Chapter 6

Metabolism I

Depending on your mathematical perspective, life is the sum or the product of the biochemical reactions that occur in cells. The collection of these reactions is known as metabolism. We break the subject into two broad areas - 1) oxidative/reductive metabolism and 2) pathways that involve little oxidation/ reduction. This chapter deals with the former.

Section 1

Metabolism I

The cost of living is energy and the producers and consumers of energy in the cell are the chemical reactions known collectively as metabolism. Metabolic processes are governed by the same laws of energy as the rest of the universe, so they must be viewed in the light of Gibbs free energy.

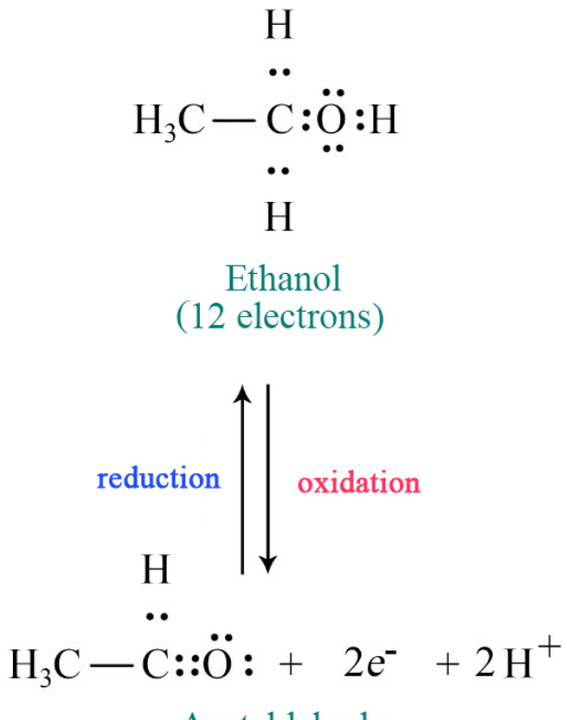
For the most part, the drivers of changes in Gibbs free energy are changes in concentration of reactants and products, but for some reactions, the concentration changes required to run a reaction in the desired direction are not practical. In such cases, cells may use alternative strategies, such as energy coupling reactions (combining an energetically unfavorable reaction with a favorable one, such as the hydrolysis of ATP) to help “drive” the unfavorable reaction. In other cases, cells use alternate pathways around energetically unfavorable reactions.

Definitions

We start by defining a few terms.

Anabolic processes refer to collections of biochemical reactions that make bigger molecules from smaller ones. Examples include the synthesis of **fatty acids** from acetyl-CoA, of proteins from amino acids, of **complex carbohydrates** from simple

A student really ought to know
The ways in which electrons flow
When running catabolically
Removal yields some energy
If anabolic bliss ensues
The tiny charges then reduce



Ethanol
(12 electrons)

Acetaldehyde
(10 electrons)

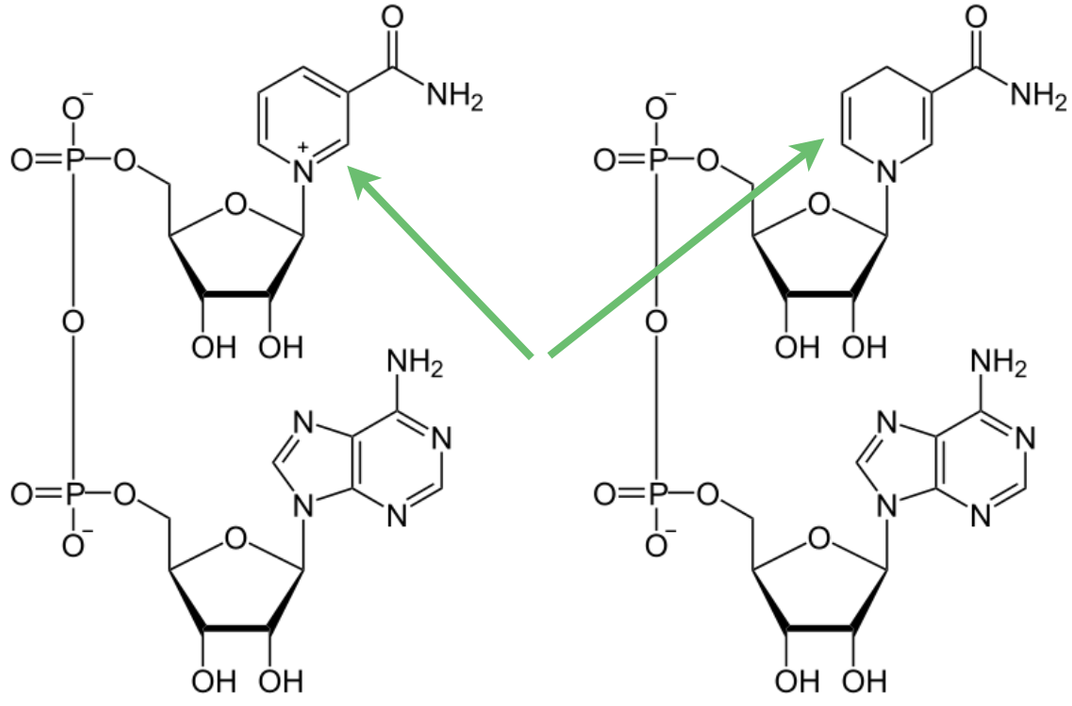
Redox Reactions

sugars, and of nucleic acids from nucleotides. Just as any construction project requires energy, so, too, do anabolic processes require input of energy. Anabolic processes tend to be reductive in nature, in contrast to **catabolic processes**, which are oxidative. Not all anabolic processes are reductive, though. Protein synthesis and nucleic acid synthesis do not involve reduction, though the synthesis of amino acids and nucleotides does.

Catabolic processes are the primary sources of energy for heterotrophic organisms and they ultimately power the anabolic processes. Examples include glycolysis (breakdown of glucose), the citric acid cycle, and fatty acid oxidation. Reductive processes require electron sources, such as **NADPH**, **NADH**, or **$FADH_2$**. Oxidative processes require electron carriers, such as **NAD^+**, **$NADP^+$**, or **FAD**. Catabolic processes are ultimately the source of **ATP** energy in cells, but the vast majority of ATP in heterotrophic organisms is not made in directly in these reactions. Instead, the electrons released by oxidation are collected by electron carriers which donate them, in the mitochondria, to complexes that make ATP (ultimately) by **oxidative phosphorylation**.

Click HERE, HERE, and HERE for Kevin's YouTube lectures on Metabolism and Glycolysis

In our tour of metabolism, we will tackle in this chapter processes that are the most oxidative/reductive in nature and in the following chapter those pathways that involve less reduction/

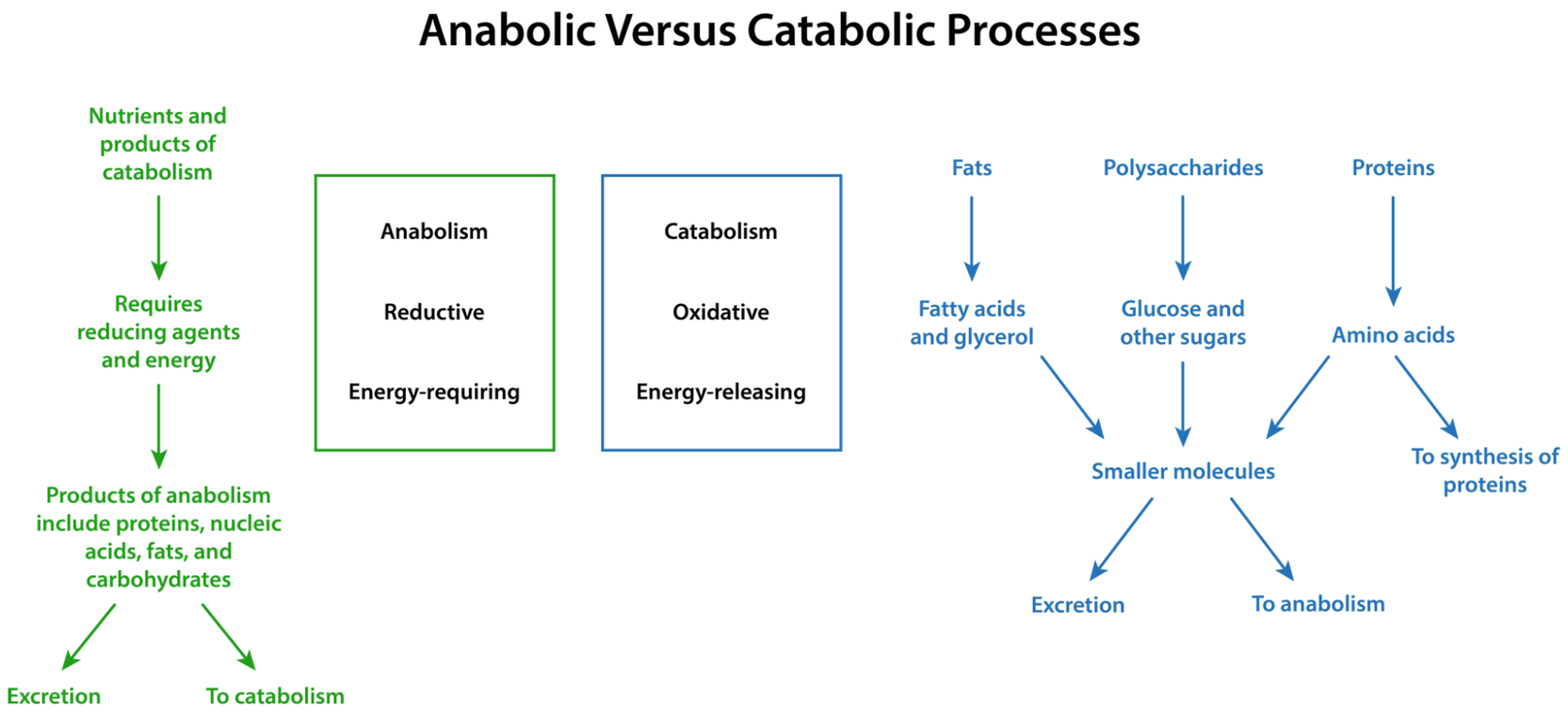


NAD^+ NADH

oxidation. The aim in this coverage is not to go through the step-by-step reactions of the pathway, but rather to focus on control points, interesting enzymes, molecules common between pathways, and how the metabolic pathways meet the organism's needs.

Perspective

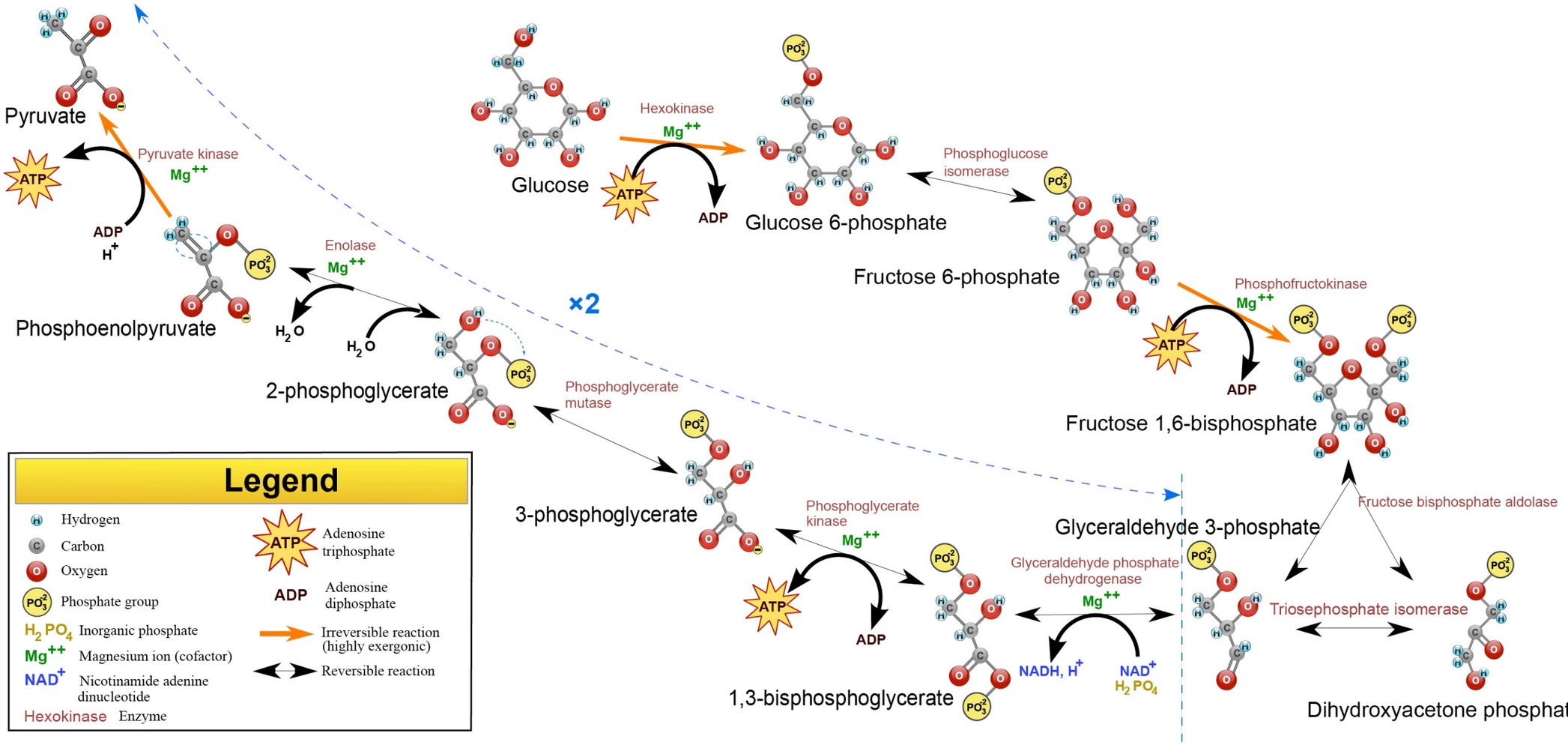
We can view metabolism at several levels. At the highest level, we have nutrients, such as sugars, fatty acids and amino acids entering cells and carbon dioxide and other waste products (such as urea) exiting. Cells use the incoming materials for energy and substance to synthesize sugars, nucleotides, and other amino acids as building blocks for the carbohydrates, nucleic acids, fatty compounds, and proteins necessary for life. As we zoom in, we can imagine pathways made up of reactions for breakdown and synthesis of each of these compounds. The figure at left shows such a simple schematic and how the pathways are not isolated from each other – molecular products of one are substrates for another. At a deeper level, we can study individual reactions and discover the enormous complexity and commonality of metabolic reactions.



In studying metabolism, we recognize that metabolic pathways are manmade concepts with artificial boundaries. Students commonly think of the molecules in the pathways being tied exclusively to those individual pathways, but with the exception of reactions that have physical barriers (such as those occurring within an organelle), **metabolic pathways** have many common intermediates used in multiple reactions occurring in the same location at the same time and thus cannot be ascribed to any one pathway. The best we can do is understand general directions of pathways in cells.

Glycolysis

The metabolic pathway traditionally covered first in biochemistry texts is



The Reactions of Glycolysis

from Wikipedia

glycolysis and there seems to be no reason to break that trend here. Glycolysis, which literally means “breakdown of sugar,” is a catabolic process in which six carbon sugars (**hexoses**) are oxidized and broken down into pyruvate molecules. The corresponding anabolic pathway by which glucose is synthesized is termed **gluconeogenesis**. Both glycolysis and gluconeogenesis are not major oxidative/reductive processes by themselves, with one step in each one involving loss/gain of electrons, but the product of glycolysis, pyruvate, can be completely oxidized to carbon dioxide. Indeed, without

Your cells may have a mounting crisis
Should they not go through glyco-lye-sis
No glucose energy releases
Unless it's fractured into pieces

production of pyruvate from glucose in glycolysis, a major energy source for the cell is not available. By contrast, gluconeogenesis can synthesize glucose reductively from very simple materials, such as pyruvate and acetyl-CoA/**glyoxylate** (at least in plants). For these reasons we include these pathways in the red/ox collection.

