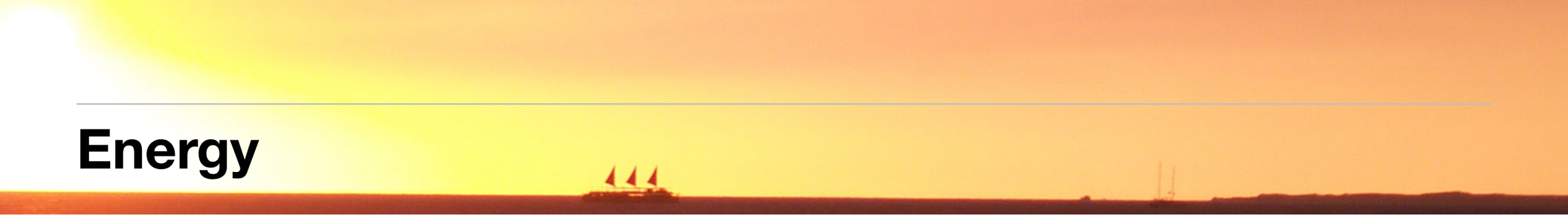
Chapter 2

Energy

In this chapter we discuss the most important factor necessary for life - energy.



Energy

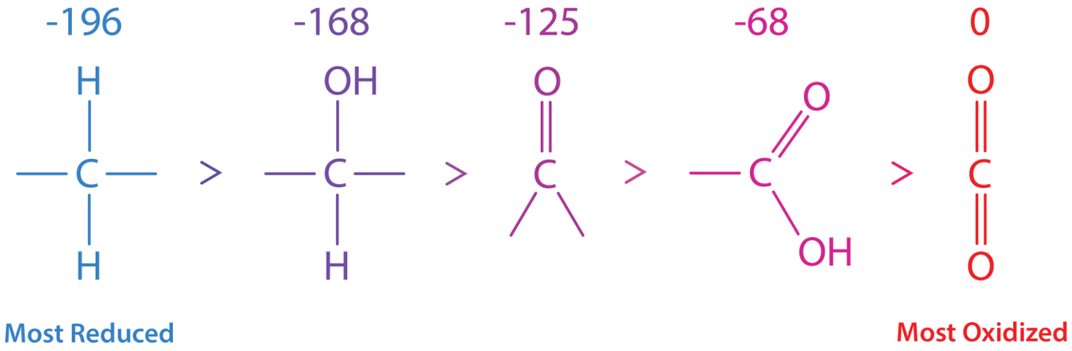
Introduction

Living organisms are made up of cells, and cells contain many biochemical components such as proteins, lipids, and carbohydrates. But, living cells are not random collections of these molecules. They are extraordinarily organized or "ordered". By contrast, in the nonliving world, there is a universal tendency to increasing disorder. Maintaining and creating order in cells takes the input of energy.

Without energy, life is not possible. It is therefore important that we consider energy first in our attempt to understand biochemistry. Where does energy come from? Photosynthetic organisms can capture energy from the sun, converting it to chemical forms usable by cells. Heterotrophic organisms like ourselves get our energy from the food we eat. How do we extract the energy from the food we eat?

Oxidative Energy

The primary mechanism used by non-photosynthetic organisms to obtain energy is oxidation and carbon is the most commonly oxidized energy source. The energy released during the oxidative steps is “captured” in ATP and can be used later for energy coupling (see below). The more reduced a carbon atom is, the more energy can be realized from its oxidation. Conversely, the more oxidized a carbon atom is, the more energy it takes to reduce it.



In this series, the most reduced form of carbon is on the left. The energy of oxidation of each form is shown above it. Fatty acids are more reduced overall than sugars. This can also be seen by their formulas.

$$\text{Palmitic acid} = C_{16}H_{34}O_2$$

$$\text{Glucose} = C_6H_{12}O_6$$

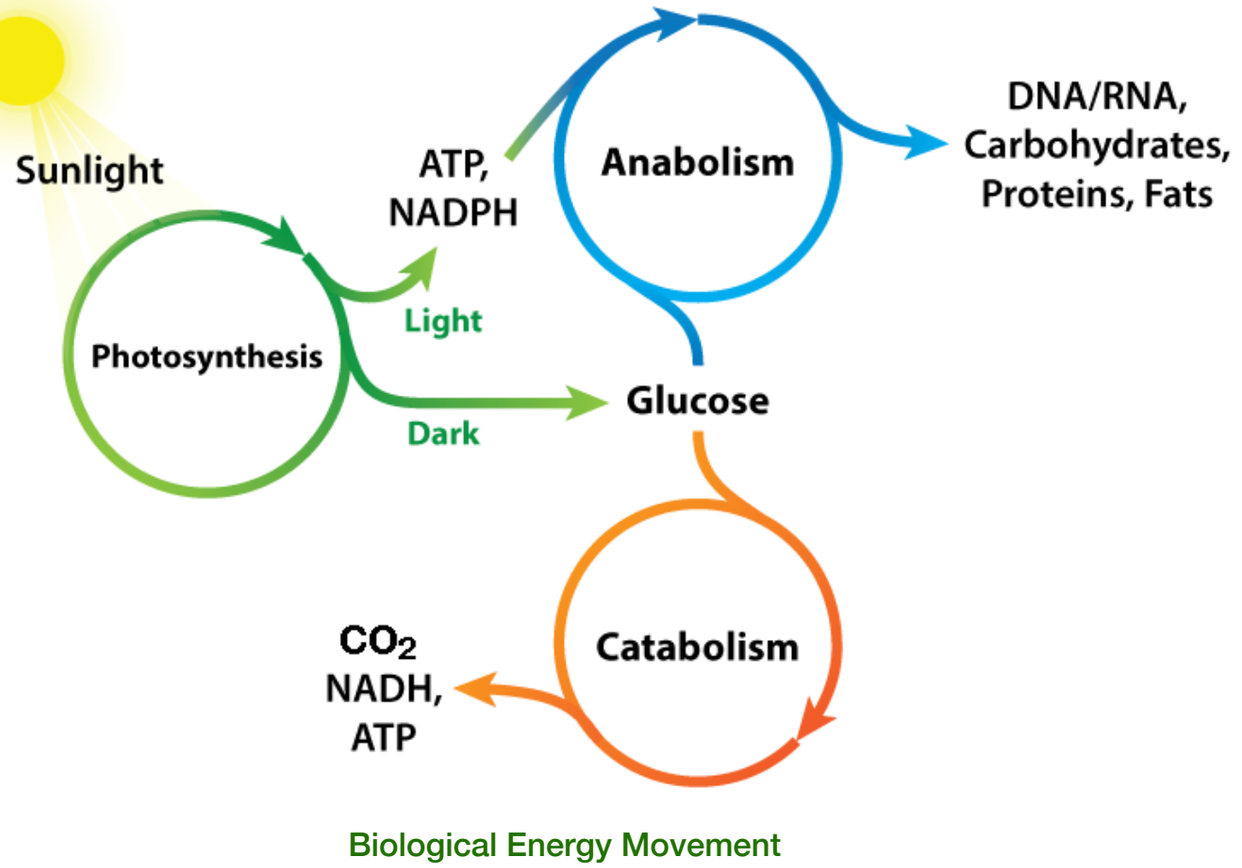
Palmitic acid only contains two oxygens per sixteen carbons, whereas glucose has six oxygen atoms per six carbons.

Consequently, when palmitic acid is fully oxidized, it generates more ATP per carbon (128/16) than glucose (38/6). It is because of this that we use fat as our primary energy storage material.

