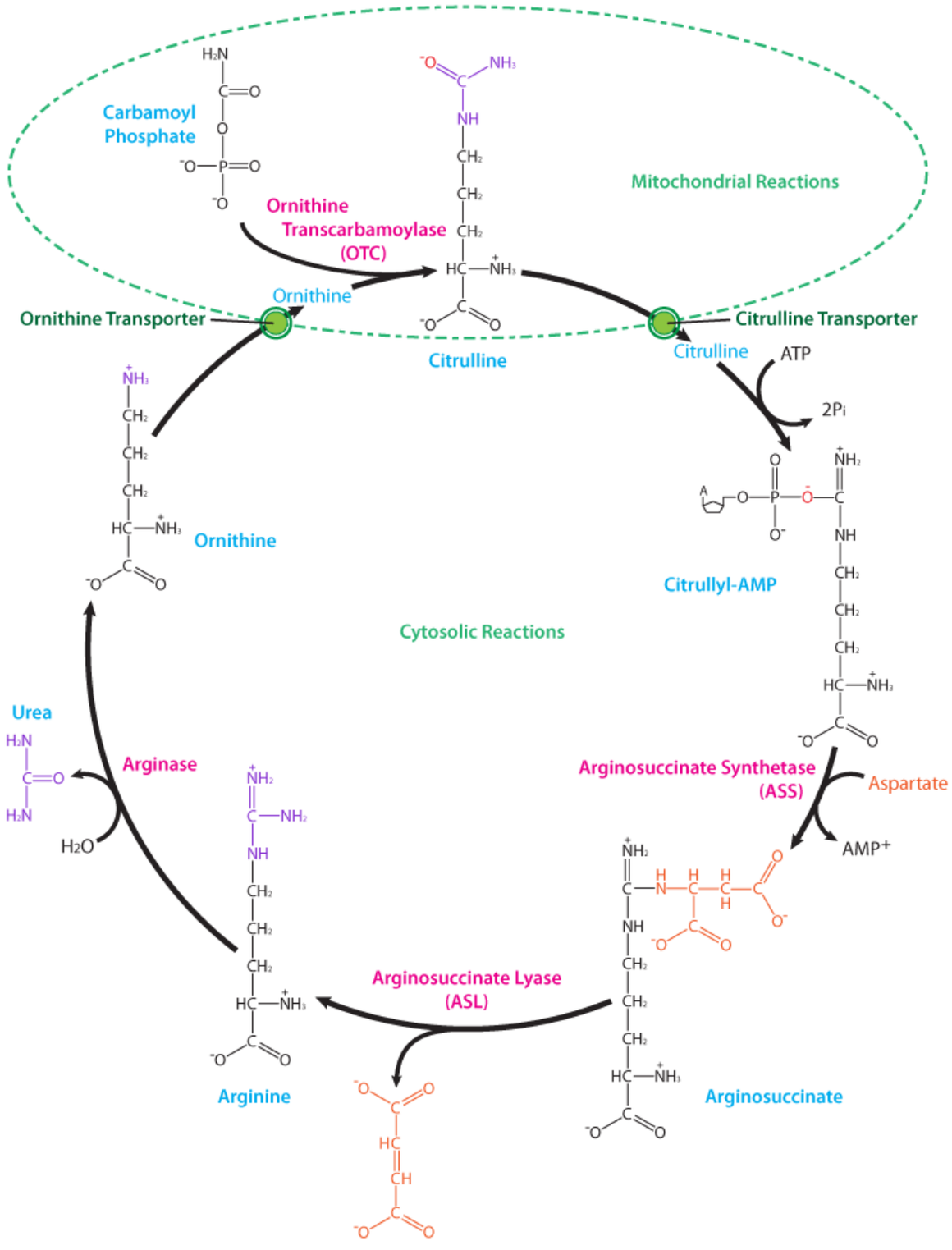
Watch Kevin's YouTube lecture on Photosynthesis HERE

CO_2 prior to assimilation. C_4 plants are generally found in hot, dry environments where conditions favor the wasteful photorespiration reactions of RUBISCO, as well as loss of water. In these plants, carbon dioxide is captured in special mesophyll cells first by phosphoenolpyruvate (PEP) to make oxaloacetate. The oxaloacetate is converted to malate and transported into bundle sheath cells where the carbon dioxide is released and it is captured by ribulose-1,5-bisphosphate, as in C_3 plants and the Calvin Cycle proceeds from there. The advantage of this scheme is that it allows concentration of carbon dioxide while minimizing loss of water and photorespiration.

Urea Cycle

Yet another cyclic pathway important in cells is the urea cycle (next page). With reactions spanning the cytoplasm and the mitochondria, the urea cycle occurs mostly in the liver and kidney. The cycle plays an important role in nitrogen balance in cells and is found in organisms that produce **urea** as a way to excrete excess amines.