Glucose is the most abundant hexose in nature and is the one people typically associate with glycolysis, but fructose (in the form of fructose-6-phosphate) is metabolized in the cell and galactose can easily be converted into glucose for catabolism in the pathway as well. The end metabolic products of the pathway are two molecules of ATP, two molecules of NADH and two molecules of pyruvate, which, in turn, can be oxidized further in citric acid cycle.

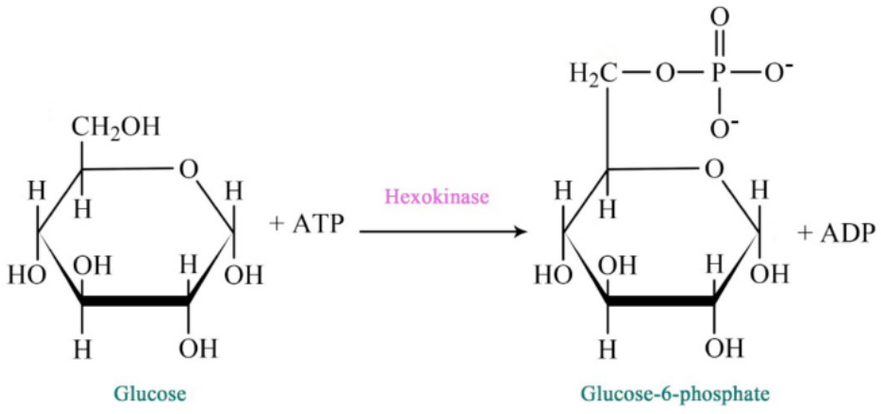
Intermediates

Glucose and **fructose** are the sugar 'funnels' serving as entry points to the glycolytic pathway. Other sugars must be converted to either of these forms to be directly metabolized. Some pathways, including the **Calvin Cycle** and the **Pentose Phosphate Pathway** (PPP, see below) contain intermediates in common with glycolysis, so in that sense, almost any cellular sugar can be metabolized here. Intermediates of glycolysis that are common to other pathways include **glucose-6-phosphate** (PPP, glycogen metabolism), F6P (PPP), **G3P** (Calvin, PPP), **DHAP** (PPP, glycerol metabolism, Calvin), 3PG (Calvin, PPP), **PEP** (C4 plant metabolism, Calvin), and **pyruvate** (fermentation, acetyl-CoA genesis, amino acid metabolism).

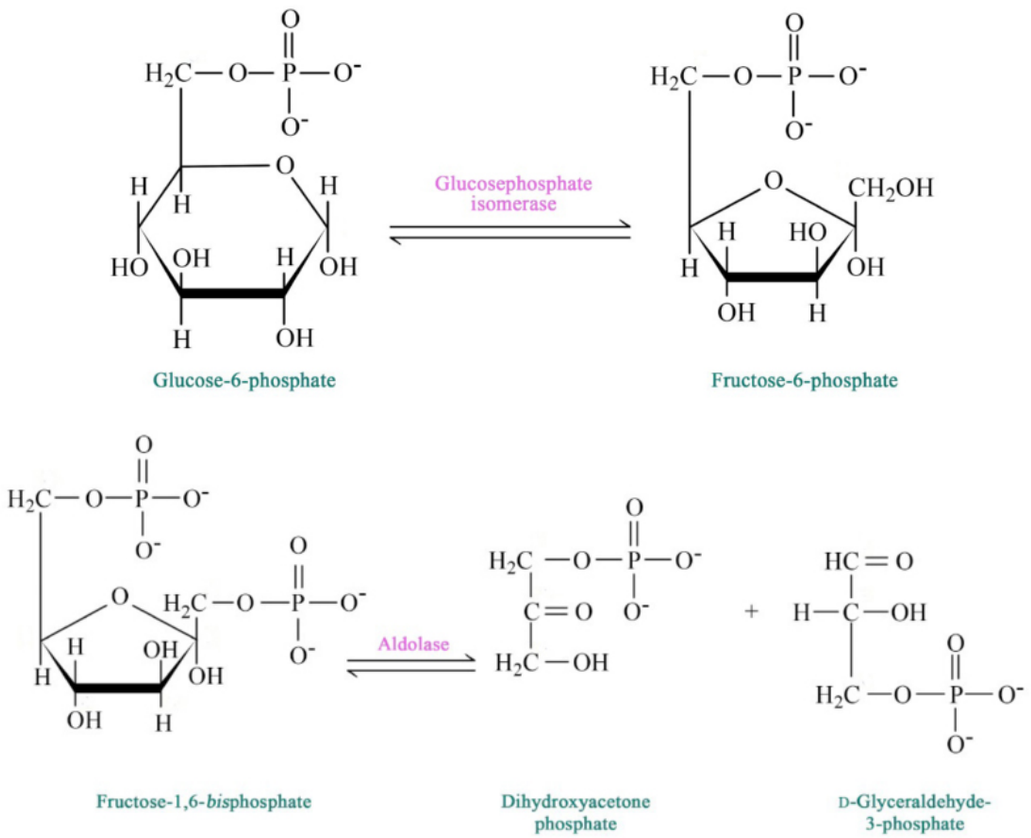
Click HERE, HERE, and HERE for Kevin's YouTube lectures on Metabolism and Glycolysis

Reactions

The pathway of glycolysis begins with two inputs of energy. First, glucose gets a phosphate from ATP to make glucose-6-phosphate (**G6P**) and later **fructose-6-phosphate** (**F6P**) gets another phosphate from ATP to make **fructose-1,6-bisphosphate** (**F1,6BP**).



Step 1 of Glycolysis

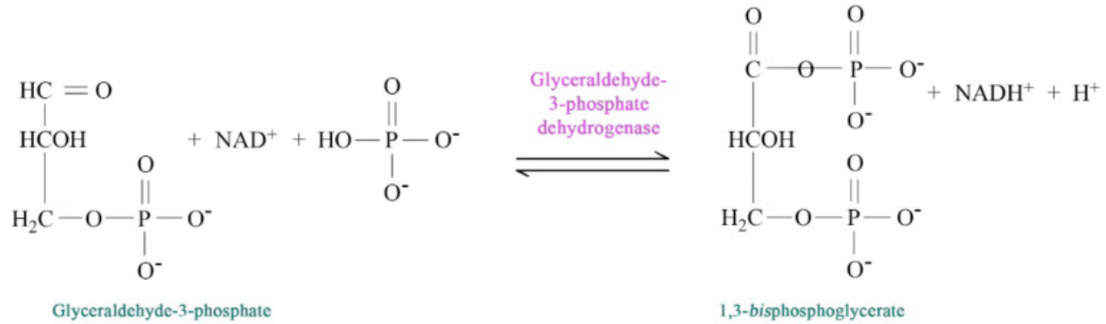


Steps 2 (top) and 4 (bottom) of Glycolysis

With the pump thus primed, the pathway proceeds first to split the F1,6BP into two 3-carbon intermediates. Later the only oxidation step in the entire pathway occurs. In that reaction, **glyceraldehyde-3-phosphate** (G3P) is oxidized and a phosphate is added, creating **1,3-bisphosphoglycerate** (**1,3 BPG**).

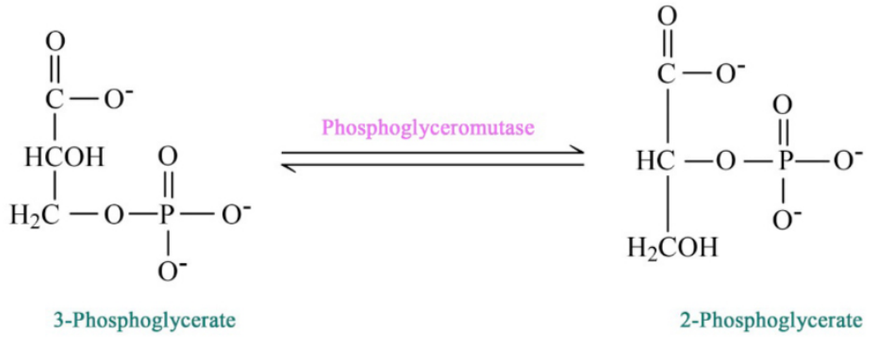
The addition of the phosphate sometimes conceals the oxidation that occurred. G3P was an aldehyde. 1,3 BGP is an acid esterified to a phosphate. The two phosphates in the tiny 1,3BPG molecule repel each other and give the molecule high energy. It uses this energy to phosphorylate ADP to make ATP.

Since there are two 1,3 BPGs produced for every glucose, the two ATP produced replenish the two ATPs used to start the cycle.

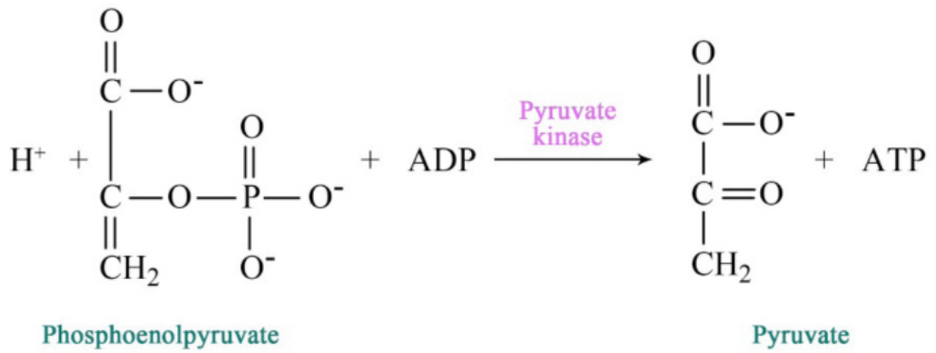


Step 5 of Glycolysis

The synthesis of ATP directly from a metabolic reaction is known as **substrate level phosphorylation** (see HERE), though it is not a significant source of ATP. Glycolysis has two reactions during which substrate-level phosphorylation occurs.



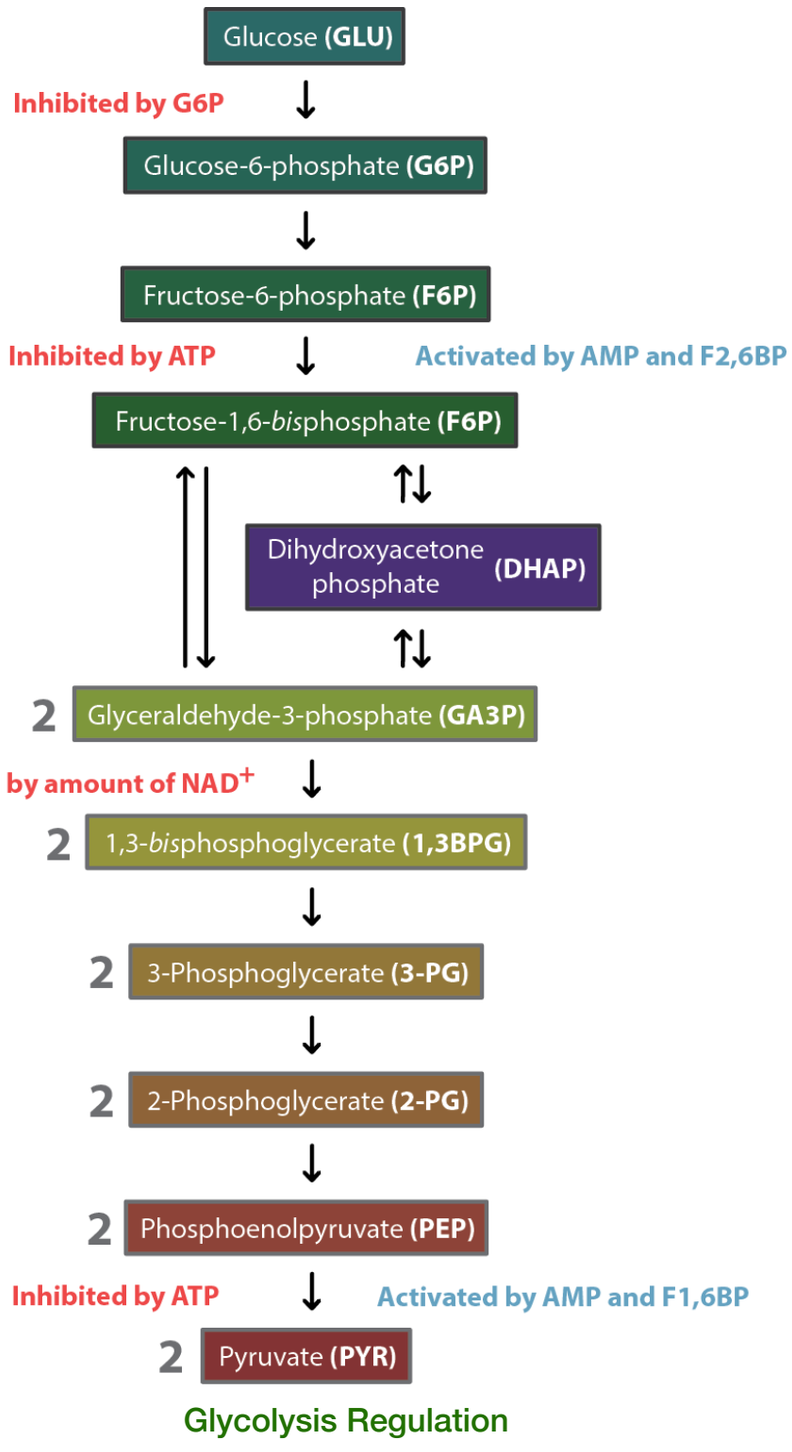
Step 8 of Glycolysis



Step 10 of Glycolysis

The transfer of phosphate from 1,3BPG to ATP creates **3-phosphoglycerate** (**3-PG**). Conversion of 3-PG to **2-PG** occurs by an important mechanism. An intermediate in the reaction (catalyzed by **phosphoglycerate mutase**) is **2,3 BPG**. This intermediate, which is stable, is released with low frequency by the enzyme instead of being converted to 2-PG. 2,3BPG is important because it binds to hemoglobin and stimulates release of oxygen (see HERE). Thus, cells which are metabolizing glucose rapidly release more 2,3BPG and, as a result, stimulate release of more oxygen, supporting their needs.

2-PG is converted to **phosphoenolpyyruvate** (PEP) by removal of water, creating a very high energy intermediate. Conversion of PEP to pyruvate is the second substrate level phosphorylation of glycolysis, creating ATP. There is almost enough energy in PEP to stimulate production of a second ATP, but it is not used. Consequently, the energy is lost as heat. If you wonder why you get hot when you exercise, the reaction that converts PEP to



Glycolysis Regulation

pyruvate is a prime culprit.

Enzymes/Control

Control of glycolysis is unusual for a metabolic pathway, in that regulation occurs at three enzymatic points – **hexokinase** (Glucose <=>G6P), **phosphofructokinase** - **PFK** (F6P <=> F1,6BP), and **pyruvate kinase** (PEP <=> pyruvate).

Glycolysis is regulated in a reciprocal fashion compared to its corresponding anabolic pathway, gluconeogenesis. **Reciprocal regulation** occurs when the same molecule or treatment (**phosphorylation**, for example) has opposite effects on catabolic and anabolic pathways. Reciprocal regulation is important when anabolic and corresponding catabolic pathways are occurring in the same cellular location.