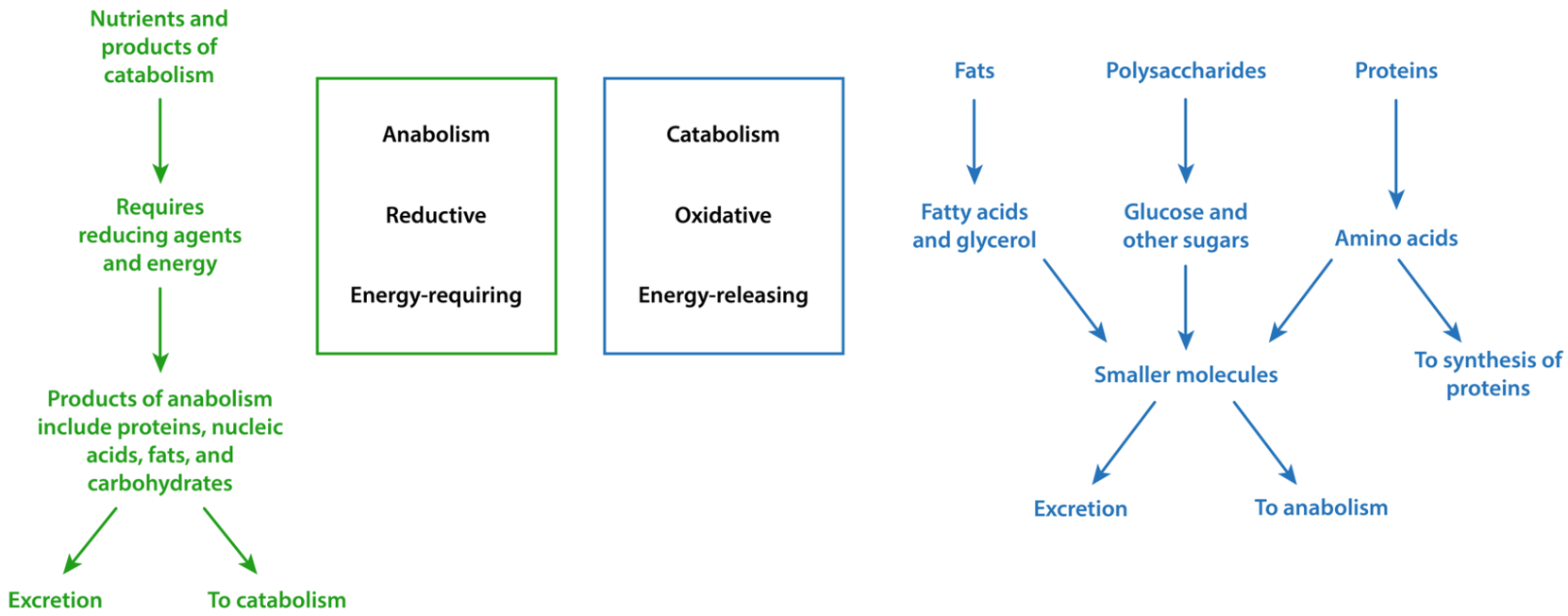
Oxidation vs. Reduction in Metabolism

Click HERE and HERE for Kevin's Metabolic Strategies Lectures on YouTube

Biochemical processes that break things down from larger to smaller are called catabolic processes. Catabolic processes are often oxidative in nature and energy releasing. Some, but not all of that energy is captured as ATP. If not all of the energy is



Biological Energy Movement



captured as ATP, what happens to the rest of it? The answer is simple. It is released as heat and it is for this reason that we get hot when we exercise. By contrast, synthesizing large molecules from smaller ones (for example, making proteins from amino acids) is referred to as anabolism. Anabolic processes are often reductive in nature and require energy input. By themselves, they would not occur, as they are reversing oxidation and decreasing entropy (making many small things into a larger one). To overcome this energy 'barrier', cells must expend energy. For example, if one wishes to reduce CO_2 to carbohydrate, energy must be used to do so. Plants do this during the dark reactions of photosynthesis. The energy source for the reduction is ultimately the sun. The electrons for the reduction ultimately come from water, and the CO_2 comes from the atmosphere and gets incorporated into a sugar.

Energy Coupling

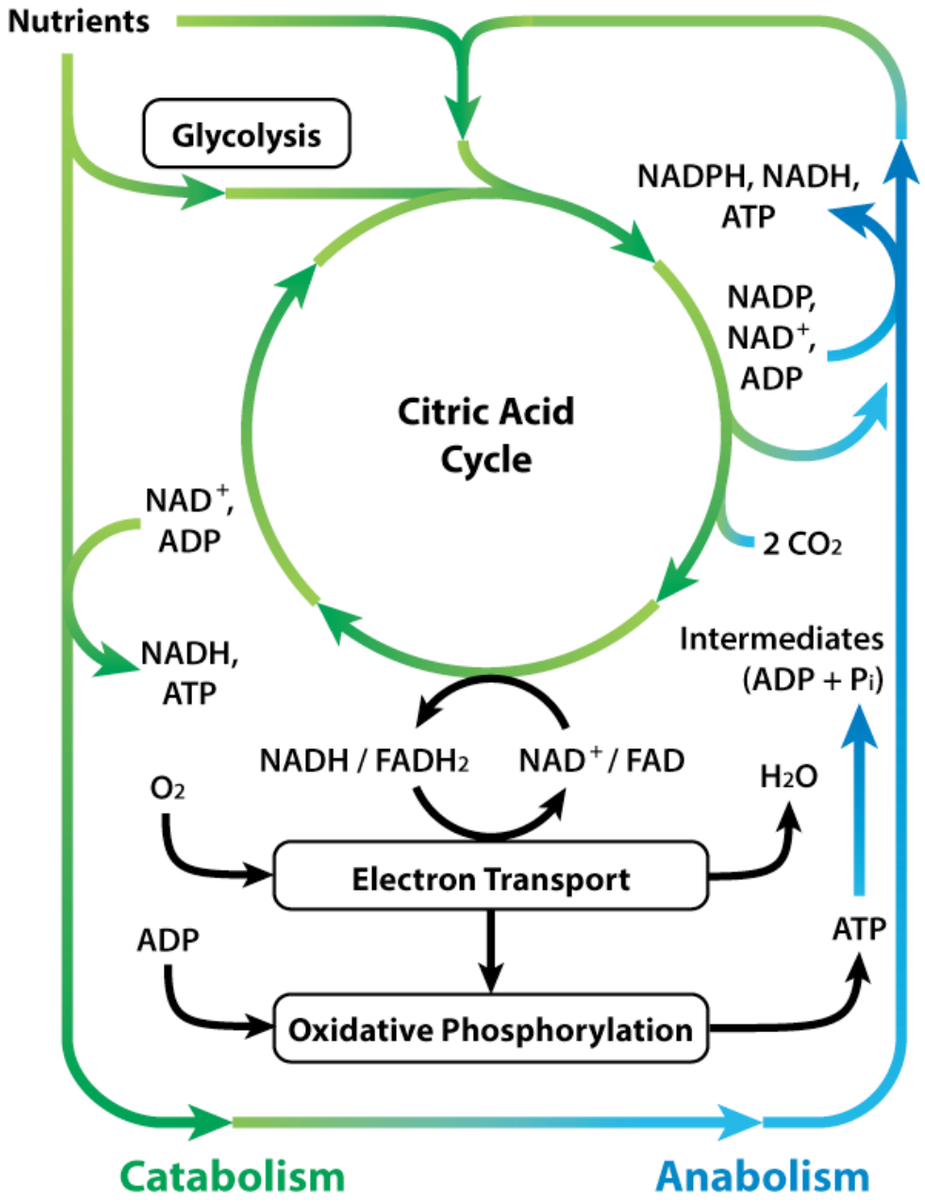
The addition of phosphate to a sugar is a common reaction that occurs in a cell. By itself, this process is not very energetically favorable (that is, it needs an input of energy to occur). Cells overcome this energy obstacle by using ATP to "drive" the reaction. The energy needed to drive reactions is harvested in very controlled conditions in the confines of an enzyme. This involves a process called 'coupling'. In coupled reactions, an enzyme binds both a high energy molecule (usually ATP) and the other molecule(s) involved in the reaction. Hydrolysis of ATP provides energy for the enzyme to stimulate the reaction on the other substance(s). Hexokinase, for example, catalyzes the phosphorylation of glucose to form