The cycle scavenges free ammonia (as



The Urea Cycle

They Call The Stuff Urea

To the tune of "*They Call The Wind Mariah*"

Get ATPs, bicarbonate,
Ammonia catalyzing
To make carba-mo-yl phosphate
And then start the synthesizing

When joined up with an ornithine
In THE mi-TOE-chon-DREE-a
It turns into a citrulline
When cycling to urea

Urea!
Urea!
They call the stuff urea!

On exit to the cytosol
There's bonding aspartat-ic
The argininosuccinate
Is produced in this schematic

Bid farewell to a fumarate
Amino panacea
Arises when the arginine
Gets lysed to form urea

Urea!
Urea!
You've just made some urea!

The body handles many things
Requiring its attention
Like balancing aminos for
Uremia prevention

So if there's excess nitrogen
It is a good idea
To rid yourself of surplus by
Producing some urea

Urea!
Urea!
Go out and take a pee, yeah!

Urea!
Urea!
Have yourself a pee, yeah!

Recorded by David Simmons
Lyrics by Kevin Ahern

ammonium ion) which is toxic if it accumulates. The capture reaction also requires ATP, and bicarbonate, and the product is **carbamoyl phosphate**. This molecule is combined with the non-protein amino acid known as **ornithine** to make another non-protein amino acid known as **citrulline**. Addition of aspartate to citrulline creates **argininosuccinate**, which splits off a fumarate, creating arginine (a source of arginine). If arginine is not needed, it can be hydrolyzed to yield urea (excreted) and ornithine, thus completing the cycle.

The first two reactions described here occur in the mitochondrion and the remaining ones occur in the cytoplasm. Molecules of the urea cycle intersecting other pathways include fumarate (citric acid cycle), aspartate (amino acid metabolism), arginine (amino acid metabolism), and ammonia (amino acid metabolism).

Nitrogen Fixation

The process of nitrogen fixation is important for life on earth, because atmospheric nitrogen is ultimately the source of amines in proteins and DNA. The enzyme playing an important role in this process is called nitrogenase and it is found in certain types of anaerobic bacteria called diazotrophs. Symbiotic relationships between some plants (legumes, for example) and the nitrogen-fixing bacteria provide the plants with access to reduced nitrogen. The

overall reduction reaction catalyzed by nitrogenase is

$$N_2 + 6H^+ + 6e^- \rightarrow 2NH_3$$

In these reactions, the hydrolysis of 16 ATP is required. The ammonia can be assimilated into glutamate and other molecules. Enzymes performing nitrogenase catalysis are very susceptible to oxygen and must be kept free of it. It is for this reason that most nitrogen-fixing bacteria are anaerobic. Movement of amines through biological systems occurs largely by the process of transmination, discussed below in amino acid metabolism.

Amino Acid Metabolism

The pathways for the synthesis and degradation of amino acids used in proteins are the most varied among the reactions synthesizing biological building blocks. We start with some terms. First, not all organisms can synthesize all the amino acids they need. Amino acids that an organism cannot synthesize (and therefore must have in their diets) are called **essential amino acids**. The remaining amino acids that the body can synthesize are called non-essential.

Four Families of Synthesis of Essential Amino Acids

1. Aspartate – lys, met, thr – proceed through aspartyl-beta-phosphate (catalyzed by aspartokinase)

2. Pyruvate – leu, ile, val – proceed through hydroxyethyl-TPP intermediate

3. Aromatic – phe, tyr, trp – precursors are PEP and erythrose-4-phosphate

4. Histidine – from 5-phosphoribosyl-pyrophosphate (from erythrose-4-phosphate), histidine (from ribose-5-phosphate),

Amino acids are also divided according to the pathways involved in their degradation. There are three general categories. Ones that yield intermediates in the glycolysis pathway are called **glucogenic** and those that yield intermediates of acetyl-CoA or acetoacetate are called **ketogenic**. Those that involve both are called glucogenic and ketogenic.

See Kevin's YouTube lectures on Nitrogen Metabolism HERE and HERE

An important general consideration in amino acid metabolism is that of transamination. In this process, an exchange of amine and oxygen between an amino acid and an alpha-ketoacid occurs (see below)

Alpha-ketoacid + amino acid <=> amino acid + alpha-ketoacid

An example reaction follows

Pyruvate + Aspartic acid <=> Alanine + Oxaloacetate

This reaction is catalyzed by an enzyme known as a transaminase. Amino acids, such as glutamate, can also gain

nitrogen directly from ammonium ion, as shown below

Alpha-ketoglutarate + NH_4^+ <=> Glutamate

This reaction can occur, for example, in nitrifying bacteria, and in places where ammonia waste is produced. Many amino acids can be synthesized from citric acid cycle intermediates. For example, synthesis of the non-essential amino acids occurs as follows: aspartic acid can be made by transamination of oxaloacetate. Glutamate comes from transamination of alpha-ketoglutarate. Pyruvate, as noted, is a precursor of alanine (*via* transamination). Amino acids that can be made from glutamate include glutamine (by addition of an additional ammonium ion), proline, and arginine, Asparagine is made from aspartate by addition of ammonium ion also. Serine is formed from 3-phosphoglycerate and is itself the precursor of both glycine and cysteine. Cysteine and serine are also made from methionine. Tyrosine is made by hydroxylation of phenylalanine.