As an example, consider regulation of PFK. It is activated by several molecules, most importantly **fructose-2,6-bisphosphate** (**F2,6BP**). This molecule

The Sound of Glucose

To the tune of "*A Few of My Favorite Things*"

Aldehyde sugars are always aldoses and
If there's a ketone we call them ketoses
Some will form structures in circular rings
Saccharides do some incredible things

Onto a glucose we add a 'P' to it
ATP energy ought to renew it
Quick rearranging creates F6P
Without requiring input energy

At a high rate
Add a phosphate
With PFK
F1,6BP is made up this way
So we can run and play

Aldolase breaks it and then it releases
DHAP and a few G3Pieces
These both turn in to 1,3 BPG
Adding electrons onto NAD

Phosphate plus ADP makes ATP
While giving cells what they need - en-er-gy
Making triphosphate's a situa-shun
Of substrate level phosphoryla-shun

3-B-P-G
2-B-P-G
Lose a water
PEP gets a high energy state
Just to make py-ru-vate

So all the glucose gets broken and bent
If there's no oxygen cells must ferment
Pyruvate / lactate our cells hit the wall
Some lucky yeast get to make ethanol

This is the end of your glucose's song
Unless you goof up and get it all wrong
Break it, don't make it to yield ATP
You'll save your cells from fu-til-i-ty

Recorded by Tim Karplus
Lyrics by Kevin Ahern

has an inhibitory effect on the corresponding gluconeogenesis enzyme, **fructose-1,6-bisphosphatase** (**F1,6BPase**).

You might wonder why pyruvate kinase, the last enzyme in the pathway, is regulated. The answer is simple. Pyruvate kinase catalyzes the most energetically rich reaction of glycolysis. The reaction is favored so strongly in the forward direction that cells must do a 'two-step' around it in the reverse direction when making glucose. In other words, it takes two enzymes, two reactions, and two triphosphates to go from pyruvate back to PEP in gluconeogenesis. When cells are needing to make glucose, they can't be sidetracked by having the PEP they have made in gluconeogenesis be converted directly back to pyruvate by pyruvate kinase. Consequently, pyruvate kinase is inhibited during gluconeogenesis, lest a "futile cycle" occur. (See HERE)

Another interesting control mechanism called **feedforward activation** involves pyruvate kinase. Pyruvate kinase is activated allosterically by F1,6BP. This molecule is a product of the PFK reaction and a substrate for the aldolase reaction. It should be noted that the aldolase reaction is energetically unfavorable (high + $\Delta G°'$), thus allowing F1,6BP to accumulate. When this happens, some of the excess F1,6BP activates pyruvate kinase, which jump-starts the conversion of PEP to pyruvate. The resulting drop in PEP levels has the effect of "pulling" on the reactions preceding pyruvate kinase. As a consequence, the concentrations of G3P and DHAP fall, helping to move the aldolase reaction forward.

For cells a glucose cycling's cost
Is energy in reams
Four ATPs each time is lost
From breaking/making schemes

So use for metabolic heat
To make it warm inside your feet
Else it's of no utility
To practice such futility

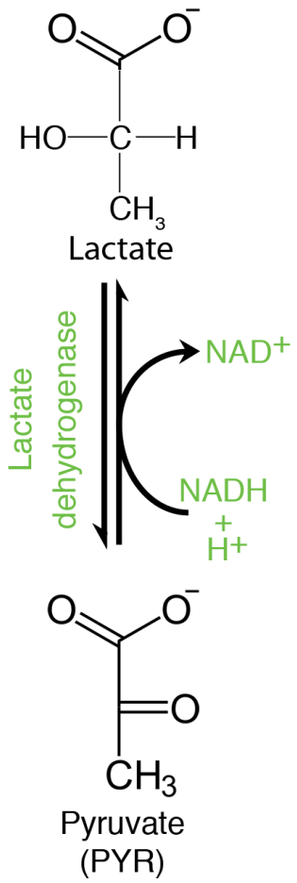
Pyruvate Metabolism

As noted, pyruvate produced in glycolysis can be oxidized to acetyl-CoA, which is itself oxidized in the citric acid cycle to carbon dioxide. That is not the only metabolic fate of pyruvate, though.

Pyruvate is a "starting" point for gluconeogenesis, being converted to oxaloacetate in the mitochondrion in the first step.

Pyruvate in animals can also be reduced to **lactate** when oxygen is limiting. This reaction, which requires NADH produces NAD^+ and is critical for generating the latter molecule to keep the **glyceraldehyde-3-phosphate dehydrogenase** reaction of glycolysis going when there is no oxygen.

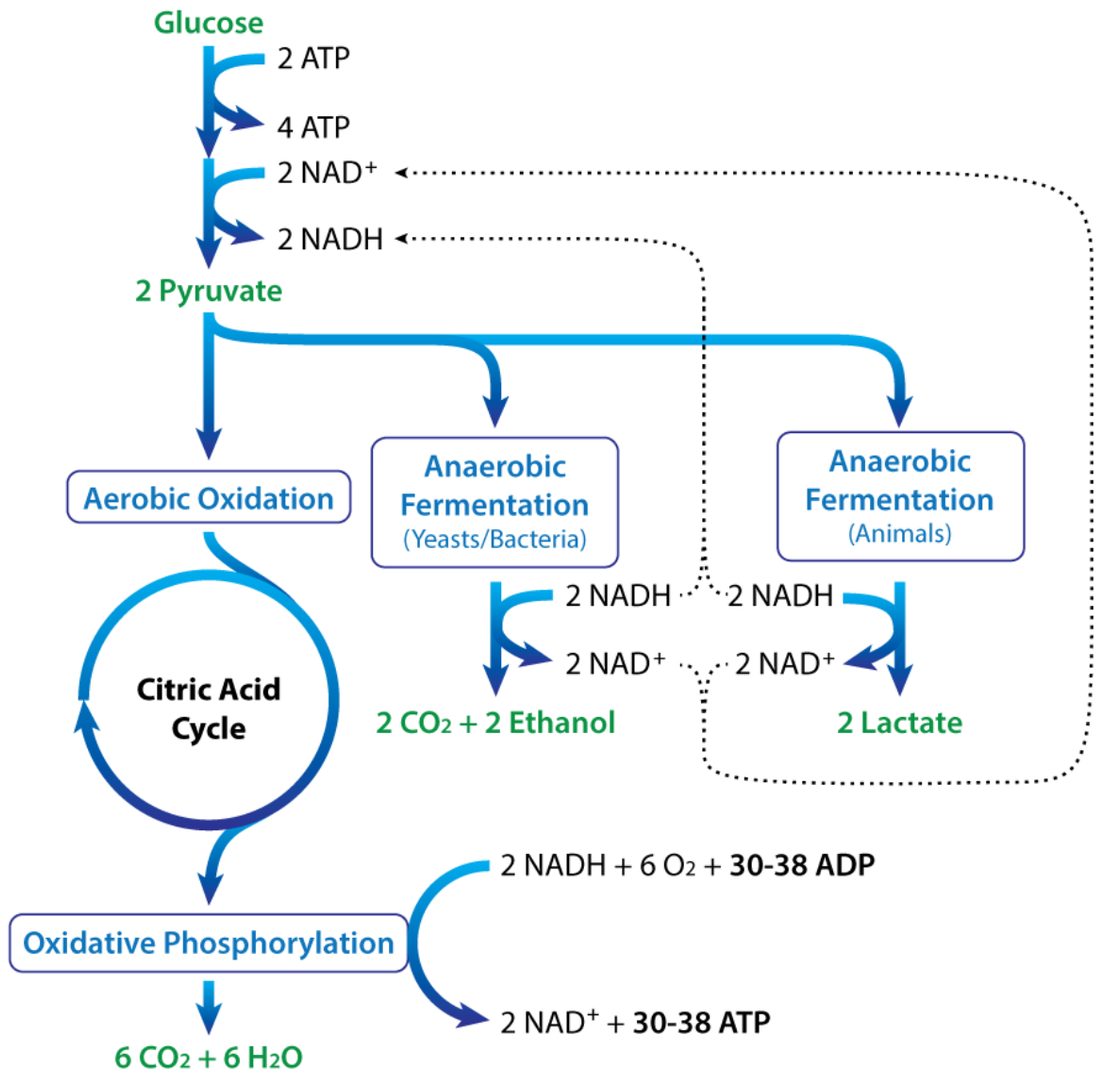
Oxygen is necessary for the electron transport system to operate and this, in turn, is what oxidizes NADH to NAD^+. In the absence of oxygen, thus, an alternative means of making NAD^+ is necessary, or else glycolysis will halt. Bacteria and yeast have NADH requiring reactions that regenerate NAD^+ while producing ethanol from pyruvate under anaerobic conditions, instead of lactic acid. Thus, **fermentation** of pyruvate is necessary to keep



glycolysis operating when oxygen is limiting. It is also for these reasons that brewing of beer (using yeast) involves depletion of oxygen and muscles low in oxygen produce lactic acid (animals).

Pyruvate is a precursor of alanine which can be easily synthesized by transfer of a nitrogen from an amine donor, such as glutamic acid. Pyruvate can also be converted into **oxaloacetate** by carboxylation in the process of gluconeogenesis (see below).

The enzymes involved in pyruvate metabolism include **pyruvate dehydrogenase** (makes acetyl-CoA), **lactate dehydrogenase** (makes lactate), **transaminases** (make alanine), and **pyruvate carboxylase** (makes oxaloacetate).



Metabolic Redox

Gluconeogenesis

The **anabolic** counterpart to glycolysis is gluconeogenesis, which occurs mostly in the cells of the liver and kidney. In seven of the eleven reactions of gluconeogenesis (starting from pyruvate), the same enzymes are used as in glycolysis, but the reaction directions are reversed. Notably, the ΔG values of these reactions in the cell are typically near zero, meaning their direction can be readily controlled by changing substrate and product concentrations.

Click HERE and HERE to see Kevin's YouTube lectures on Gluconeogenesis

The three regulated enzymes of glycolysis all catalyze reactions whose **ΔG** values are not close to

Gluconeogenesis

To the tune of "*Supercalifragelisticexpialidocious*"

When cells have lots of ATP and NADH too
They strive to store this energy as sugar yes they do
Inside of mitochondria they start with pyruvate
(slow) Carboxylating it to make oxaloacetate

Oh gluconeogenesis is so exhilarating
Memorizing it can really be exasperating
Liver cells require it so there's no need for debating
Gluconeogenesis is so exhilarating

Oh, glucose, glucose come to be
Glucose, glucose come to be

Oxaloacetate has got to turn to PEP
Employing energy that comes from breaking GTP
From there it goes to make a couple phosphoglycerates
(slow) Exploiting ee-nolase and mutase' catalytic traits

Oh gluconeogenesis is liver's specialty
Producing sugar for the body most admirably
Six ATPs per glucose is the needed energy*
Gluconeogenesis is liver's specialty

Oh glucose, glucose joy to me
Glucose, glucose joy to me

Converting phosphoglycerate to 1,3BPG
Requires a phosphate that includes A-T-P energy
Reduction with electrons gives us all an N-A-D
(slow) And G3P's isomerized to make D-H-A-P

Oh gluconeogenesis is anabolic bliss
Reversing seven mechanisms of glycolysis
To do well on the final students have to learn all this
Gluconeogenesis is anabolic bliss

Oh, glucose, glucose factory
Glucose, glucose factory

The aldolase reaction puts together pieces so
A fructose molecule is made with two phosphates in tow
And one of these gets cleaved off by a fructose phosphatase
(slow) Unless F2,6BP's acting blocking path-a-ways

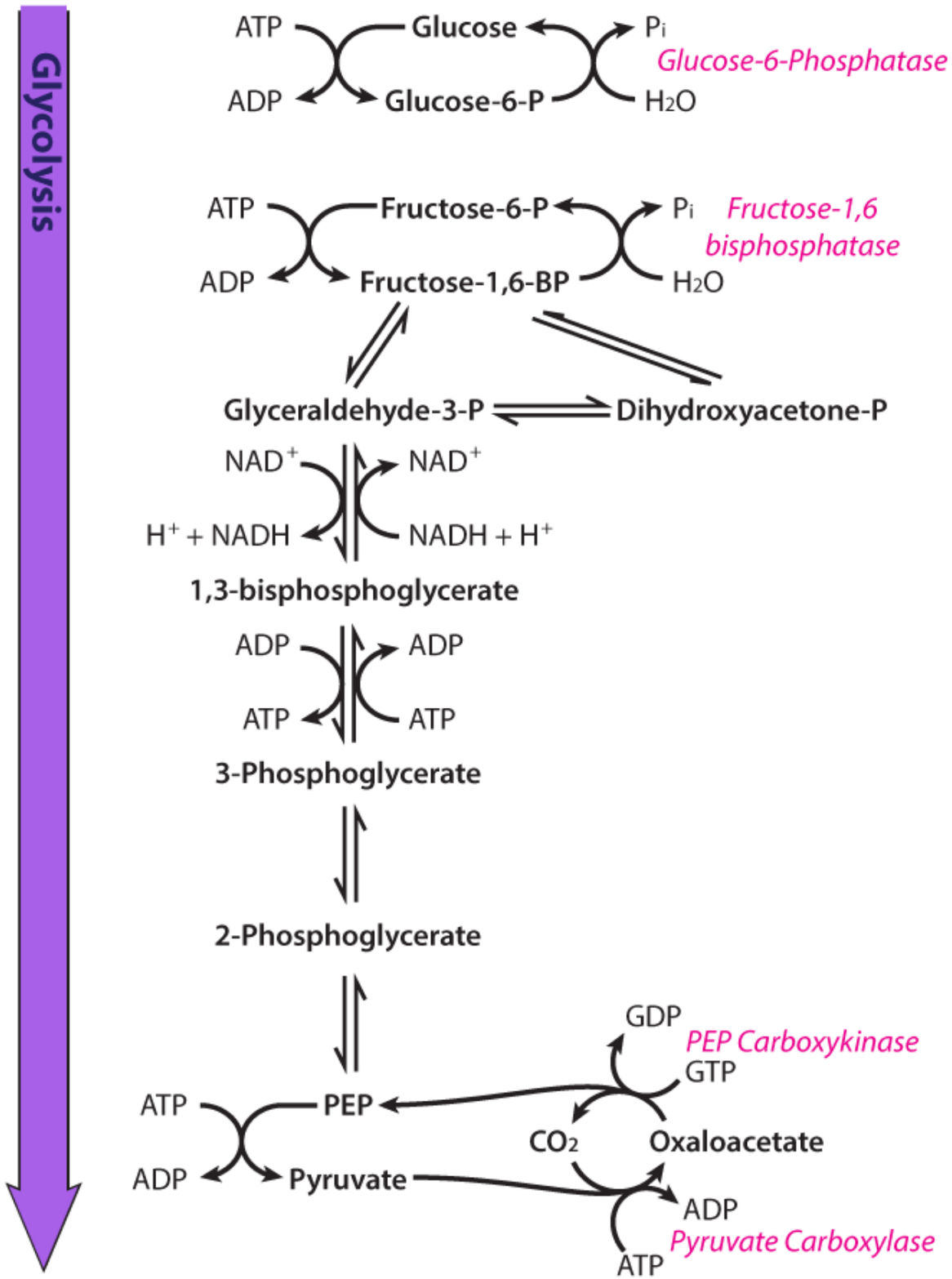
Oh gluconeogenesis a pathway to revere
That makes a ton of glucose when it kicks into high gear
The cell's a masterminding metabolic engineer
Gluconeogenesis a pathway to revere

Oh glucose, glucose jubilee
Glucose, glucose jubilee

From F6P to G6P, that is the final phase
The enzyme catalyzing it is an isomerase
Then G6P drops phosphate and a glucose it becomes
(slow) Inside the tiny endoplasmic-al reticulums

Oh gluconeogenesis is not so very hard
I know that on the final we will not be caught off guard
Cuz our professor lets us use a filled out index card
Gluconeogenesis is not so very hard

Recorded by Tim Karplus
Lyrics by Kevin Ahern



zero, making manipulation of reaction direction non-trivial. Consequently, cells employ "work-around" reactions catalyzed by four different enzymes to favor gluconeogenesis, when appropriate.