One reason we need ATP
Is the high cost of living, you see
'Cause the chaotic entity
Known as the entropy
Requires cells to burn energy

glucose-6-phosphate. In the absence of ATP, the reaction has a fairly positive ΔG°' (described later), but hydrolysis of ATP provides excess energy, giving the coupled reaction a fairly negative ΔG°' value.

Entropy and Energy

Most students who have had some chemistry know about the principle of the Second Law of Thermodynamics with respect to increasing disorder of a system. Cells are very organized or ordered structures, leading some to mistakenly conclude that life somehow violates the second law. In fact, that notion is incorrect. The second law doesn't say that entropy always increases, just that, left alone, it tends to do so, in an isolated system. Cells are not isolated systems, in that they obtain energy, either from the sun, if they are autotrophic, or food, if they are heterotrophic.



To counter the universal tendency towards disorder on a local scale requires energy. As an example, take a fresh deck of cards which is neatly aligned with Ace-King-Queen . . . . 4,3,2 for each suit. Throw the deck into the air, letting the cards scatter. When you pick them up, they will be more disordered than when they started. However, if you spend a few minutes (and expend a bit of energy), you can reorganize the same deck back to its previous, organized state. If entropy always increased everywhere, you could not do this. However, with the input of energy, you overcame the disorder. The cost of fighting disorder is energy.

There are, of course, other reasons that organisms need energy. Muscular contraction, synthesis of molecules, neurotransmission, signaling, thermoregulation, and subcellular movements are examples. Where does this energy come from? The currencies of energy are generally high-energy phosphate-containing molecules. ATP is the best known and most abundant, but GTP is also an important energy source (required for protein synthesis). CTP is involved in synthesis of glycerophospholipids and UTP is used for synthesis of glycogen. In each of these cases, the

energy is in the form of potential chemical energy stored in the multi-phosphate bonds. Hydrolyzing those bonds releases the energy in them.

Of the triphosphates, ATP is the primary energy source, acting to facilitate the synthesis of the others by action of the enzyme NDPK. ATP is made by three distinct types of phosphorylation – oxidative phosphorylation (in mitochondria), photophosphorylation (in chloroplasts of plants), and substrate level phosphorylation (in enzymatically catalyzed reactions).

Gibbs Free Energy

Most of the time, ATP is the “storage battery” of cells (See also ‘Molecular Battery Backups for Muscles below). In order to understand how energy is captured, we must first understand Gibbs free energy and in doing so, we begin to see the role of energy in determining the directions chemical reactions take.

Oh Delta G

To the tune of “*Danny Boy*”

Oh Delta G - the change in Gibbs free energy
Can tell us if a process will advance
'Cause if the value's less than naught it translates that
Reverse reactions haven't got a chance

But when the sign is plus it is the opposite
And then the backwards happens all the time
A factor is the standard Gibbs free energy
So don't forget about the Delta G naught prime

Recording by Tim Karplus
Lyrics by Kevin Ahern

Wikipedia defines Gibbs free energy as “a thermodynamic potential that measures the "useful" or process-initiating work obtainable from an isothermal, isobaric thermodynamic system,” and further points out that it is “the maximum amount of non-expansion work that can be extracted from a closed system; this maximum can be attained only in a completely reversible process.”

Mathematically, the Gibbs free energy is given as

$$G = H - TS$$

where H is the enthalpy, T is the temperature in Kelvin, and S is the entropy.

At standard temperature and pressure, every system seeks to achieve a minimum of free energy. Thus, increasing entropy will reduce Gibbs free energy. Similarly, if excess heat is available (reducing the enthalpy), the free energy can also be reduced. Cells must work within the laws of thermodynamics, as noted, so all of their

biochemical reactions, too, have limitations. Now we shall consider energy in the cell. The change in Gibbs free energy (ΔG) for a reaction is crucial, for it, and it alone, determines whether or not a reaction goes forward.

$$\Delta G = \Delta H - T\Delta S,$$

There are three cases

ΔG < 0 - the reaction proceeds as written

ΔG = 0 - the reaction is at equilibrium

ΔG > 0 - the reaction runs in reverse

For a reaction aA <=> bB (where 'a' and 'b' are integers and A and B are molecules) at pH 7, ΔG can be determined by the following equation,

$$\Delta G = \Delta G^{\circ\prime} + RT\ln([B]^b/[A]^a)$$

For multiple substrate reactions, such as aA + cC <=> bB + dD

$$\Delta G = \Delta G^{\circ\prime} + RT\ln\{([B]^b[D]^d)/([A]^a[C]^c)\}$$

The ΔG°' term is called the change in Standard Gibbs Free energy, which is the change in energy that occurs when all of the products and reactants are at standard conditions and the pH is 7.0. It is a constant for a given reaction.

In simple terms, if we collect all of the terms of the numerator together and call them {Products} and all of the terms of the denominator together and call them {Reactants},

$$\Delta G = \Delta G^{\circ\prime} + RT\ln(\{Products\}/\{Reactants\})$$

For most biological systems, the temperature, T, is a constant for a given reaction. Since ΔG°' is also a constant for a given reaction, the ΔG is changed almost exclusively as the ratio of {Products}/{Reactants} changes. If one starts out at standard conditions, where everything except protons is at 1M, the RTln({Products}/{Reactants}) term is zero, so the ΔG°' term determines the direction the reaction will take. This is why people say that a negative ΔG°' indicates an energetically favorable reaction, whereas a positive ΔG°' corresponds to an unfavorable one.

When reactions have product largesse
They will act to address the excess
Henry Le Chatelier
Showed conversion's the way
To suppress the excess by redress

Increasing the ratio of {Products}/{Reactants} causes the value of the natural log (ln) term to become more positive (less negative), thus making the value of ΔG more positive. Conversely, as the ratio of {Products}/{Reactants} decreases, the value of the natural log term becomes less positive (more negative), thus making the value of ΔG more negative.

Intuitively, this makes sense and is consistent with Le Chatelier's Principle – a system responds to stress by acting to alleviate the

stress. If we examine the ΔG for a reaction in a closed system, we see that it will always move to a value of zero (equilibrium), no matter whether it starts with a positive or negative value.