Glucogenic and Ketogenic Amino Acids

Glucogenic	Both Glucogenic and Ketogenic	Ketogenic
Aspartate	Isoleucine	Leucine
Asparagine	Phenylalanine	Lysine
Alanine	Tryptophan	
Glycine	Tyrosine	
Serine		
Threonine		
Cysteine		
Glutamate		
Glutamine		
Arginine		
Proline		
Histidine		
Valine		
Methionine		

Amino Acid Catabolism

Breakdown of glutamine by glutaminase is a source of ammonium ion in the cell. The other product is glutamate. Glutamate, of course, can be converted by a transamination reaction to alpha-ketoglutarate, which can be oxidized in the citric acid cycle.

Asparagine can similarly be broken to ammonium and aspartate by **asparaginase** and aspartate can be converted by transamination to oxaloacetate for oxidation in the citric acid cycle.

Alanine is converted to pyruvate in a transamination reaction, making it glucogenic.

Arginine is hydrolzyed in the urea cycle to yield urea and ornithine.

Proline is catabolized to glutamate in a reversal of its synthesis pathway.

Serine donates a carbon to form a folate and the other product of the reaction is glycine, which is itself oxidized to carbon dioxide and ammonia. Glycine can also be converted back to serine, which can also be converted back to 3-phosphoglycerate or pyruvate.

Threonine can be broken down in three pathways, though only two are relevant for humans. One pathway leads to acetyl-CoA and glycine. The other leads to pyruvate.

Cysteine can be broken down in several ways. The simplest occurs in the liver, where a desulfurase can act on it to yield hydrogen sulfide and pyruvate.

Methionine can be converted to cysteine for further metabolism. It can be converted to succinyl-CoA for oxidation in the citric acid cycle. It can also be converted to **S-Adenosyl-Methionine** (SAM), a carbon donor.

Isoleucine and valine can also be converted to succinyl-CoA after conversion first to propionyl-CoA. Since conversion of propionyl-CoA to succinyl-CoA requires vitamin B_{12}, catabolism of these amino acids also requires the vitamin.

Phenylalanine is converted during catabolism to tyrosine, which is degraded ultimately to fumarate and acetoacetate. Thus, both of these amino acids are glucogenic and ketogenic. Tyrosine can also be converted to **dopamine**, **norepinephrine**, and **epinephrine**.

Leucine and lysine can be catabolized to acetoacetate and acetyl-CoA. Lysine is also an important precursor of carnitine.

Histidine can be catabolized by bacteria in intestines to **histamine**, which causes construction or dilation of various blood vessels when in excess.

Tryptophan's catabolism is complex, but can proceed through alanine, acetoacetate and acetyl-CoA

In summary, the following are metabolized to pyruvate – alanine, cysteine, glycine, serine, and threonine