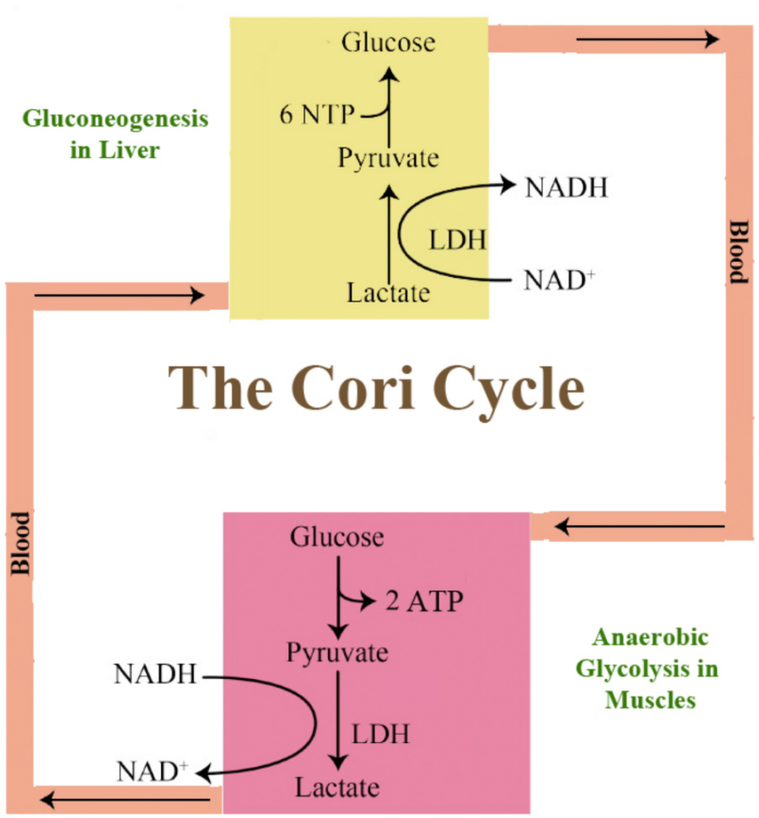
Directional velocity
Inverts with reciprocity
If glycolysis is flowing
Glucose synthesis awaits
But when the latter is a-going
Sugar breakdown then abate

Two of the enzymes (pyruvate carboxylase and PEP carboxykinase - **PEPCK**) catalyze reactions that bypass pyruvate kinase. F1,6BPase bypasses PFK and **G6Pase** bypasses hexokinase. Notably, pyruvate carboxylase and G6Pase are found in the mitochondria and endoplasmic reticulum, respectively, whereas the other two are found in the cytoplasm along with all of the enzymes of glycolysis. As a result, all of glycolysis and most of gluconeogenesis occurs in the cytoplasm. Controlling these pathways then becomes of critical importance because cells generally need to minimize the extent to which paired anabolic and catabolic pathways are occurring simultaneously, lest they waste energy and make no tangible product except heat. The mechanisms of controlling these pathways work, in some ways, in opposite fashions, called reciprocal regulation (see above).

Besides reciprocal regulation, other mechanisms help control gluconeogenesis. First, PEPCK is controlled largely at the level of synthesis. Overexpression of PEPCK (stimulated by glucagon, glucocorticoids, and cAMP and inhibited by insulin) causes symptoms of diabetes. Pyruvate carboxylase is sequestered in the mitochondrion and is sensitive to acetyl-CoA, which is an allosteric activator. Acetyl-CoA concentrations increase as the citric acid cycle activity decreases. **Glucose-6-phosphatase** is present in low concentrations in many tissues, but is found most abundantly and importantly in the major gluconeogenic organs – the liver and kidney cortex.

Cori Cycle

With respect to energy, the liver and muscles act complementarily. The liver is the major organ in the body for the synthesis of glucose. Muscles are major users of ATP. Actively exercising muscles generate lactate as a result of running glycolysis faster than the blood can deliver oxygen during periods of heavy exercise. As a consequence, the muscles go anaerobic and produce lactate. This lactate is of no use to muscle cells, so they dump it into the blood. Lactate travels in the blood to the liver, which takes it up and reoxidizes it back to pyruvate, catalyzed by the enzyme lactate dehydrogenase. Pyruvate in the liver is then converted to glucose by gluconeogenesis. The glucose thus made by the liver is dumped into the bloodstream where it is taken up by muscles and used for energy, completing a very important intercellular pathway known as the Cori cycle.



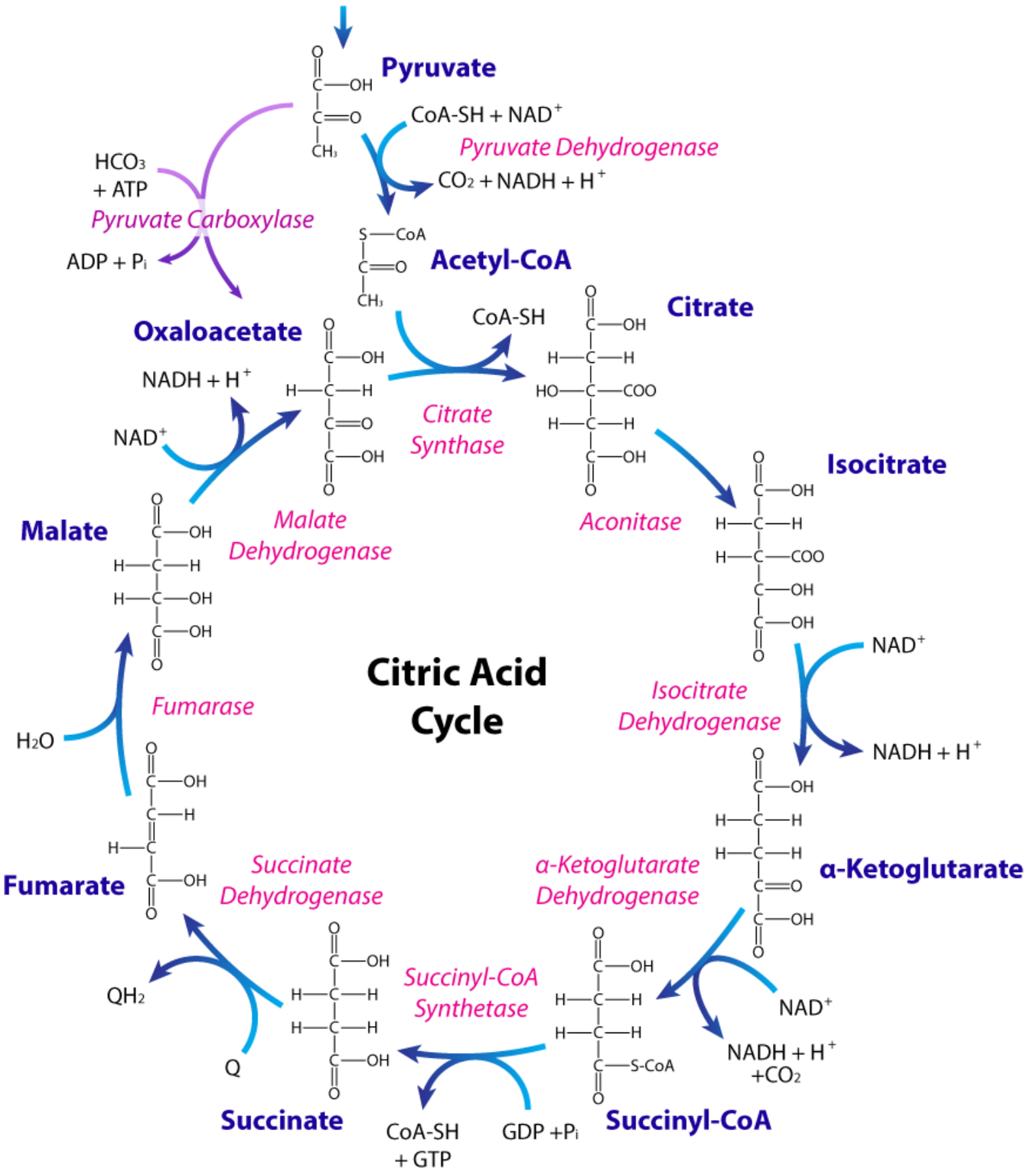
The Cori Cycle

Citric Acid Cycle

The primary catabolic pathway in the body is the **citric acid cycle** because it is here that oxidation to carbon dioxide occurs for breakdown products of the cell's major building blocks - sugars, fatty acids, amino acids. The pathway is cyclic (next page) and thus, doesn't really have a starting or ending point. All of the reactions occur in the mitochondrion, though one enzyme is embedded in the organelle's membrane. As needs change, cells may use a subset of the reactions of the cycle to produce a desired molecule rather than to run the entire cycle (see below for examples).

Focusing on the pathway itself, the traditional point to start discussion is addition

Click HERE and HERE for Kevin's YouTube lectures on the Citric Acid and Glyoxylate Cycles



The Citric Acid Cycle Reactions

of acetyl-CoA to oxaloacetate (OAA) to form citrate. Acetyl-CoA for the pathway can come from a variety of sources. They include pyruvate oxidation (from glycolysis and amino acid metabolism), fatty acid oxidation, and amino acid metabolism. The reaction joining it to OAA is catalyzed by citrate synthase and the ΔG°' is fairly negative. This, in turn, helps to "pull" the reaction preceding it in the cycle (catalyzed by malate dehydrogenase).

In the next reaction, citrate is isomerized to **isocitrate** by action of the enzyme called **aconitase**. Isocitrate is a branch point in plants and bacteria for the **glyoxylate cycle**. Oxidative decarboxylation of isocitrate by isocitrate dehydrogenase produces the first NADH and yields **alpha-ketoglutarate**. This five carbon intermediate is a branch point for synthesis of glutamate. In addition, glutamate can also be made easily into this citric acid cycle intermediate. Decarboxylation of alpha-ketoglutarate yields **succinyl-CoA** and is catalyzed by **alpha-ketoglutarate dehydrogenase**. This enzyme is structurally very similar to pyruvate dehydrogenase and employs the same five coenzymes – **NAD**, FAD, **CoASH**, **TPP**, and **lipoic acid**.

The remainder of the citric acid cycle involves

I love my citrate synthase
It really is first rate
Adds O-A-A to Ac-Co-A
Producing a citrate

Aconitase is picky
Binds substrates specially
Creating isocitrate
Which has no symmetry

Then CO_2 gets lost from it
Released in the next phase
The secret weapon - Isocitrate
Dehydrogenase

The alpha K–D-H is next
It gets my admiration
For clipping CO_2 in one more
Decarboxylation

Succ-CoA synthetase steps up
Reacting most absurd
It's named for a catalysis
That really goes backward

Suc -CIN-ate de-hyd-ROG-en-ase
Pulls H from succinate
Creating $FADH_2$
As well as fumarate

The fumarate gains water
OH-configured L
The fumarase's product?
Some malate for the cell

With the last oxidation
Malate de-hyd-ROG-en-ase
Expels its two creations
N-A-D-H / O-A-A

conversion of the four carbon succinyl-CoA into oxaloacetate. Succinyl-CoA is a branch point for the synthesis of heme. Succinyl-CoA is converted to **succinate** in a reaction catalyzed by **succinyl-CoA synthetase** (named for the reverse reaction) and a GTP is produced, as well – the only substrate level phosphorylation in the cycle. The energy for the synthesis of the GTP comes from hydrolysis of the high energy **thioester** bond between succinate and the CoA. Evidence for the high energy of a thioester bond is also evident in the citrate synthase reaction, which is also very energetically favorable. Succinate is also produced by metabolism of odd-chain fatty acids (see below).

Oxidation of succinate occurs in the next step, catalyzed by **succinate dehydrogenase**. This interesting enzyme both catalyzes this reaction and participates in the electron transport system, funneling electrons from the $FADH_2$ it gains in the reaction to **coenzyme Q**. The product of the reaction, **fumarate** gains a water across its *trans* double bond in the next reaction, catalyzed by **fumarase** to form **malate**. Fumarate is also a byproduct of nucleotide metabolism and of the **urea cycle**. Malate is important also for transporting electrons across membranes in the **malate aspartate shuttle** and in ferrying carbon dioxide in **C_4 plants**.

Conversion of malate to OAA is a rare biological oxidation that has a $\Delta G^{\circ\prime}$ with a positive value. The reaction product includes NADH and the reaction is 'pulled' by the energetically favorable conversion of OAA to citrate in what was described above as the first reaction of the cycle. OAA intersects other important pathways, including amino acid metabolism (readily converted to

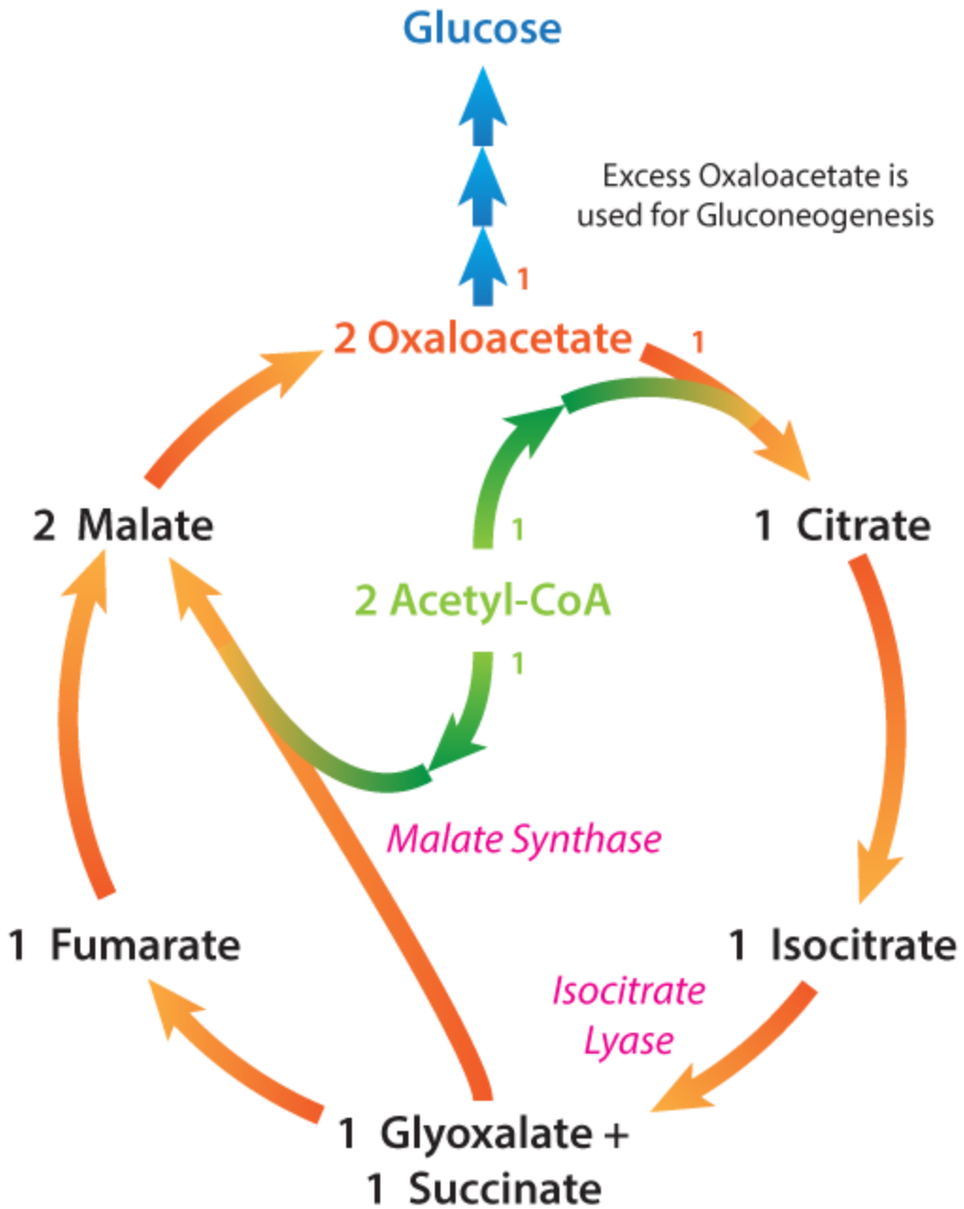
I'm thinking I could lose some weight
If I could make glyoxylate
Combined with acetyl-CoA
Malate would then form OAA
The excess OAA in turn
Would give more glucose to be burned
Converting fat to glucose, see
Expends it glycolytically

aspartic acid), **transamination** (nitrogen movement) and gluconeogenesis.