Another type of free energy available to cells is that generated by electrical potential. For example, mitochondria and chloroplasts partly use Coulombic energy (based on charge) from a proton gradient across their membranes to provide the necessary energy for the synthesis of ATP. Similar energies drive the transmission of nerve signals (differential distribution of sodium and potassium) and the movement of some molecules in secondary active transport processes across membranes (e.g., H^+ differential driving the movement of lactose). From the Gibbs free energy change equation,

$$\Delta G = \Delta H - T\Delta S$$

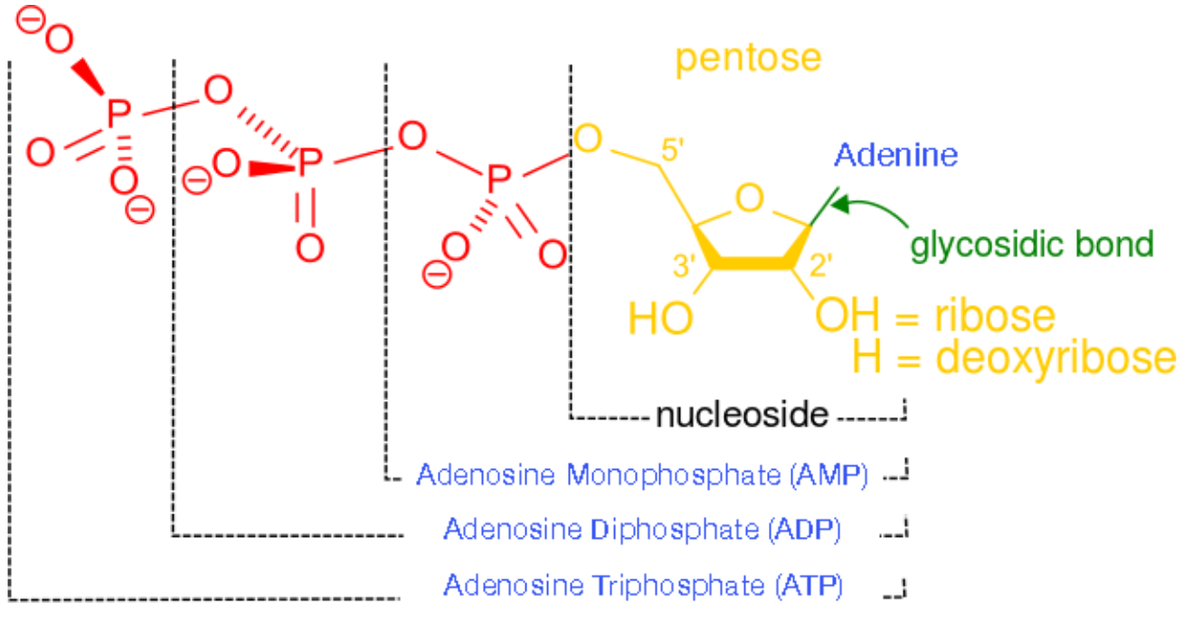
it should be noted that an increase in entropy will help contribute to a decrease in ΔG. This happens, for example when a large molecule is being broken into smaller pieces or when the rearrangement of a molecule increases the disorder of molecules around it. The latter situation arises in the hydrophobic effect, which helps drive the folding of proteins.

Cellular Phosphorylations

Formation of triphosphates is essential to meet the cell's immediate energy needs for synthesis, motion, and signaling. In a given day, an average human being uses more than their body weight in triphosphates. Since triphosphates are the "currency" that meet immediate needs of the cell, it is important to understand how triphosphates are made. There are three phosphorylation mechanisms – 1) substrate level; 2) oxidative; and 3) photophosphorylation. We consider them here individually.

Substrate Level Phosphorylation

The easiest type of phosphorylation to understand is that which occurs at the substrate level. This type of phosphorylation involves the direct synthesis of ATP from ADP and a reactive intermediate, typically a high energy phosphate-containing molecule. Substrate level phosphorylation is a relatively minor contributor to the total synthesis of triphosphates by cells. An example substrate phosphorylation comes from glycolysis.



Adenine Nucleotides

Phosphoenolpyruvate (PEP) + ADP <=> Pyuvate + ATP

This reaction has a very negative ΔG°' (-31.4 kJ/mol), indicating that the PEP contains more energy than ATP, thus energetically favoring ATP's synthesis. Other triphosphates can be made by substrate level phosphorylation, as well. For example, GTP can be synthesized by the following citric acid cycle reaction

Succinyl-CoA + GDP + Pi <=> Succinate + GTP + CoA-SH

Triphosphates can be interchanged readily in substrate level phosphorylations catalyzed by the enzyme Nucleoside Diphosphate Kinase (NDPK). A generalized form of the reactions catalyzed by this enzyme is as follows:

XTP + YDP <=> XDP + YTP

Where X = adenosine, cytidine, uridine, thymidine, or guanosine and Y can be any of these as well. Last, an unusual way of synthesizing ATP by substrate level phosphorylation is that catalyzed by adenylate kinase

2 ADP <=> ATP + AMP

This reaction is an important means of generating ATP when the cell doesn't have other sources of energy. Accumulation of AMP resulting from this reaction activates enzymes, such as phosphofructokinase, of glycolysis, that will catalyze reactions to give the cell additional, needed energy.

Electron Transport / Oxidative Phosphorylation

Mitocohondria are called the power plants of the cell because most of a cell's ATP is produced there, in a process referred to as oxidative phosphorylation. The mechanism by which ATP is made in oxidative phosphorylation is one of the most interesting processes in all of biology. It has three primary considerations. The first is electrical – electrons from

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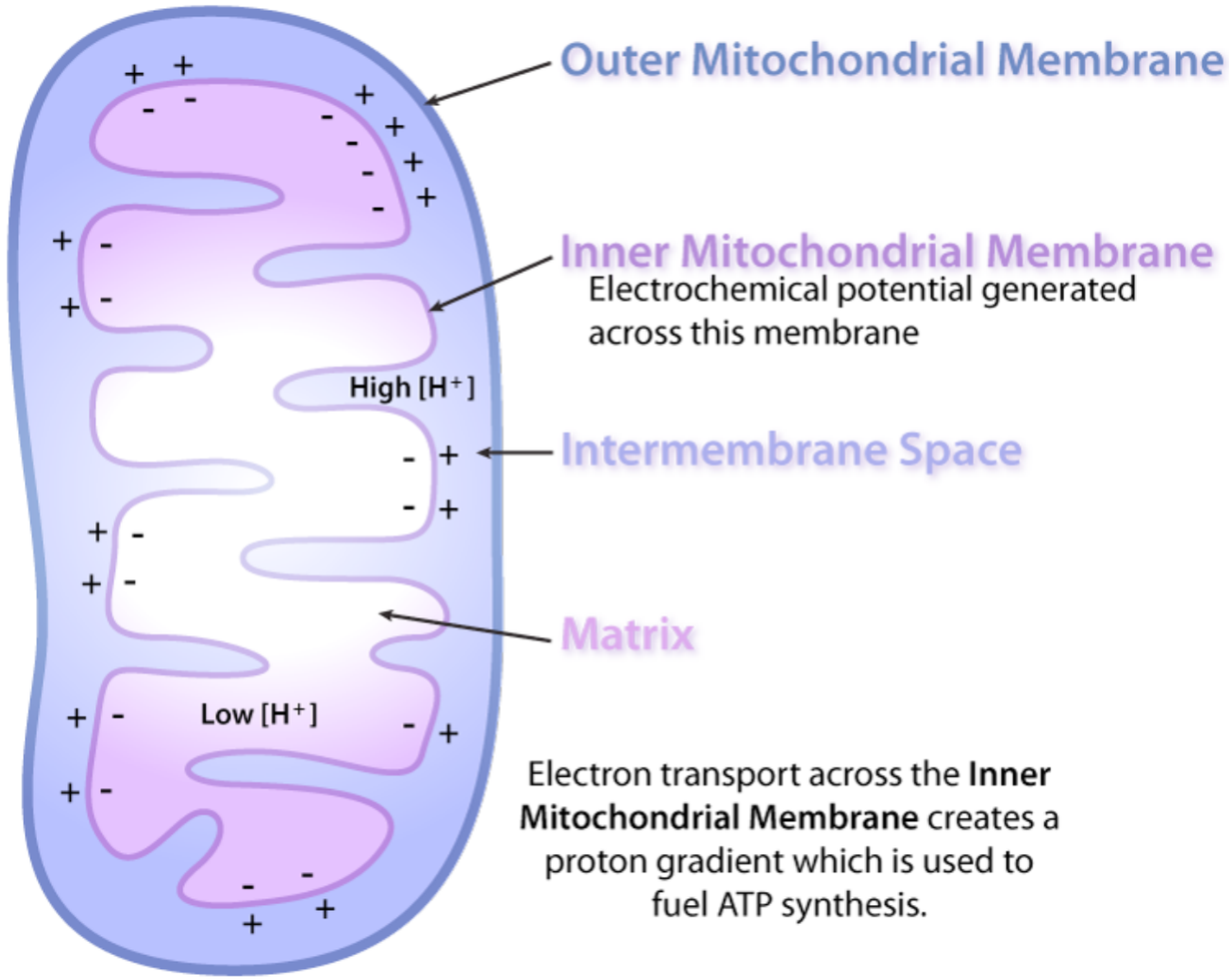