Oxaloacetate is produced from aspartate and asparagine

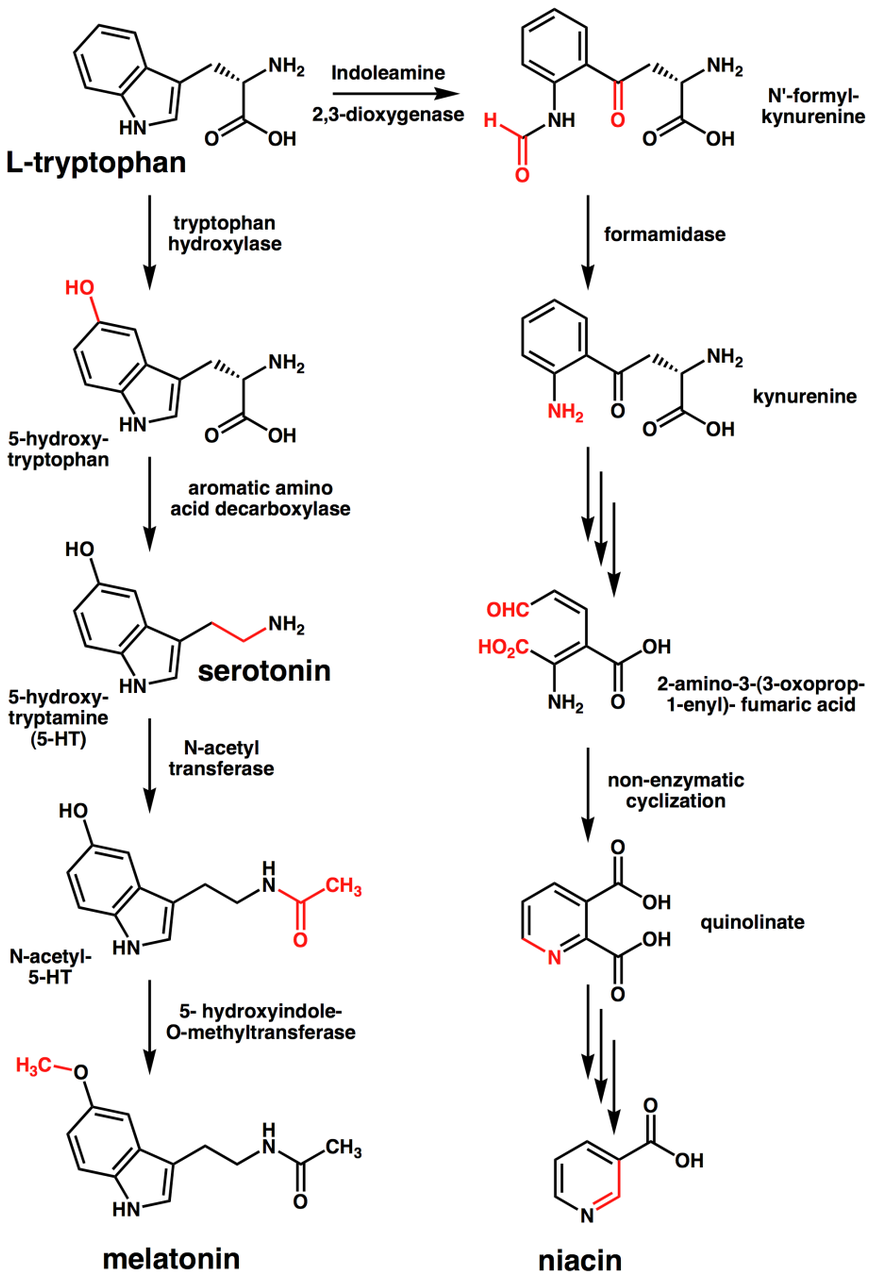
Succinyl-CoA is produced from isoleucine, valine, and methionine

Alpha-ketoglutarate is produced from arginine, glutamate, glutamine, histidine and proline.

Phenylalanine and tyrosine are broken down to fumarate and acetoacetate

Leucine and lysine yield acetoacetate and acetyl-CoA.

Tryptophan leads to alanine, acetoacetate and acetyl-CoA



Conversion of L-tryptophan into Serotonin, Melatonin, and Niacin.

from Wikipedia

Last, amino acids, besides being incorporated into proteins, serve as precursors of important compounds, including **serotonin** (from tryptophan), porphyrin heme (from glycine), **nitric oxide** (from arginine), and nucleotides (from aspartate, glycine, and glutamine).