Glyoxylate Pathway

A pathway related to the Citric Acid Cycle (CAC) is the **glyoxylate pathway** (right). This pathway, which overlaps all of the non-decarboxylation reactions of the CAC does not operate in animals, because they lack two enzymes necessary for the pathway – **isocitrate lyase** and **malate synthase**. Isocitrate lyase catalyzes the conversion of isocitrate into succinate and glyoxylate. Because of this, all six carbons of the CAC survive and do not end up as carbon dioxide.

Succinate continues through the remaining reactions of the CAC to produce oxaloacetate. Glyoxylate combines with another acetyl-CoA (one acetyl-CoA was used to start the cycle) to create malate (catalyzed by malate synthase).



The Glyoxylate Cycle

Malate can, in turn, be oxidized to oxaloacetate.

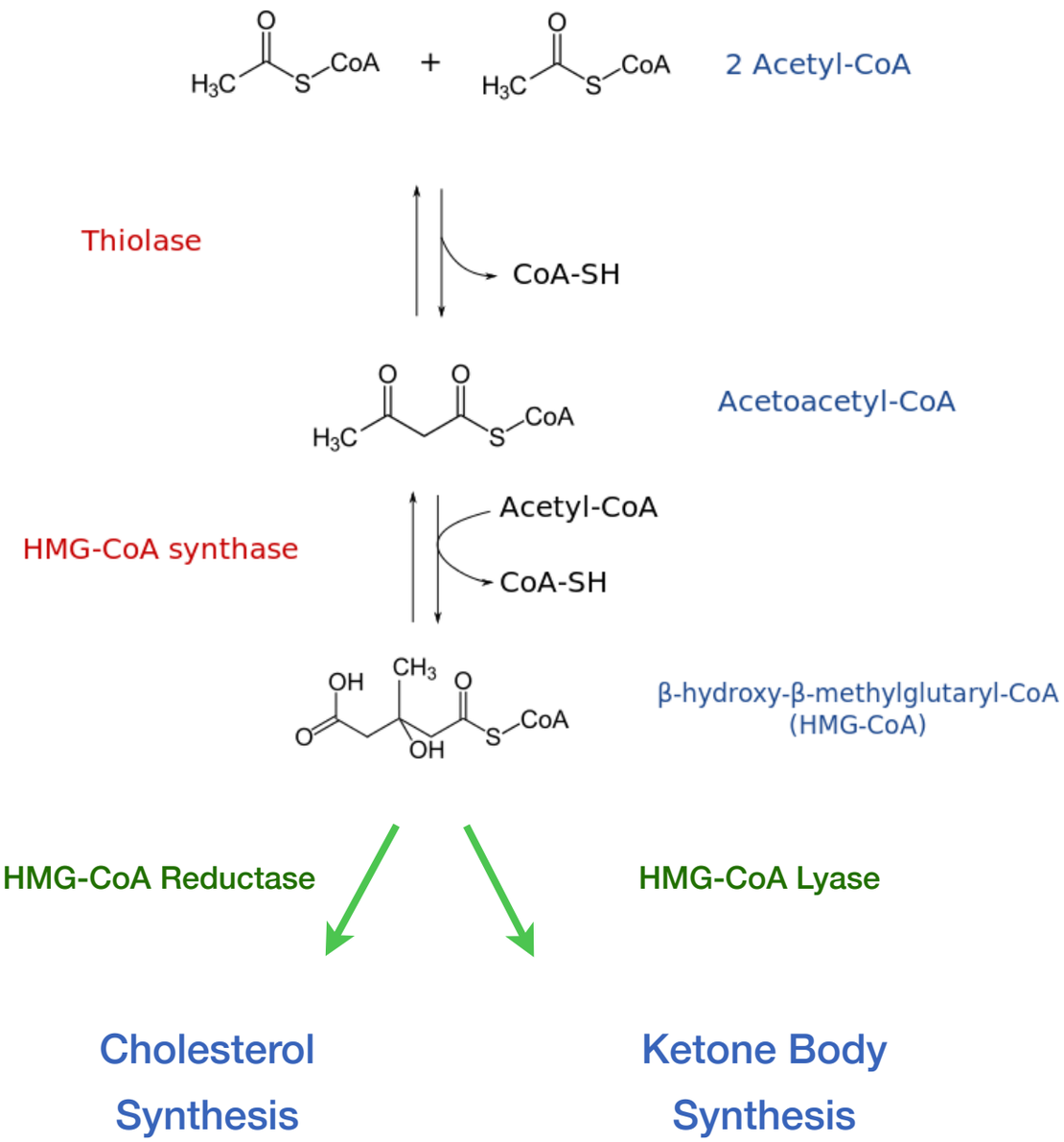
It is at this point that the pathway's contrast with the CAC is apparent. After one turn of the CAC, a single oxaloacetate is produced and it balances the single one used in the first reaction of the cycle. Thus, in the CAC, no net production of oxaloacetate is realized. By contrast, at the end of a turn of the glyoxylate cycle, two oxaloacetates are produced, starting with one. The extra oxaloacetate can then be used to make other molecules, including glucose in gluconeogenesis.

Because animals do not run the glyoxylate cycle, they cannot produce glucose from acetyl-CoA in net amounts, but plants and bacteria can. As a result, these organisms can turn acetyl-CoA from fat into glucose, while animals can't. Bypassing the decarboxylations (and substrate level phosphorylation) has its costs, however. Each turn of the glyoxylate cycle produces one $FADH_2$

and one NADH instead of the three NADHs, one $FADH_2$, and one GTP made in each turn of the CAC.

Acetyl-CoA Metabolism

Acetyl-CoA is one of the most “connected” metabolites in biochemistry, appearing in fatty acid oxidation/ reduction, pyruvate oxidation, the citric acid cycle, amino acid anabolism/catabolism, ketone body metabolism, steroid/bile acid synthesis, and (by extension from fatty acid metabolism) prostaglandin synthesis. Most of these pathways will be dealt with separately. Here we will cover the last three.



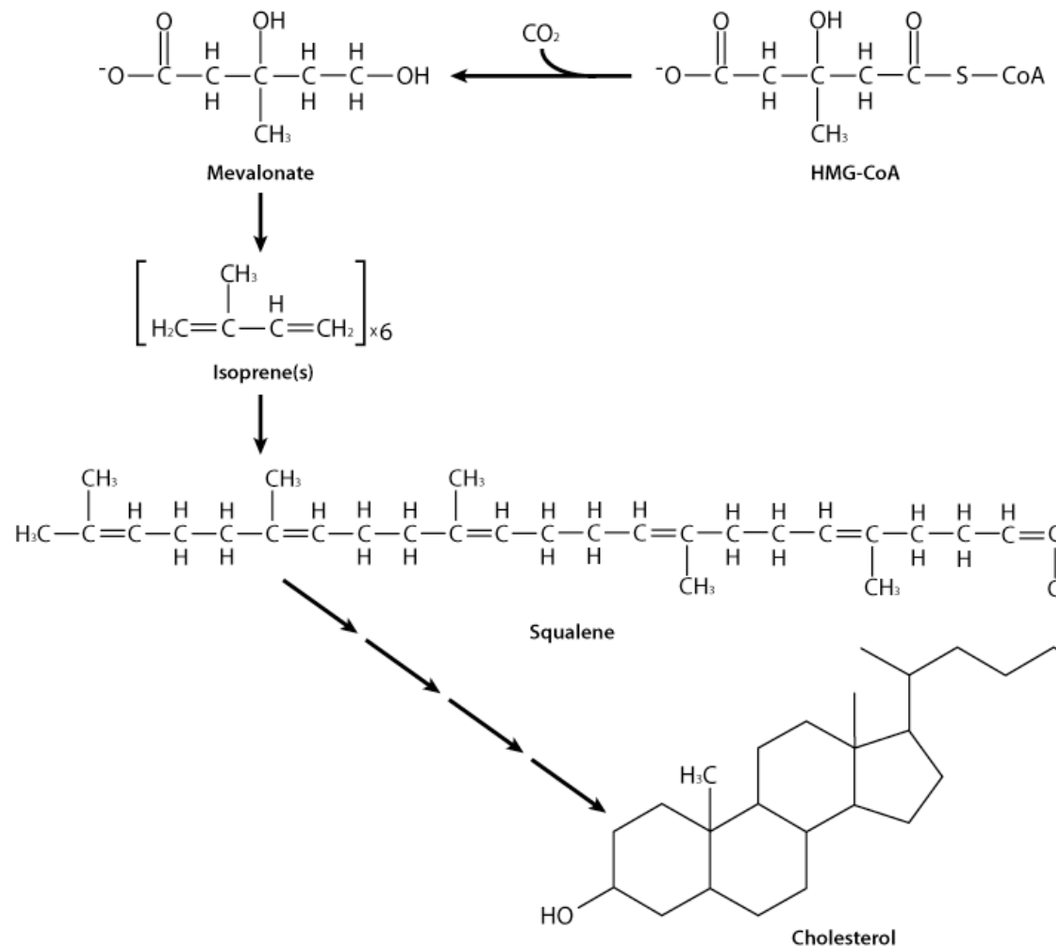
The pathways for ketone body synthesis and cholesterol biosynthesis overlap at the beginning. Each of these starts by combining two acetyl-CoAs together to make acetoacetyl-CoA. Not coincidentally, that is the next to last product of oxidation of fatty acids with even numbers of carbons (see below). In fact, the enzyme that catalyzes the joining is the same as the one that catalyzes its breakage in fatty acid oxidation – **thiolase**. Thus, these pathways start by reversing the last step of the last round of fatty acid oxidation. Both pathways also include addition of two more carbons from a third acetyl-CoA to form **Hydroxy-Methyl-Glutaryl-CoA**, or **HMG-CoA**, as it is more commonly known. It is at this point that the two pathways diverge.

Cholesterol Metabolism

The cholesterol biosynthesis pathway is a long one and it requires significant amounts of reductive and ATP energy, which is why it

is included here. Cholesterol has important roles in the body in membranes. It as also a precursor of **steroid hormones** and **bile acids** and its immediate metabolic precursor, **7-dehydrocholesterol**, is a precursor of **Vitamin D**. The pathway leading to cholesterol is known as the **isoprenoid pathway** and branches of it lead to other molecules including other fat-soluble vitamins.

From HMG-CoA, the enzyme HMG-CoA reductase catalyzes the formation of **mevalonate**. The reaction requires NADPH and results in release of coenzyme A and appears to be one of the most important regulatory steps in the synthesis pathway. The enzyme is regulated both by feedback inhibition (cholesterol inhibits it) and by covalent modification (phosphorylation inhibits it). The enzyme's synthesis is also regulated transcriptionally. When cholesterol levels fall, transcription of the gene increases.



The Pathway to Cholesterol

See Kevin's YouTube lectures on Steroid and Lipid Metabolism and Lipid Movement in the Body HERE and HERE

Mevalonate gets phosphorylated twice and then decarboxylated to yield the five carbon intermediate known as **isopentenyl-pyrophosphate** (IPP). IPP is readily converted to **dimethylallylpyrophosphate** (DMAPP). These two five carbon compounds, also called isoprenes, are the building blocks for the synthesis of cholesterol and related compounds. This pathway is known as the isoprenoid pathway. It proceeds in the direction of cholesterol starting with the joining of IPP and DMAPP to form **geranyl-pyrophosphate**. Geranyl-pyrophosphate combines with another IPP to make **farnesyl-pyrophosphate**, a 15-carbon compound. Two farnesyl-pyrophosphates join to create the 30-carbon

To Make a Cholesterol

To the tune of "*When Johnny Comes Marching Home*"

Some things that you can build with acetyl-CoAs
Are joined together partly thanks to thiolase
They come together 1-2-3
Six carbons known as H-M-G
And you're on your way
To make a cholesterol

To synthesize a mevalonate in the cell
Requires reducing HMG-CoA, as well
The enzyme is a RE-ductase
Controlled in allosteric ways
When the cell's impelled
To make a cholesterol.

The mevalonate made in metabolic schemes
Gets decarboxylated down to isoprenes
They're linked together willy-nil
To build a PP-geranyl
In the cells' routinesTo make a cholesterol

A single step links farnesyls but that's not all
The squalene rearranges to lanosterol
From that there's nineteen steps to go
Before the sterol's apropos
Which you must recall
To make a cholesterol

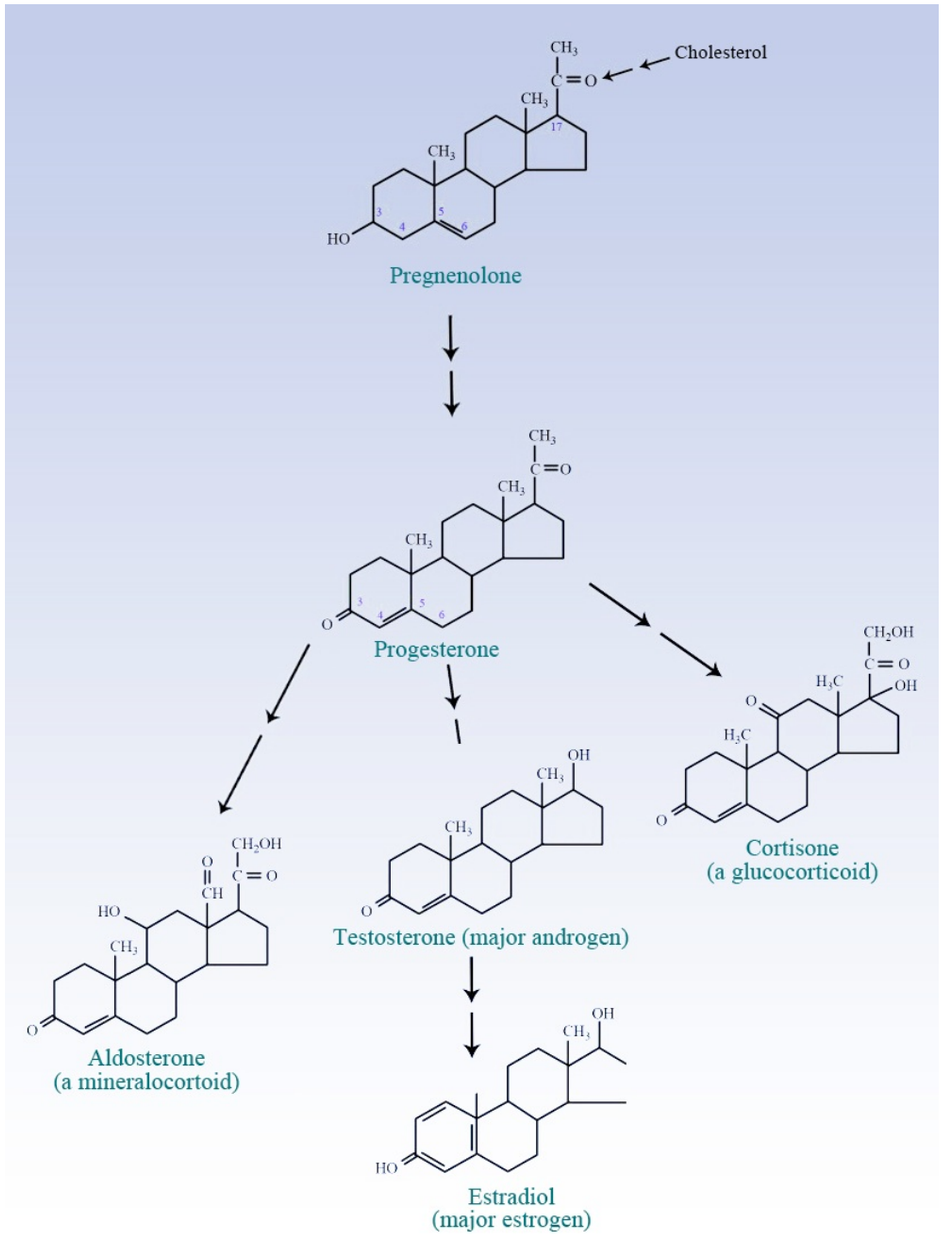
The regulation of the scheme's complex in ways
Inhibited by feedback of the RE-duc-tase
And statins mimic so they say
The look of HMG-CoA
So we sing their praise
And not make cholesterol

Recorded by David Simmons
Lyrics by Kevin Ahern

compound known as **squalene**. Squalene, in a complicated rearrangement involving reduction and molecular oxygen forms a cyclic intermediate known as lanosterol that resembles cholesterol. Conversion of lanosterol to cholesterol is a lengthy process involving 19 steps that occur in the endoplasmic reticulum.