I’m a Little Mitochondrion

To the tune of “*I’m a Lumberjack*”

I'm a little mitochondrion
Who gives you energy
I use my proton gradient
To make the ATPs

He's a little mitochondrion
Who gives us energy
He uses proton gradients
To make some ATPs

Electrons flow through Complex II
To traffic cop Co-Q
Whenever they arrive there in
An FADH-two

Electrons flow through Complex II
To traffic cop Co-Q
Whenever they arrive there in
An FADH-two

Tightly coupled is my state
Unless I get a hole
Created in my membrane by
Some di-ni-tro-phe-nol

Yes tightly coupled is his state
Unless he gets a hole
Created in his membrane by
Some di-ni-tro-phenol

Both rotenone and cyanide
Stop my electron flow
And halt the calculation of
My "P" to "O" ratio

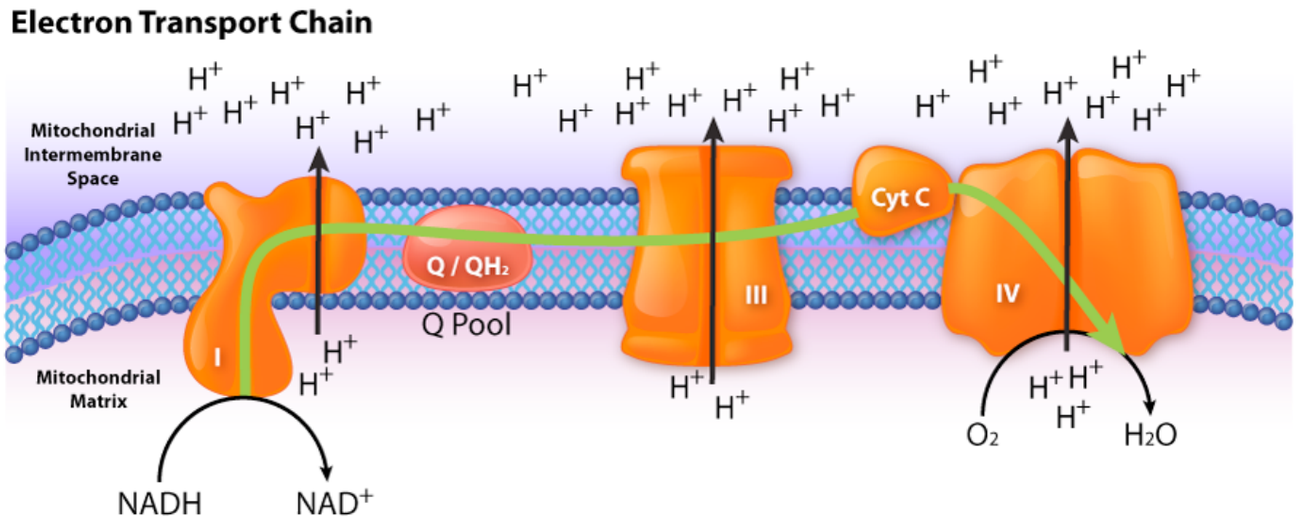
Both rotenone and cyanide
Stop his electron flow
And halt the calculation of
His "P" to "O" ratio

Recording by Tim Karplus
Lyrics by Kevin Ahern

reduced energy carriers, such as NADH and $FADH_2$, enter an electron transport system *via* protein complexes containing iron. As seen in the figure on the following page, electrons move from one complex to the next, not unlike the way they might move through an electrical circuit.

The next consideration arises as a secondary phenomenon. When electrons pass through complexes I, III, and IV, protons are moved from the mitochondrial matrix (inside of mitochondrion) and deposited in the intermembrane space (between the inner and outer membranes of the mitochondrion). The effect of this redistribution is to increase the electrical and chemical potential across the membrane. Students may think of the process as “charging the battery.”

Just like a charged battery, the potential arising from the proton differential across the membrane can be used to do things. This is the third consideration. In the mitochondrion, the “thing” that the proton gradient does is create ATP from ADP and Pi (inorganic phosphate). This process requires energy and is accomplished by movement of protons through a protein complex in the inner mitochondrial membrane. The protein complex is an enzyme that has several names, including Complex V, PTAS (Proton Translocating ATP Synthase), and ATP Synthase. Central to its function is the movement of protons through it (from outside back into the matrix). Protons will *only* move through ATP Synthase if their concentration is greater outside the inner membrane than in the matrix.



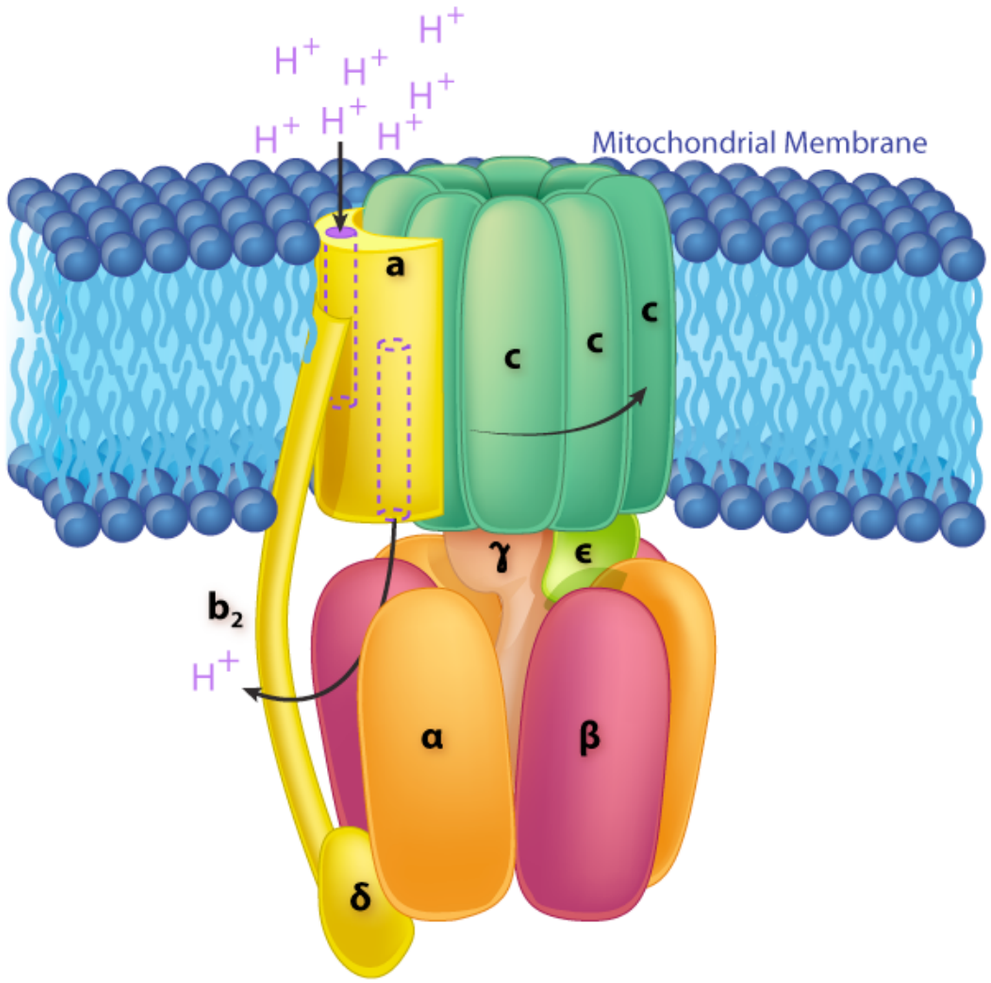
Electron Transport Starting With Complex I