Nucleotide Metabolism

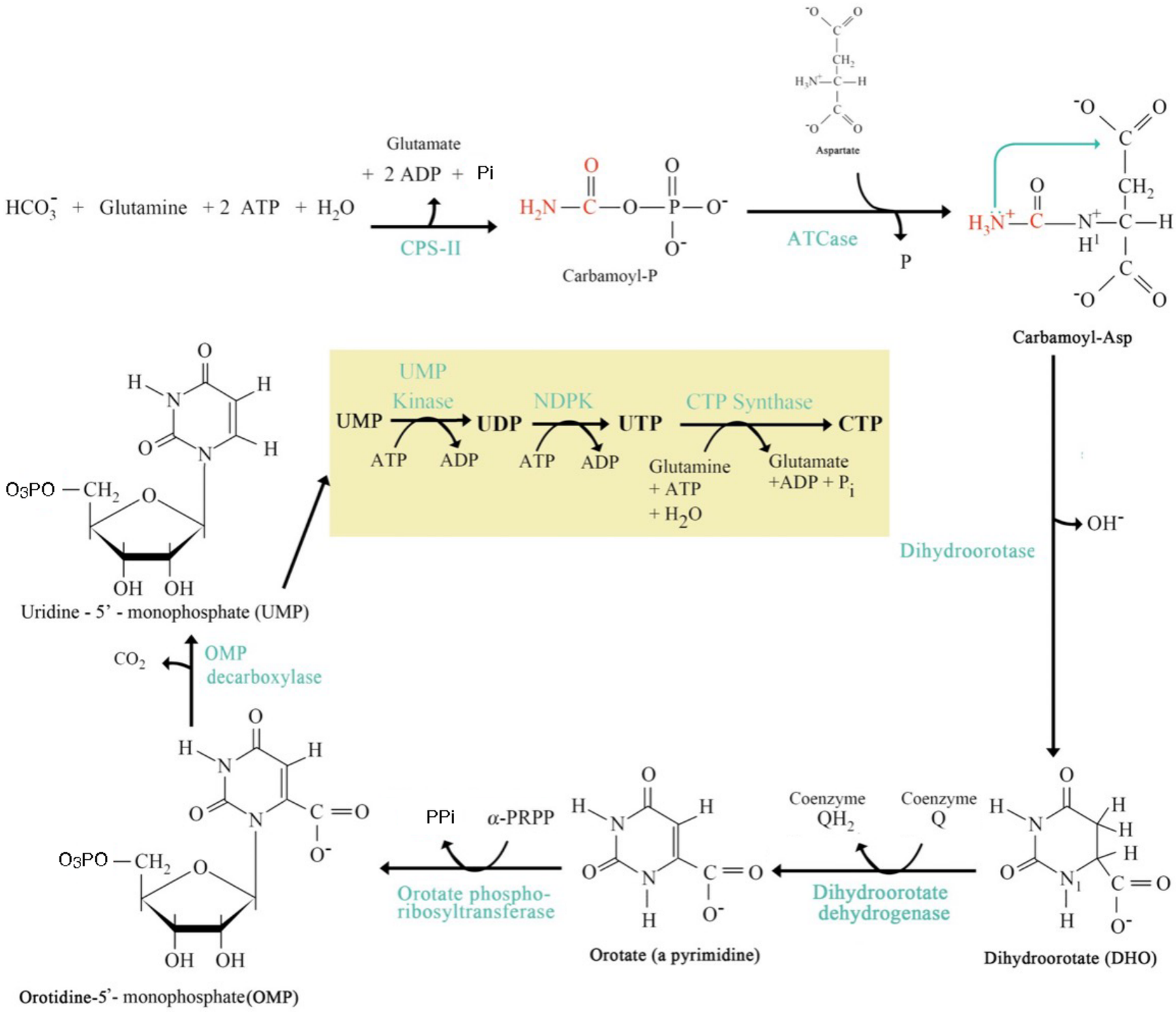
Arguably, the most interesting metabolic pathways from the perspective of regulation are those leading to the synthesis of nucleotides. We shall consider **ribonucleotide** synthesis from from scratch (*de novo* synthesis). **Deoxyribonucleotide** synthesis from ribonucleotides will be considered separately.

Synthesis of ribonucleotides by the *de novo* method occurs in two pathways – one for purines and one for pyrimidines. What is notable about both of these pathways is that nucleotides are built from very simple building blocks.

Pyrimidine *de novo* Biosynthesis

Starting materials for pyrimidine biosynthesis (shown in the figure) include bicarbonate, amine from glutamine, and phosphate from ATP to make **carbamoyl-phosphate** (similar to the reaction of the urea cycle). Joining of carbamoyl phosphate to aspartic acid (forming carbamoyl aspartate) is catalyzed by the most important regulatory enzyme of the cycle, aspartate transcarbamoylase (also called aspartate carbamoyltransferase or ATCase).

ATCase is regulated by three compounds. One of these (aspartate) is a substrate and it activates the enzyme by binding



De novo Synthesis of Pyrimidine Ribonucleotides

to the catalytic site and favoring the enzyme's R state. The other two regulators bind to regulatory subunits of the enzyme and either inhibit (CTP) or activate (ATP) the enzyme.

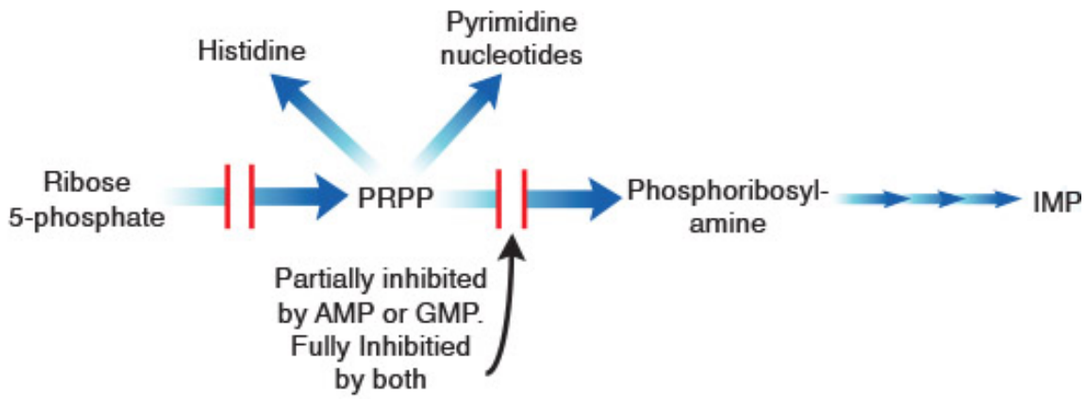
The reaction product, carbamoyl aspartate, is transformed in two reactions to **orotic acid**, which is, in turn combined with **phosphoribosylpyrophosphate** (**PRPP**). The product of that reaction, orotidyl monophosphate (**OMP**) is decarboxylated to form the first pyrimidine nucleotide, **UMP**. Conversion of UMP to **UDP** is catalyzed by **nucleoside monophosphate kinases** (NMPs) and UDP is converted to **UTP** by **nucleoside diphosphokinase** (**NDPK**).