Branching from cholesterol, one can form Vitamin D or the steroid hormones, which include the progestagens, androgens, estrogens, mineralocorticoids, and the glucocorticoids (pictured on the previous page). The branch molecule for all of these is the cholesterol metabolite (and progestagen) known as pregnenalone. The progestagens are precursors of all of the other classes.

The estrogens are derived from the androgens in an interesting reaction that required formation of an aromatic ring. The enzyme catalyzing this reaction is known as an aromatase and it is of medical significance. The growth of some tumors is stimulated by estrogens, so aromatase inhibitors are prescribed to prevent the formation of estrogens and slow tumor growth.

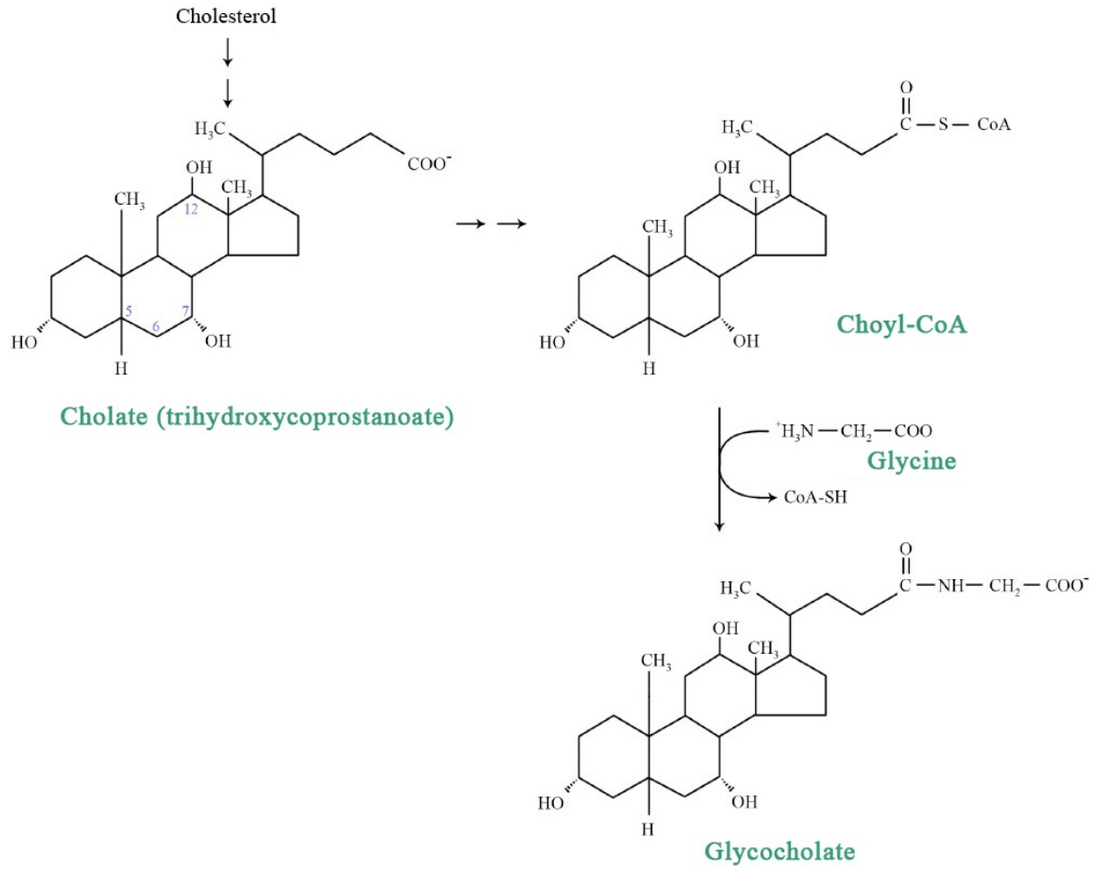


Steroid Hormone Synthesis

It is worth noting that synthesis of other fat soluble vitamins and **chlorophyll** also branches from the isoprenoid synthesis pathway at geranylpyrophosphate. Joining of two geranylgeranylpyrophosphates occurs in plants and bacteria and leads to synthesis of **lycopene**, which, in turn is a precursor of **beta-carotene**, the final precursor of **Vitamin A**. Vitamins E and K, as well as chlorophyll are all also synthesized from geranylgeranylpyrophosphate.

Bile Acid Metabolism

Another pathway from cholesterol leads to the polar bile acids, which are important for the solubilization of fat during digestion. Converting the very non-polar cholesterol to a bile acid involves



Bile Salts

oxidation of the terminal carbon on the side chain off the rings. Other alterations to increase the polarity of these compounds include hydroxylation of the rings and linkage to other polar compounds.

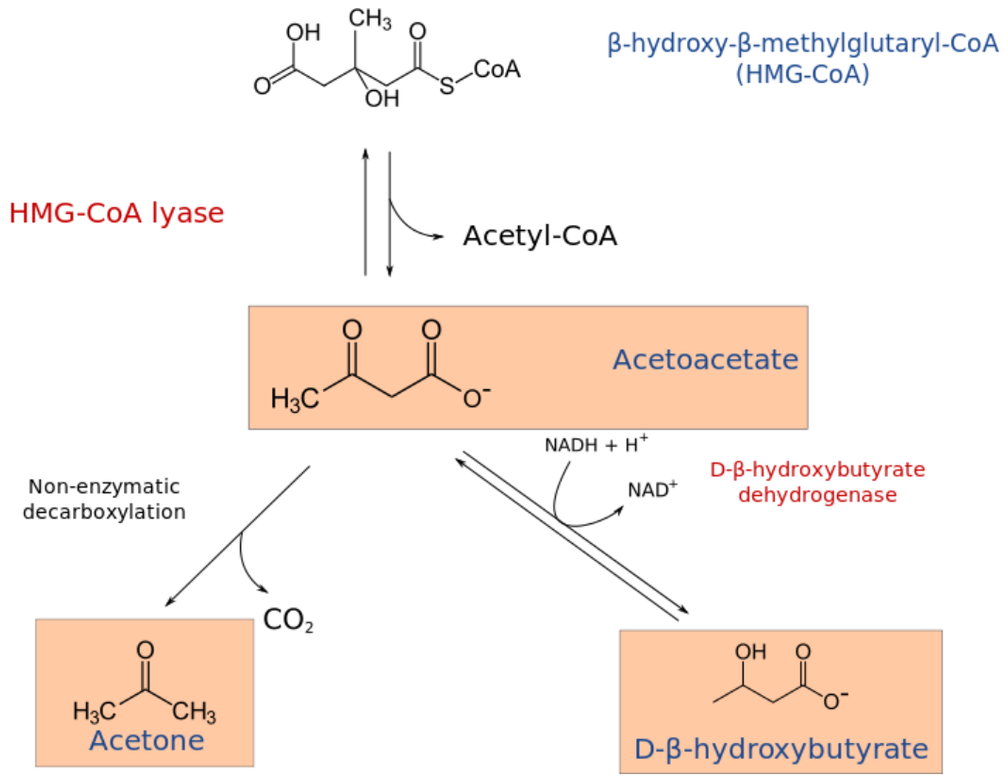
Common bile acids include **cholic acid**, **chenodeoxycholic acid**, **glycocholic acid**, **taurocholic acid**, and **deoxycholic acid**. Another important fact about bile acids is that their synthesis reduces the amount of cholesterol available and promotes uptake of LDLs by the liver. Normally bile acids are recycled efficiently resulting in limited reduction of cholesterol levels. However, inhibitors of the recycling promote reduction of cholesterol levels.

Ketone Body Metabolism

In ketone body synthesis, an acetyl-CoA is split off from HMG-CoA, yielding acetoacetate, a four carbon ketone body that is somewhat unstable, chemically. It will decarboxylate spontaneously to some extent to yield acetone.

Ketone bodies are made when the blood levels of glucose fall very low. Ketone bodies can be converted to acetyl-CoA, which can be used for ATP synthesis via the citric acid cycle. People who are very hypoglycemic (including some diabetics) will produce ketone bodies and these are often first detected by the smell of acetone on their breath.

Acetone is of virtually no use for energy production since it is not readily converted to acetyl-CoA.



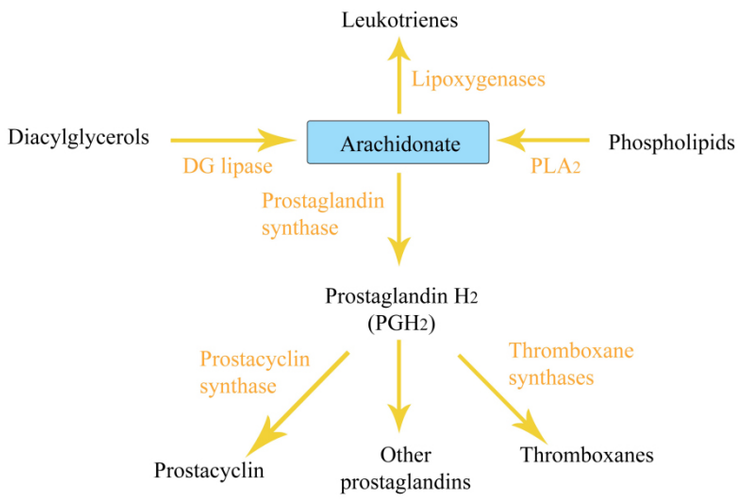
Ketone Body Reactions

See Kevin's lecture on Ketone Bodies HERE

Consequently, cells convert acetoacetate into beta-hydroxybutyrate, which is more chemically stable. Though technically not a ketone, beta-hydroxybutyrate is frequently referred to as a ketone body. Upon arrival at a target cell, it can be oxidized back to acetoacetate with conversion to acetyl-CoA. Both **acetoacetate** and **beta-hydroxybutyrate** can cross the blood-brain barrier and provide important energy for the brain when glucose is limiting.

Prostaglandin Synthesis

The pathway for making **prostaglandins** is an extension of the fatty acid synthesis pathway



Prostaglandin Metabolism

(below). Prostaglandins, molecules associated with localized pain, are synthesized in cells from **arachidonic acid** (see previous page) which has been cleaved from membrane lipids. The enzyme catalyzing their synthesis is known as p**rostaglandin synthase**, but is more commonly referred to as a **cyclooxygenase** (or COX) enzyme. Inhibition of the action of this enzyme is a strategy of non-steroidal pain relievers (also called **NSAID**s), such as **aspirin** or **ibuprofen**. Inhibition of the release of arachidonic acid from membranes is the mechanism of action of steroidal anti-inflammatories, which inhibit the phospholipase A_2 (PLA_2) that catalyzes the cleavage reaction.

See Kevin's YouTube lectures on Fat, Fatty Acid, and Prostaglandin Metabolism HERE, HERE, and HERE

Fatty Acid Oxidation

Breakdown of fats yields fatty acids and **glycerol**. Glycerol can be readily converted to DHAP for oxidation in glycolysis or synthesis into glucose in gluconeogenesis. Fatty acids are broken down in two carbon units of acetyl-CoA. To be oxidized, they must be transported

Prostaglandins

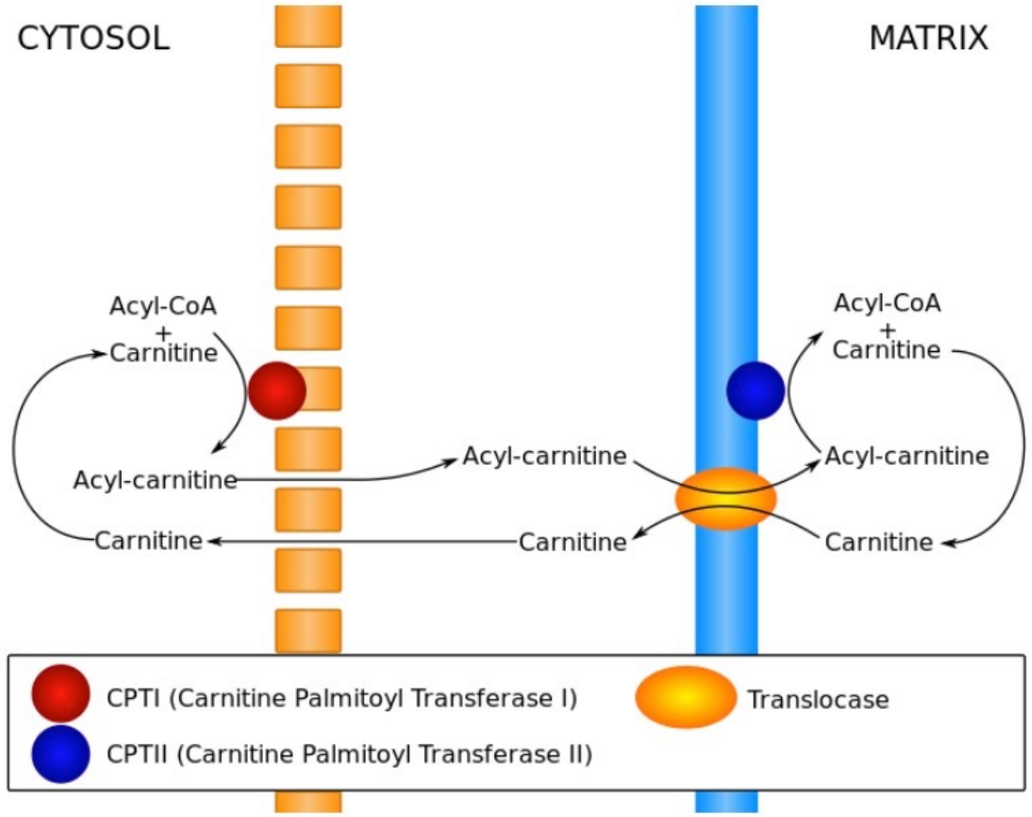
To the tune of "*Oklahoma*"

Prossss-taglandins
The ei-co-sa-noids creating pain
Are the ones to blame - when you get inflamed
And ouch(!) - they hurt inside your brain

Prossss-taglandins
Every throb and ache gets magnified
If you hope to win, cyclo-oxygen's
Generation's got to be denied
The Vioxx has all been recalled
So go get yourself Tylenol-ed

And if you aaaaaaaaaaaache
Blame PGH synthaaaaaaaaase!
We must complain that
You make the aches prostaglandins
Prostaglandin - D2, F1, G2, E2
Prostaglandin, it's you