In summary, the electron transport system charges the battery for oxidative phosphorylation by pumping protons out of the mitochondrion. The intact inner membrane of the mitochondrion keeps the protons out, except for those that re-enter through ATP Synthase. The ATP Synthase allows protons to re-enter the mitochondrial matrix and harvests their energy to make ATP.

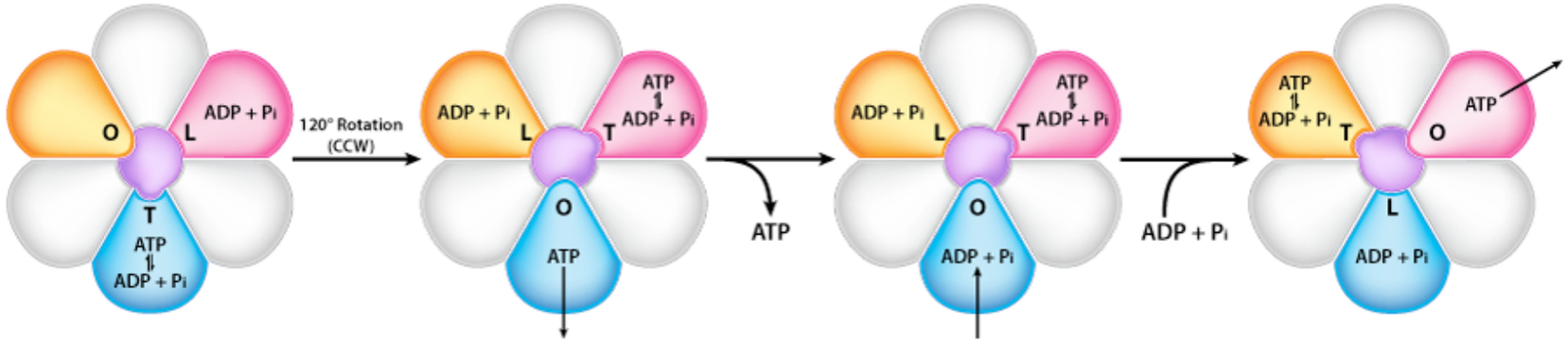
ATP Synthase

The ATP Synthase itself is an amazing nanomachine that makes ATP using a gradient of protons flowing through it from the intermembrane space back into the matrix. It is not easy to depict in a single image what the synthase does. The figure at the right illustrates the multi-subunit nature of this membrane protein, which acts like a turbine at a hydroelectric dam. The movement of protons through the ATP Synthase causes it to spin like a turbine, and the spinning is necessary for making ATP.

In ATP Synthase, the spinning component is the membrane portion (c ring) of the F_0 stalk. The c ring proteins are linked to the gamma-epsilon stalk, which projects into the F_1 head of the mushroom structure. The F_1 head contains the catalytic ability to make ATP.



ATP Synthase (Complex V)