UDP (like all of the nucleoside diphosphates) is a branch point to deoxyribonucleoside diphosphates, catalyzed by ribonucleotide reductases, which are discussed later. UTP is converted to **CTP** by **CTP synthase**. This enzyme, which uses an amino group from glutamine for the reaction, serves to balance the relative amounts of CTP and UTP, thanks to inhibition by excess CTP.

Purine *de novo* Biosynthesis

Synthesis of purine nucleotides differs fundamentally from that of pyrimidine nucleotides in that the bases are built on the ribose ring. The starting material is ribose-5-phosphate, which is phosphorylated by **PRPP synthetase** to PRPP using two phosphates from ATP. **PRPP amidotransferase** catalyzes the transfer of an amine group to PRPP, replacing the **pyrophosphate** on carbon 1. Thus begins the synthesis of the purine ring.

PRPP amidotransferase is regulated partly by **GMP** and partly by **AMP**. The presence of either of these can reduce the enzyme's activity. Only when both are present is the enzyme fully inactivated. Subsequent reactions include adding glycine, adding carbon (from N_{10}-formyltetrahydrofolate), adding amine (from



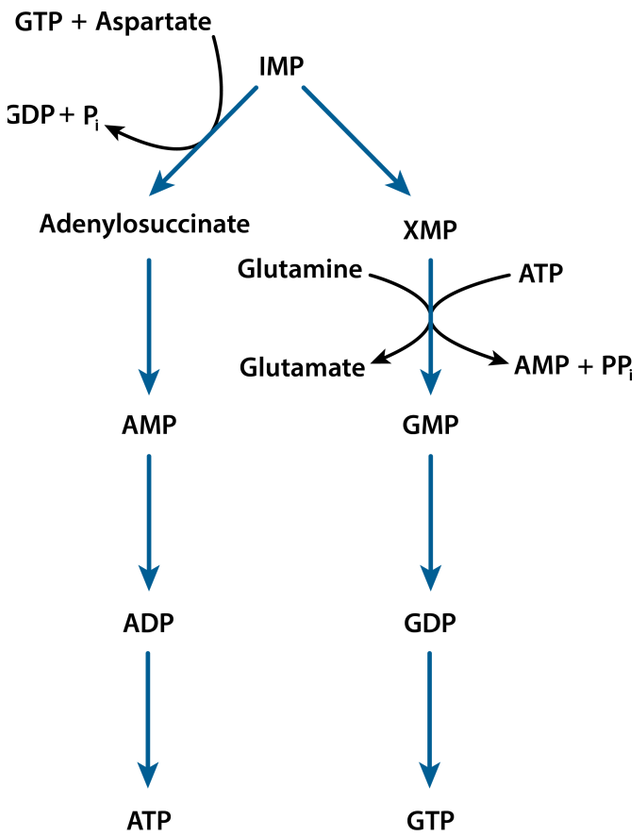
Beginning of Purine Metabolism

glutamine), closing of the first ring, addition of carboxyl (from CO_2), addition of aspartate, loss of fumarate (a net gain of an amine), addition of another carbon (from N_{10}-formyltetrahydrofolate), and closing of the second ring to form **inosine monophosphate** (**IMP**).

IMP is a branch point for the

See Kevin's Nucleotide Metabolism lectures on YouTube HERE and HERE

synthesis of the **adenine** and **guanine** nucleotides. The pathway leading from IMP to AMP involves addition of amine from asparate and requires energy from **GTP**. The pathway from IMP to GMP involves an oxidation and addition of an amine from glutamine. It also requires energy from ATP. The pathway leading to GMP is inhibited by its end product and the pathway to AMP is inhibited by its end product.