Recorded by Tim Karplus
Lyrics by Kevin Ahern



Movement of Acyl-CoAs Into the Mitochondrial Matrix

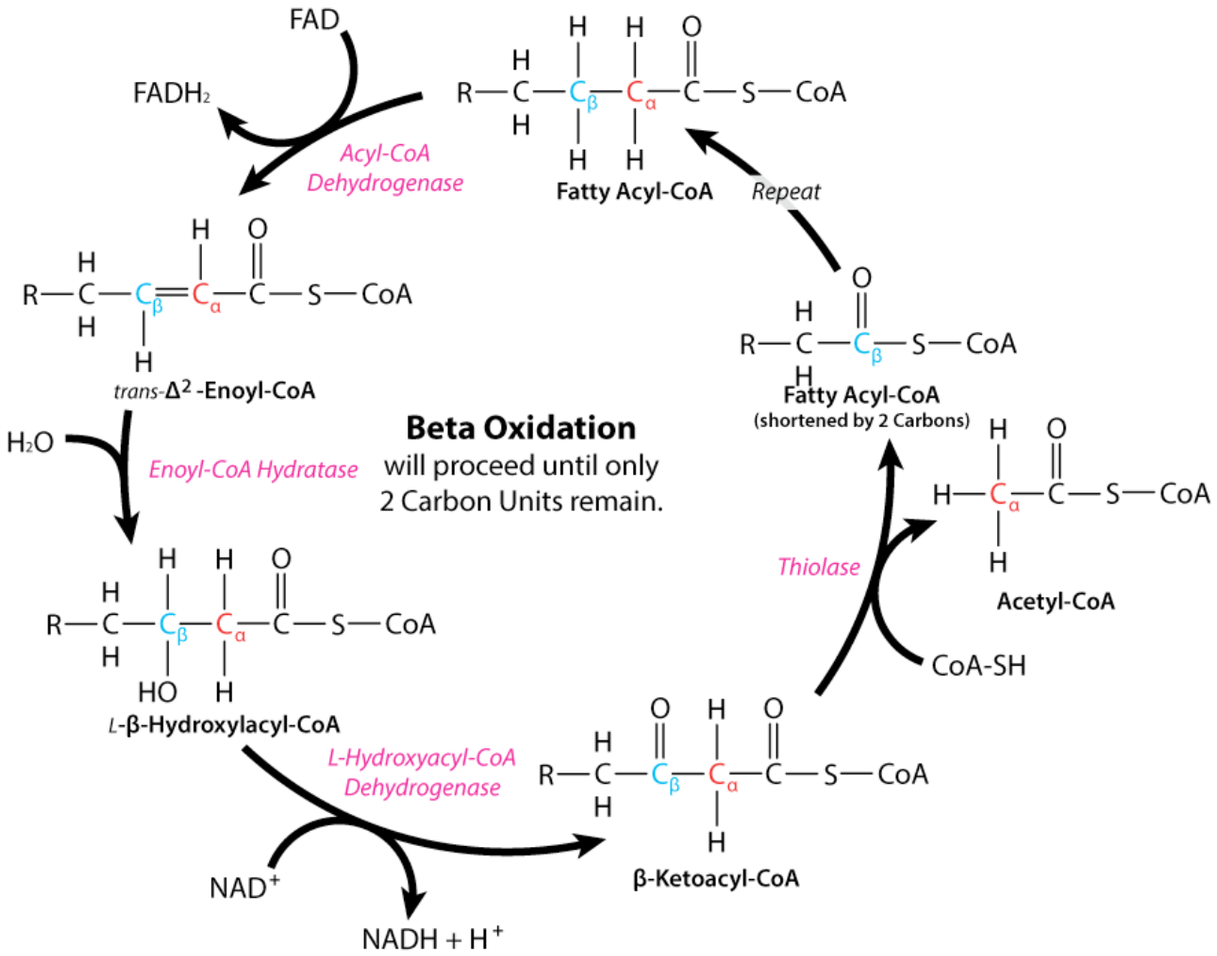
through the cytoplasm attached to coenzyme A and moved into mitochondria. The latter step requires removal of the CoA and attachment of the fatty acid to a molecule of **carnitine**. The carnitine complex is transported across the inner membrane of the mitochondrion after which the fatty acid is reattached to coenzyme A in the mitochondrial matrix.

The process of fatty acid oxidation, called **beta oxidation**, is fairly simple. The reactions all occur between carbons 2 and 3 (with #1 being the one linked to the CoA) and sequentially include the following 1) dehydrogenation to create $FADH_2$ and a fatty acyl group with a double bond in the *trans* configuration; 2) hydration across the double bond to put a hydroxyl group on carbon 3 in the L configuration; 3) oxidation of the hydroxyl group to make a ketone; and 4) thiolytic cleavage to release acetyl-CoA and a fatty acid two carbons shorter than the starting one.

Unsaturated fatty acids complicate the picture a bit (see below), primarily because they have *cis* bonds, for the most part, if they are of biological origin and these must be converted to the relevant trans intermediate made in step 1. Sometimes the bond must be moved down the chain, as well, in order to be positioned properly. Two enzymes (described below) handle all the necessary isomerizations and moves necessary to oxidize all of the unsaturated fatty acids.

Enzymes of Beta Oxidation

The reactions of fatty acid oxidation are notable in mirroring the oxidations in the latter half of the citric acid cycle – dehydrogenation of succinate to make a *trans* double bond (fumarate), hydration across the double bond to make L-malate and oxidation of the hydroxyl to make a ketone (oxaloacetate). Two of the enzymes of beta-oxidation are notable. The first is **acyl-CoA dehydrogenase**, which catalyzes the initial dehydrogenation and yields $FADH_2$. It comes in three different forms – ones that work on long, medium, or short chain length fatty acids. The first of these is sequestered in the peroxisome of



Beta Oxidation of Fatty Acids

animals whereas the others are found in the mitochondria. Plants and yeast perform beta oxidation exclusively in the peroxisome. The most interesting of the acyl-CoA dehydrogenases is the one that works on medium length fatty acids. This one, which is the one most commonly deficient in animals, has been linked to sudden infant death syndrome.

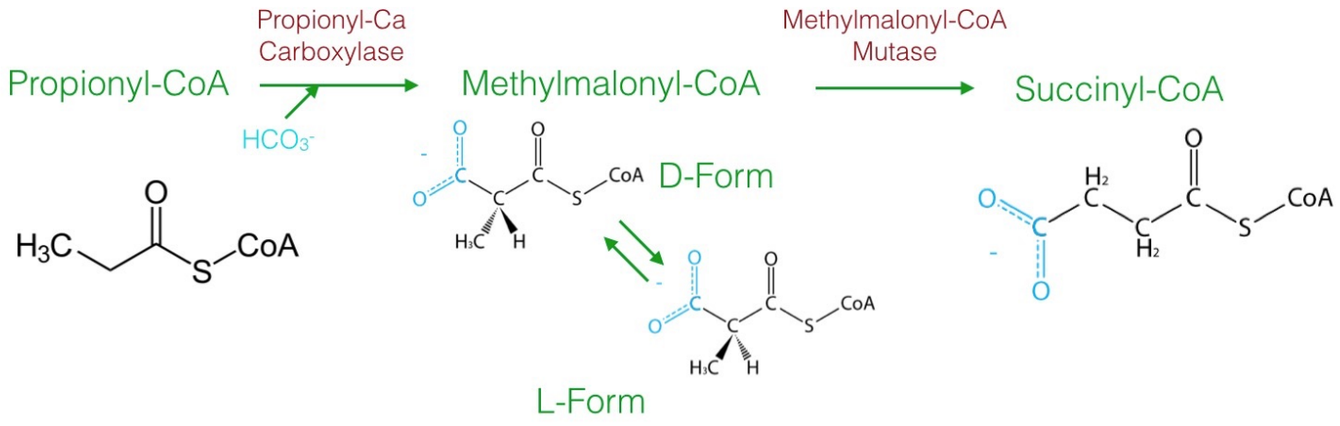
See Kevin's lectures on fat, Fatty Acid, and Prostaglandin Metabolism HERE, HERE, and HERE

Reactions two and three in beta oxidation are catalyzed by enoyl-CoA hydratase and 3-hydroxyacyl-CoA dehydrogenase, respectively. The latter reaction yields an NADH. The final enzyme of beta oxidation is thiolase and this enzyme is notable in not only catalyzing the formation of acetyl-CoAs in beta oxidation, but also catalyzing the joining of two acetyl-CoAs (essentially the reversal of the last step of beta oxidation) to form **acetoacetyl-CoA** – essential for the pathways of ketone body synthesis and cholesterol biosynthesis.

Oxidation of Odd Chain Fatty Acids

Though most fatty acids of biological origin have even numbers of carbons, not all of them do. Oxidation of fatty acids with odd numbers of carbons ultimately produces an

In beta oxidation, it just occurred to me
The process all takes place 'tween carbons two and three
Some hydrogens are first removed to $FADH_2$
Then water adds across the bond, the H to carbon two
Hydroxyl oxidation's next, a ketone carbon three
Then thiolase catalysis dissects the last two C's
The products of the path, of course, are acetyl-CoAs
Unless there were odd carbons, hence propionyl-CoA

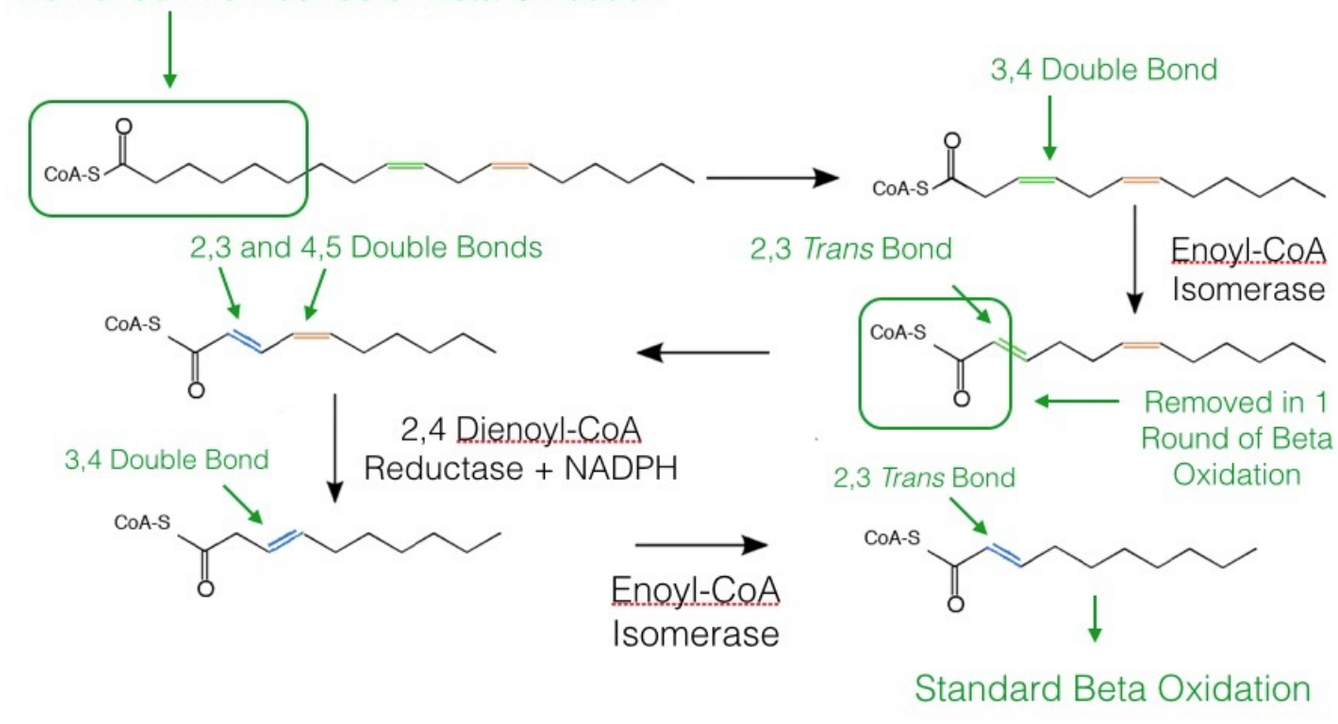


Odd-Chain Fatty Acid Oxidation

intermediate with three carbons called **propionyl-CoA**, which cannot be oxidized further in the beta-oxidation pathway. Metabolism of this intermediate is odd. Sequentially, the following steps occur – 1) carboxylation to make D-methylmalonyl-CoA; 2) isomerization to L-methylmalonyl-CoA; 3) rearrangement to form succinyl-CoA. The last step of the process utilizes the enzyme methylmalonyl-CoA mutase, which uses the B_{12} coenzyme in its catalytic cycle. Succinyl-CoA can then be metabolized in the citric acid cycle.

Unsaturated Fatty Acid Oxidation

As noted above, oxidation of unsaturated fatty acids requires two additional enzymes to the complement of enzymes for beta oxidation. If the beta oxidation of the fatty acid produces an intermediate with a *cis* bond between carbons three and four, cis-Δ3-Enoyl-CoA Isomerase will convert the bond to a *trans* bond between carbons two and three and beta oxidation can



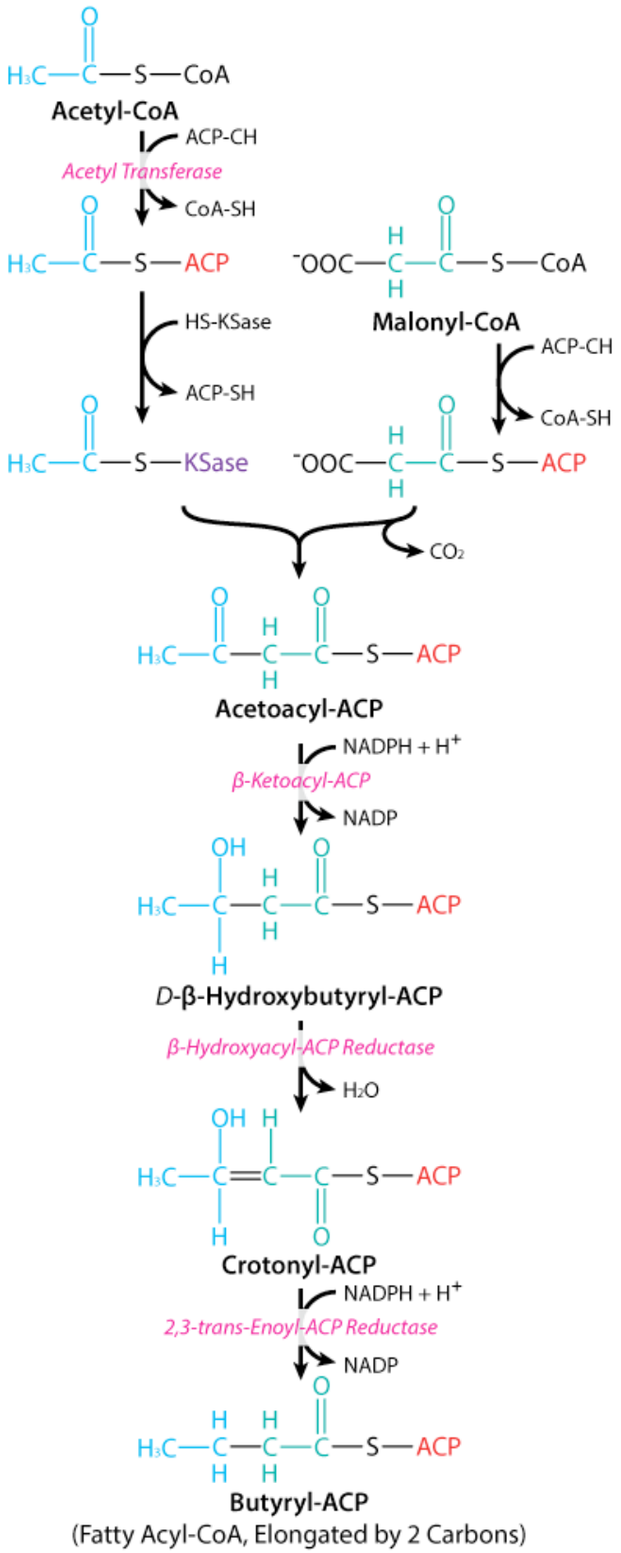
Unsaturated Fatty Acid Oxidation

proceed as normal.

On the other hand, if beta oxidation produces an intermediate with a *cis* double bond between carbons four and five, the first step of beta oxidation (dehydrogenation between carbons two and three) occurs to produce an intermediate with a *trans* double bond between carbons two and three and a *cis* double bond between carbons four and five. The enzyme 2,4 dienoyl CoA reductase reduces this intermediate (using NADPH) to one with a single *cis* bond between carbons three and four. This intermediate is then identical to the one acted on by cis-Δ3-Enoyl-CoA Isomerase above, which converts it into a regular beta oxidation intermediate, as noted above.