Three States of ATP Synthase

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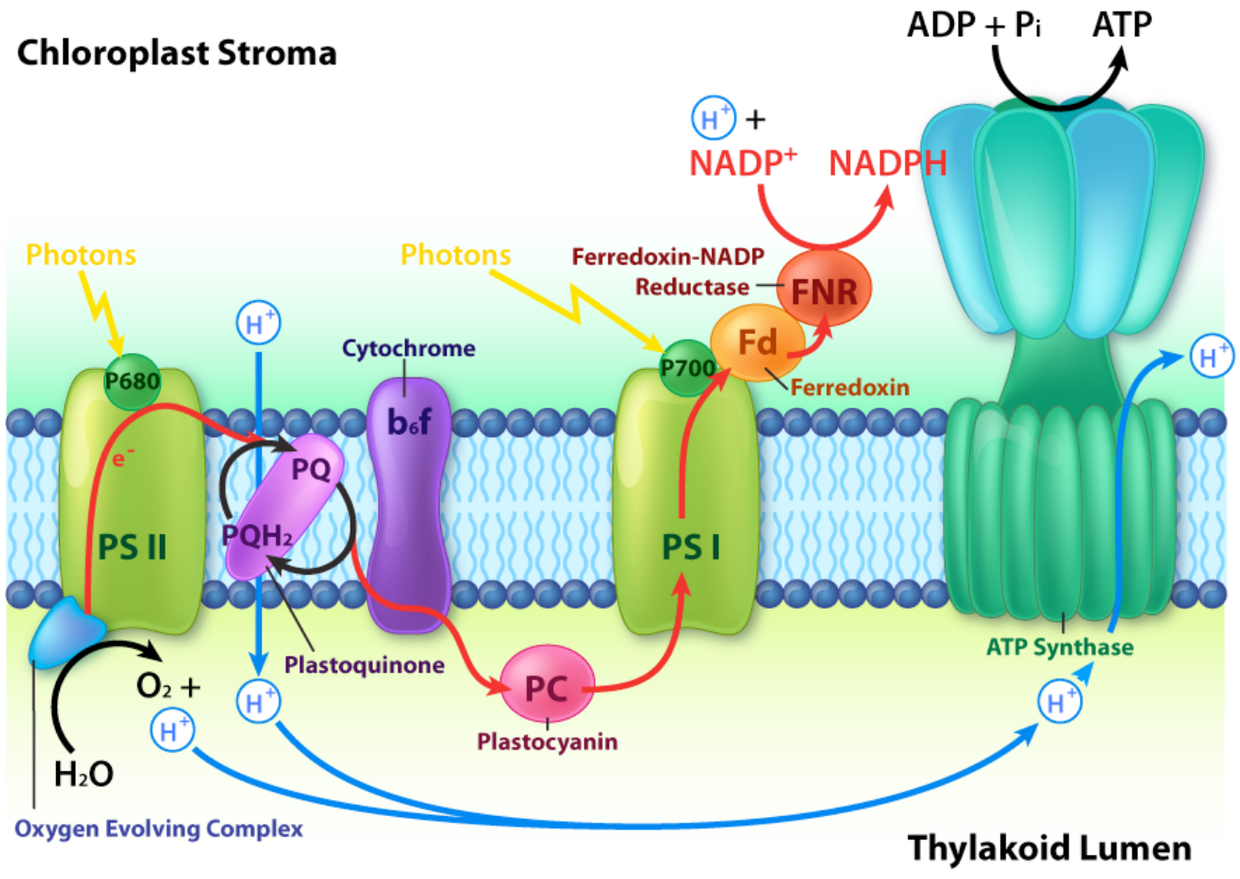
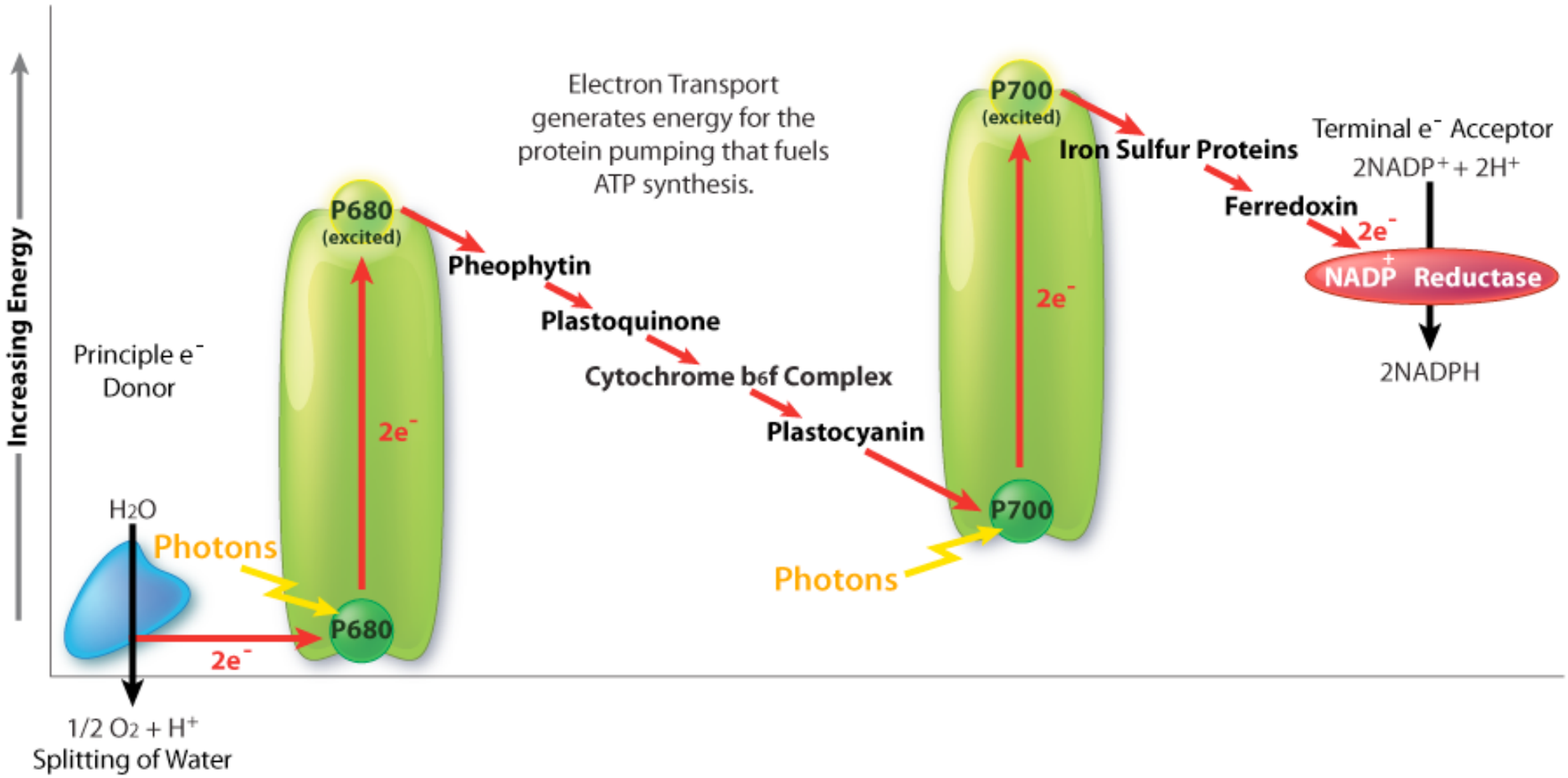
The F_1 head is hexameric in structure with paired alpha and beta proteins arranged in a trimer of dimers. Movement of the gamma protein inside the alpha-beta trimer causes each set of beta proteins to change structure slightly into three different forms called Loose, Tight, and Open (L,T,O). Each of these forms has a function. The Loose form binds ADP + P_i. The tight form "squeezes" them together to form the ATP. The open form releases the ATP into the mitochondrial matrix. Thus, as a result of the proton excess in the intermembrane space, ATP is made.



Photophosphorylation

Photophosphorylation

The third type of phosphorylation to make ATP is found only in cells that carry out photosynthesis. This process is similar to oxidative phosphorylation in several ways. A primary difference is the ultimate source of the energy for ATP synthesis. In oxidative phosphorylation, the energy comes from electrons produced by oxidation of biological molecules. In the case of photosynthesis, the energy comes from the light of the sun.



Electron Movement in Photosynthesis

Photons from the sun interact with chlorophyll molecules in reaction centers in the chloroplasts of plants or membranes of photosynthetic bacteria. A schematic of the process is shown above.

The similarities of photophosphorylation to oxidative phosphorylation include:

- an electron transport chain
- creation of a proton gradient
- harvesting energy of the proton gradient by making ATP with the help of an ATP synthase.

Some of the differences include :

- the source of the electrons – H_2O for photosynthesis versus NADH/ $FADH_2$ for oxidative phosphorylation
- direction of proton pumping – into the thylakoid space of the chloroplasts versus outside the matrix of the mitochondrion
- movement of protons during ATP synthesis – out of the thylakoid space in photosynthesis versus into the mitochondrial matrix
- nature of the terminal electron acceptor – $NADP^+$ in photosynthesis versus O_2 in oxidative phosphorylation.

Electron Transport in Chloroplasts versus Mitochondria

In some ways, the movement of electrons in chloroplasts during photosynthesis is opposite that of electron transport in mitochondria. In photosynthesis, water is the source of electrons and their final destination is NADPH. In mitochondria, NADH/$FADH_2$ are electron sources and H_2O is their final destination. How do biological systems get electrons to go both ways? It would seem to be the equivalent of going to and from a particular place while always going downhill, since electrons will move according to potential. The answer is the captured energy of the photons, which elevates electrons in photosynthesis to an energy where they move "downhill" to their NADPH destination in a Z-shaped scheme (previous page). The movement of electrons through this scheme in plants requires energy from photons in two places to "lift" the energy of the electrons sufficiently.