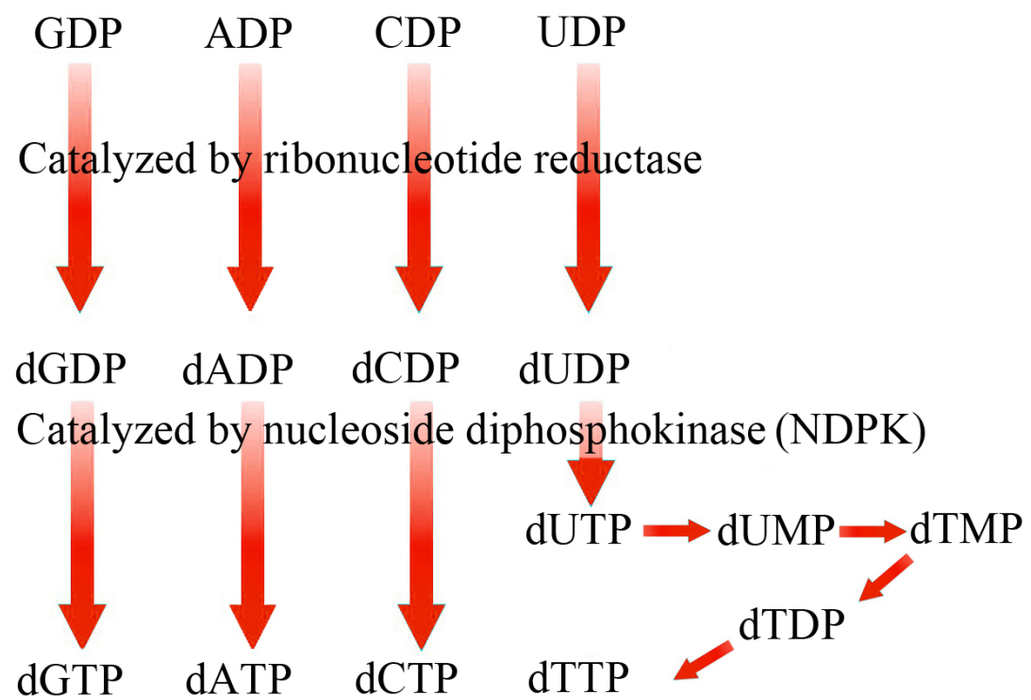


Synthesis of ATP and GTP

Thus, balance of the purine nucleotides is achieved from the IMP branch point forward. It is at this point that the significance of the unusual regulation of PRPP amidotransferase becomes apparent. If there is an imbalance of AMP or GMP, the enzyme is slowed, but not stopped, thus allowing the reactions leading to IMP to proceed, albeit slowly. At IMP, the nucleotide in excess feedback inhibits its own synthesis, thus allowing the partner purine nucleotide to be made and balance to be achieved. When both nucleotides are in abundance, then PRPP amidotransferase is fully inhibited and the production of purines is stopped, thus preventing them from over-accumulating.