Alpha Oxidation

Yet another consideration for oxidation of fatty acids is alpha oxidation. This pathway is necessary for catabolism of fatty acids that have branches in their chains. For example, breakdown of chlorophyll's phytol group yields phytanic acid, which undergoes hydroxylation and oxidation on carbon number two (in contrast to carbon three of beta oxidation), followed by decarboxylation and production of a branched intermediate that can be further oxidized by the beta oxidation pathway. Though alpha oxidation is a relatively minor metabolic pathway, the inability to perform the reactions of the pathway leads to Refsum's disease where accumulation of phytanic acid leads to neurological damage.



Fatty Acid Synthesis

Fatty Acid Synthesis

Synthesis of fatty acids occurs in the cytoplasm and endoplasmic reticulum of the cell and is chemically similar to the beta-oxidation process, but with a couple of key differences. The first of these occur in preparing substrates for the reactions that grow the fatty acid. Transport of acetyl-CoA from the mitochondria occurs when it begins to build up. Two molecules can play roles in moving it to the cytoplasm – citrate and **acetylcarnitine**. Joining of oxaloacetate with acetyl-CoA in the

mitochondrion creates citrate which moves across the membrane, followed by action of citrate lyase in the cytoplasm of the cell to release acetyl-CoA and oxaloacetate. Additionally, when free acetyl-CoA accumulates in the mitochondrion, it may combine with carnitine and be transported out to the cytoplasm.

Starting with two acetyl-CoA, one is converted to **malonyl-CoA** by carboxylation catalyzed by the enzyme **acetyl-CoA carboxylase** (ACC), the only regulatory enzyme of fatty acid synthesis (see below). Next, both molecules have their CoA portions replaced by a carrier protein known as **ACP** (**acyl-carrier protein**) to form acetyl-ACP and malonyl-ACP. Joining of a fatty acyl-ACP (in this case, acetyl-ACP) with **malonyl-ACP** splits out the carboxyl that was added and creates the intermediate at the upper right in the figure at left.

From this point forward, the chemical reactions resemble those of beta oxidation reversed. First, the ketone is reduced to a hydroxyl using NADPH. In contrast to the hydroxylated intermediate of beta oxidation, the beta intermediate here is in the D-configuration. Next, water is removed from carbons 2 and 3 of the hydroxyl intermediate to produce a *trans* doubled bonded molecule. Last, the double bond is hydrogenated to yield a saturated intermediate. The process cycles with the addition of another malonyl-ACP to the growing chain until ultimately an intermediate with 16 carbons is produced (palmitoyl-CoA). At this point, the cytoplasmic synthesis ceases.

Enzymes of Fatty Acid Synthesis

Acetyl-CoA carboxylase, which catalyzes synthesis of malonyl-CoA, is the only regulated enzyme in fatty acid synthesis. Its regulation involves both allosteric control and covalent modification. The enzyme is known to be phosphorylated by both AMP Kinase and Protein Kinase A. Dephosphorylation is stimulated by **phosphatases** activated by **insulin** binding. Dephosphorylation activates the enzyme and favors its assembly into a long polymer, while phosphorylation reverses the process.

For fatty acid synthesis, I must reverse the path
Of breaking fatty acids, though you'll wonder 'bout the math

Each cycle of addition starts with carbons one two three
Yet products of reactions number carbons evenly

The answer is that CO_2 plays peek-a-boo like games
By linking to an Ac-CoA then popping off again

Reactions are like oxidations 'cept they're backwards here
Reduction, dehydration, then two hydrogens appear

The product of the process is a 16 carbon chain
The bonds are saturated, no double ones remain

For them desaturases toil to put in links of *cis*
In animals to delta nine, but no more go past this

And last there's making longer ones eicosanoidic fun
They're made by elongases in the e. reticulum

When Acids Are Synthesized

To the tune of "*When Johnny Comes Marching Home*"

The 16 carbon fatty acid, palmitate
Gets all the carbons that it needs from acetate
Which citric acid helps release
From mitochondri - matrices
Oh a shuttle's great
When acids are synthesized

Carboxylase takes substrate and it puts within
Dioxy carbon carried on a biotin
CoA's all gain a quick release
Replaced by larger ACPs
And it all begins
When acids are synthesized

A malonate contributes to the growing chain
Two carbons seven times around again, again
For saturated acyl-ates
There's lots of N-A-DPH
That you must obtain
When acids are synthesized

Palmitic acid made this way all gets released
Desaturases act to make omega-threes
The finished products big and small
Form esters with a glycerol
So you get obese
When acids are synthesized

Recorded by Tim Karplus
Lyrics by Kevin Ahern

Citrate acts as an allosteric activator and may also favor polymerization. **Palmitoyl-CoA** allosterically inactivates it.

In animals, six different catalytic activities necessary for the remaining catalytic actions to fully make palmitoyl-CoA are contained in a single complex called **Fatty Acid Synthase**. These include transacylases for swapping CoA with ACP on acetyl-CoA and malonyl-CoA; a synthase to catalyze addition of the two carbon unit from the three carbon malonyl-ACP in the first step of the elongation process; a reductase to reduce the ketone; a dehydrase to catalyze removal of water, and a reductase to reduce the *trans* double bond. In bacteria, these activities are found on separate enzymes and are not part of a complex.

Fatty Acid Elongation

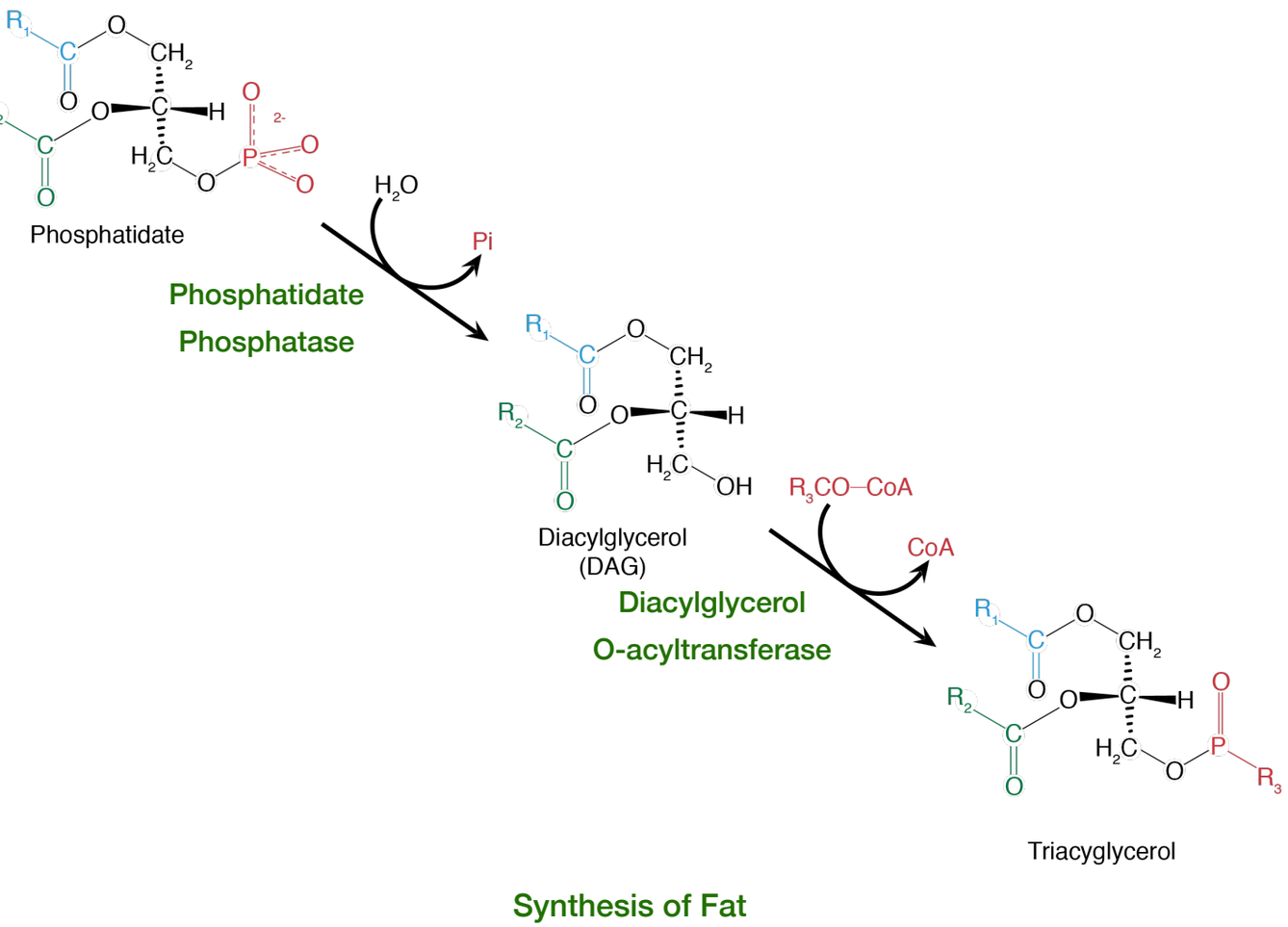
Elongation to make fatty acids longer than 16 carbons occurs in the endoplasmic reticulum and is catalyzed by enzymes described as **elongases**. Mitochondria also can elongate fatty acids, but their starting materials are generally shorter than 16 carbons long. The mechanisms in both environments are similar to those in the cytoplasm (a malonyl group is used to add two carbons, for example), but CoA is attached to the intermediates, not ACP. Further, whereas cytoplasmic synthesis employs an enzyme complex called fatty acid synthase, the enzymes in these organelles are separable and not part of a complex.

Desaturation of Fatty Acids

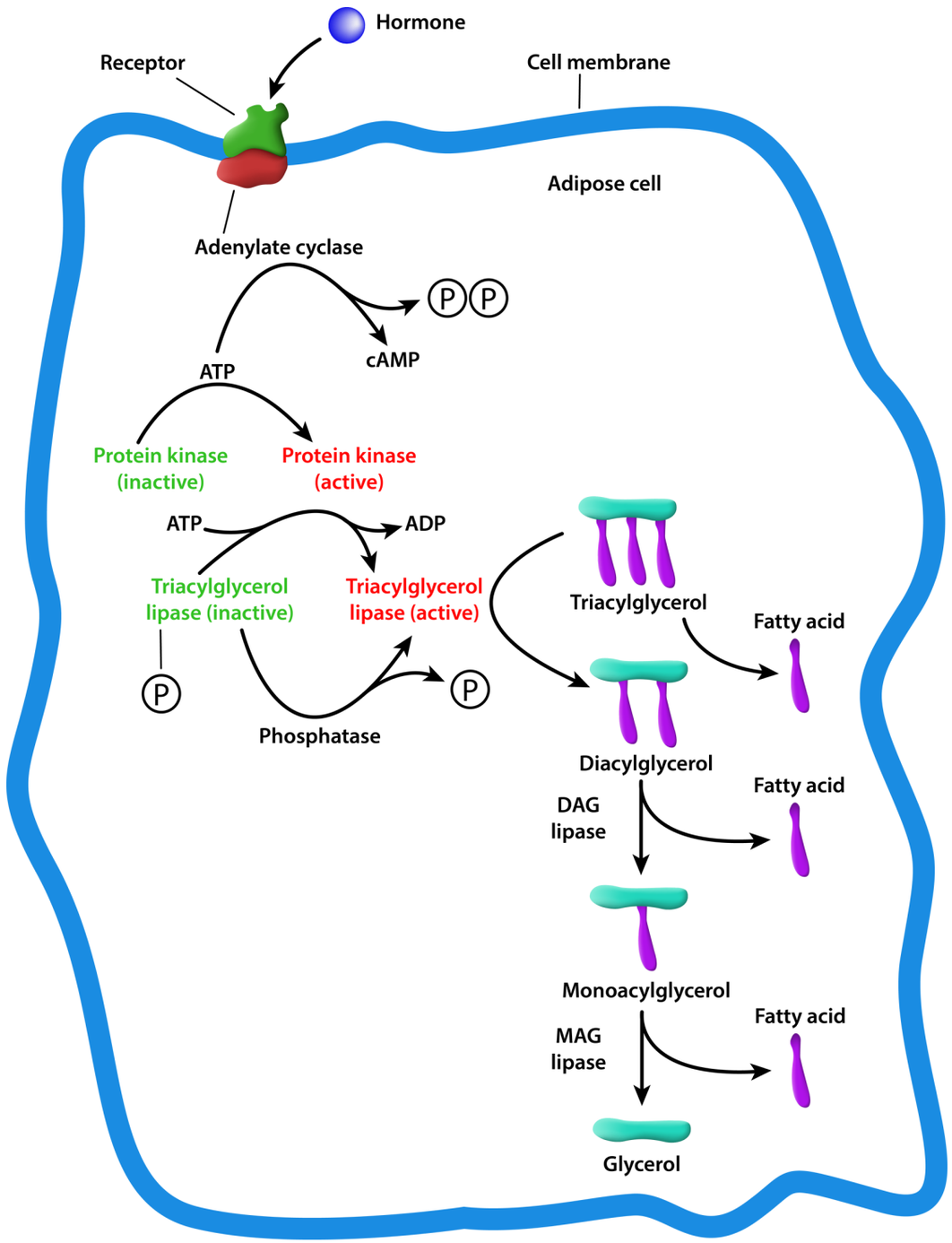
Fatty acids are synthesized in the saturated form and desaturation occurs later. Enzymes called **desaturases** catalyze the formation of *cis* double bonds in mature fatty acids. These enzymes are found in the endoplasmic reticulum. Animals are limited in the desaturated fatty acids they can make, due to an inability to catalyze reactions beyond carbons 9 and 10. Thus, humans can make **oleic acid**, but cannot synthesis **linoleic acid** or **linolenic acid**. Consequently, these two must be provided in the diet and are referred to as **essential fatty acids**.

Metabolism of Fat

Breakdown of fat in adipocytes requires catalytic action of three enzymes, **hormone sensitive triacylglycerol lipase** (called LIPE) to remove the first fatty acid from the fat, diglyceride lipase to remove the second one and monoglyceride lipase to remove the third. Of these, only LIPE is regulated and it appears to be the rate limiting reaction. Synthesis of fat starting with glycerol-3-phosphate requires action of acyl transferase enzymes, such as glycerol-3-phosphate acyl transferase, which catalyze addition of fatty acids to the glycerol backbone.



Interestingly, there appear to be few controls of the metabolism of fatty acids. The primary control of their oxidation is availability. One way to control that is by control of the breakdown of fat. This process, which can be stimulated by the epinephrine kinase cascade, is controlled through LIPE, found in adipocytes (fat-containing cells). Breakdown of fat in apidocytes requires action of three enzymes, each



Activation of Fat Hydrolysis

hydrolyzing one fatty acid from the glycerol backbone. As noted earlier, only HSTL, which catalyzes the first hydrolysis, is regulated.

Synthesis of fat requires **glycerol-3-phosphate** (or DHAP) and three fatty acids. In the first reaction, glycerol-3-phosphate is esterified at position 1 with a fatty acid, followed by a duplicate reaction at position 2 to make **phosphatidic acid**. This molecule, which is an intermediate in the synthesis of both fats and phosphoglycerides, gets dephosphorylated to form **diacylglycerol** before the third esterification to make a fat.

Glycerophospholipid Metabolism

Phosphatidic acid, as noted above, is an important intermediate in the metabolism of glycerophospholipids. These compounds, which are important membrane constituents, can be synthesized in several ways.

Connections to Other Pathways

There are several connections between metabolism of fats and fatty acids to other metabolic pathways. As noted, phosphatidic acid is an intermediate in the synthesis of triacylglycerols, as well as of other lipids, including phosphoglycerides. Diacylglycerol (DAG), which is an intermediate in fat synthesis, also acts as a messenger in some signaling systems. Fatty acids twenty carbons long based on arachidonic acid (also called eicosanoids) are precursors of the classes of molecules known as leukotrienes and prostaglandins. The latter, in turn, are precursors of the class of molecules known as thromboxanes. The ultimate products of beta oxidation are acetyl-CoA molecules and these can be

assembled by the enzyme thiolase to make acetoacetyl-CoA, which is a precursor of both ketone bodies and the isoprenoids, a broad category of compounds that include steroid hormones, cholesterol, bile acids, and the fat soluble vitamins, among others.

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