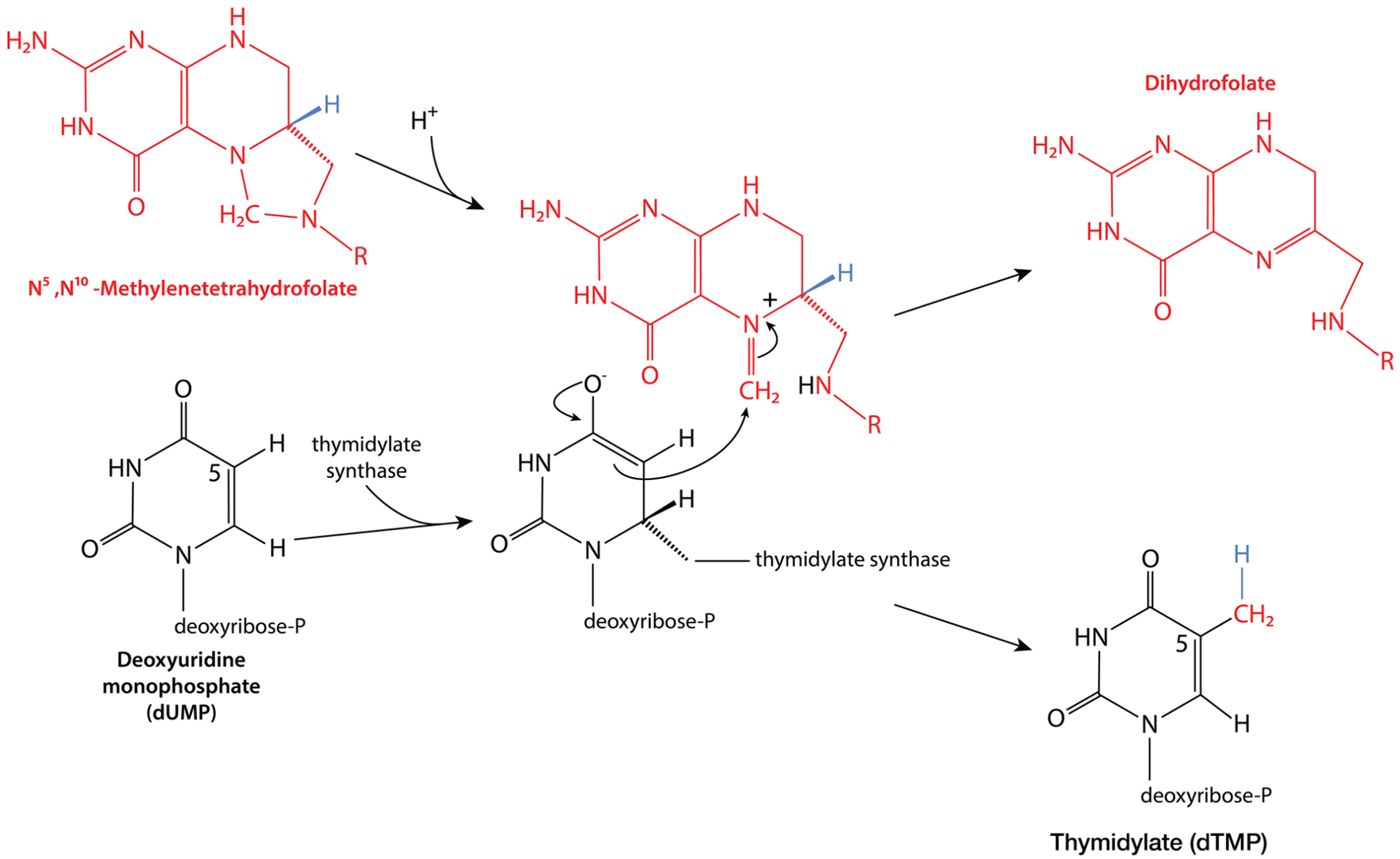
Deoxyribonucleotide *de novo* Synthesis

Synthesis of deoxyribonucleotides *de novo* requires an interesting enzyme called ribonucleotide reductase (RNR). RNR catalyzes the formation of deoxyribonucleotides from ribonucleotides. The most common form of RNR is the Type I enzyme, whose substrates are ribonucleoside diphosphates (ADP, GDP, CDP, or UDP) and the products are deoxyribonucleoside diphosphates



dNTP *de novo* Synthesis

(dADP, dGDP, dCDP, or dUDP). Thymidine nucleotides are synthesized from dUDP. RNR has two pairs of two identical subunits - R1 (large subunit) and R2 (small subunit). R1 has two allosteric binding sites and an active site. R2 forms a tyrosine radical necessary for the reaction mechanism of the enzyme.



Thymidylate (dTMP) Synthesis

Because a single enzyme, RNR, is responsible for the synthesis of all four deoxyribonucleotides, it is necessary to have mechanisms to ensure that the enzyme produces the correct amounts of each dNDP. This means that the enzyme must be responsive to the levels of the each deoxynucleotide, selectively making more of those that are in short supply, and preventing synthesis of those that are abundant. These demands are met by having two separate control mechanisms, one that determines which

substrate will be acted on, and another that controls the enzyme's catalytic activity.

Ribonucleotide reductase is allosterically regulated *via* two binding sites - a *specificity* binding site (binds dNTPs and controls which substrates the enzyme binds and thus, which deoxyribonucleotides are made) and an *activity* binding site (controls whether or not enzyme is active - ATP activates, dATP inactivates).

When a deoxypyrimidine triphosphate, dTTP is abundant, it binds to the specificity site and inhibits binding and reduction of pyrimidine diphosphates (CDP and UDP) but stimulates binding and reduction of GDP by the enzyme. Conversely, binding of the deoxypurine triphosphate, ATP stimulates reduction of pyrimidine diphosphates, CDP and UDP.

Students sometimes confuse the active site of RNR with the activity site. The *active site* is where the reaction is catalyzed, and could also be called the catalytic site, whereas the *activity site* is the allosteric binding site for ATP or dATP that controls whether the enzyme is active.

Synthesis of dTTP by the *de novo* pathway takes a convoluted pathway from dUDP to dUTP to dUMP to dTMP, then dTDP, and finally dTTP. Conversion of dUMP to dTMP, requires a tetrahydrofolate derivative and the enyzme thymidylate synthase. In the process, dihydrofolate is produced and must be converted back to tetrahyrdolate in order to keep nucleotide synthesis occurring. The enzyme involved in the conversion of dihydrofolate to tetrahydrofolate, dihydrofolate reductase (DHFR), is a target of anticancer drugs like methotrexate or aminopterin, which inhibit the enzyme,.

Jump to Chapter

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