N-A-D

To the tune of "*Penny Lane*"

In the catabolic pathways that our cells employ
Oxidations help create the ATP
While they lower Gibbs free energy
Thanks to enthalpy

If a substrate is converted from an alcohol
To an aldehyde or ketone it is clear
Those electrons do not disappear
They just rearrange – very strange

N-A-D is in my ears and in my eyes
Help-ing mol-e-cules get oxidized
Making N-A-D-H then

And the latter is a problem anaerobically
'Cuz accumulations of it muscles hate
They respond by using pyruvate
To produce lactate

Catalyzing is essential for the cells to live
So the enzymes grab their substrates eagerly
If they bind with high affinity
Low K_m you see, just as me

N-A-D is in my ears and in my eyes
Help-ing mol-e-cules get oxidized
Making N-A-D-H then

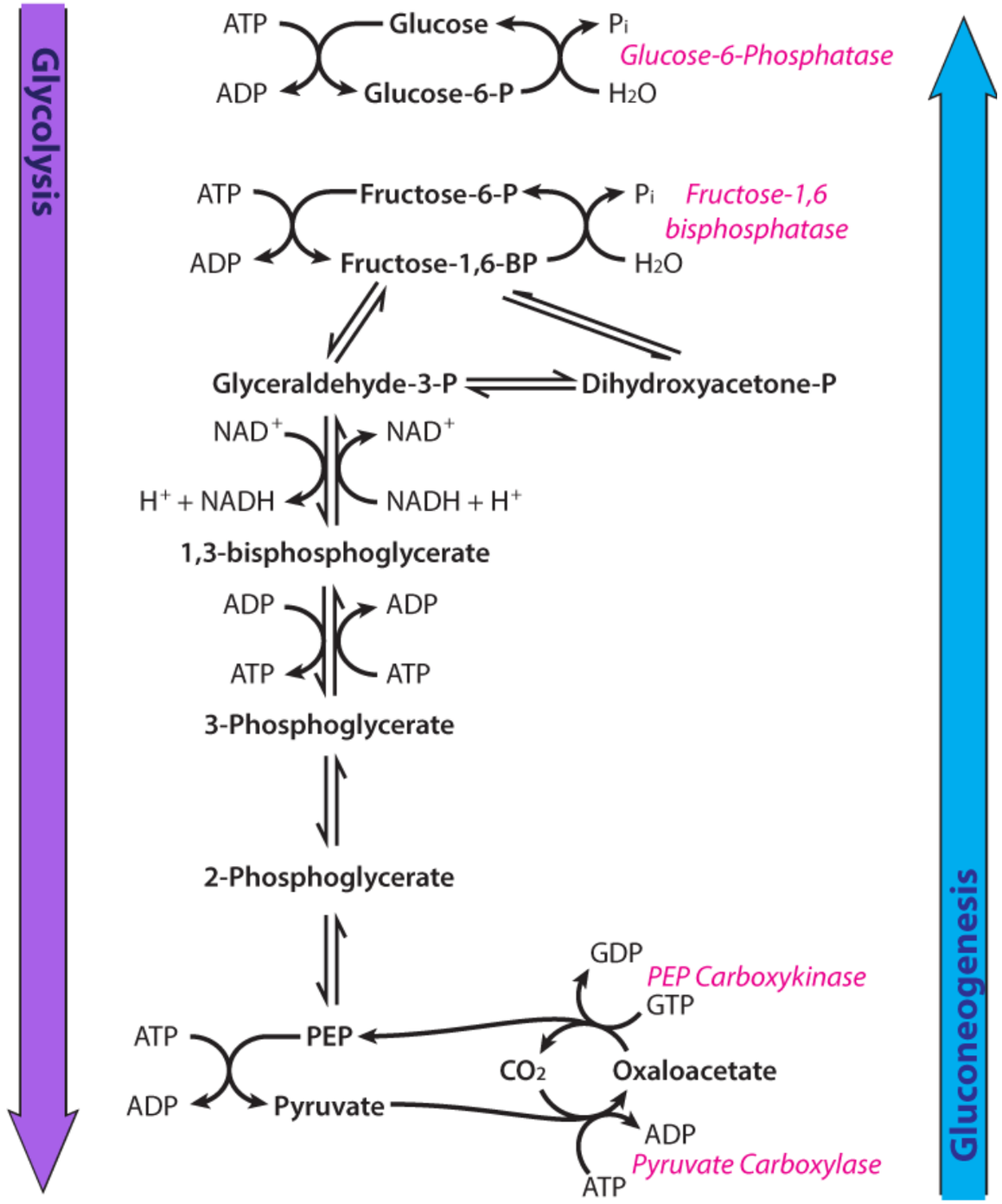
Recorded by Tim Karplus
Lyrics by Kevin Ahern

Photosynthesis left me aghast
As electrons all went whizzing past
Leaving water at start
They trace the Z chart
In the membranes of each chloroplast

Last, it should be noted that photosynthesis actually has two phases, referred to as the light cycle (described above) and the dark cycle, which is a set of chemical reactions that captures CO_2 from the atmosphere and "fixes" it, ultimately into glucose. The dark cycle is also referred to as the Calvin Cycle and is discussed HERE.

Energy Efficiency

Cells are not 100% efficient in energy use. Nothing that we know of is. Consequently, cells do not get as much energy out of catabolic processes as they put into anabolic processes. A good example is the synthesis and breakdown of glucose, something liver cells are frequently doing. The complete conversion of glucose to pyruvate in glycolysis (catabolism) yields two pyruvates plus 2 NADH plus 2 ATPs. Conversely, the complete conversion of two pyruvates into glucose by gluconeogenesis (anabolism) requires 4 ATPs, 2 NADH, and 2 GTPs. Since the energy of GTP is essentially equal to that of ATP, gluconeogenesis requires a net of 4 ATPs more than glycolysis yields. This difference must be made up in order for the organism to balance everything. It is for this reason that we eat. In addition, the inefficiency of our capture of energy in reactions results in the production of heat and helps to keep us warm. You can read more about glycolysis and gluconeogenesis HERE.



Glycolysis and Gluconeogenesis

Metabolic Controls of Energy

It is also noteworthy that cells do not usually have both catabolic and anabolic processes for the same molecules (for example, breakdown of glucose and synthesis of glucose, shown on the previous page) occurring simultaneously inside of them because the cell would see no net production of anything but heat and a loss of ATPs with each turn of the cycle. Such cycles are called futile cycles and cells have controls in place to limit the extent to which they occur. Since futile cycles can, in fact, yield heat, they are sources of heat in some types of tissue. See also HERE for more on futile cycles.

Molecular Backups for Muscles

For plants, the needs for energy are different than for animals. Plants do not need to access energy sources as rapidly as animals do, nor do they have to maintain a constant internal temperature. Plants can neither flee predators, nor chase prey. These needs of animals are much more immediate and require that energy stores be accessible on demand. Muscles, of course, enable the motion of animals and the energy required for muscle contraction is ATP. To have stores of energy readily available, muscles have, in addition

The Muscle Energy Song

To the tune of "*I Will*"

For running and for jumping
You need some energy
Chemically the body stores it
In the form of ATP

If backup should be needed
Reserves are there in wait
Muscles brimming with supplies of
Tiny creatine phosphate

Ready whenever you are ever
Wanting to exercise
Steady as ever when whatever
Energy needs arise

The action is exacting
For leaping in the air
Myofibrils all contracted
Using energy extracted

From reactions that react in me
Using A-T-P
You see

Recorded by David Simmons
Lyrics by Kevin Ahern

to ATP, creatine phosphate and glycogen for quick release of glucose from glycogen. The synthesis of creatine phosphate is a prime example of the effects of concentration on the synthesis of high energy molecules. For example, creatine phosphate has an energy of hydrolysis of -43.1 kJ/mol whereas ATP has an energy of hydrolysis of -30.5 kJ/mol Creatine phosphate, however, is made from creatine and ATP in the reaction shown below. How is this possible?

Creatine + ATP <=> Creatine phosphate + ADP

The ΔG°' of this reaction is +12.6 kJ/mol, reflecting the energies noted above. In a resting muscle cell, ATP is abundant and ADP is low, driving the reaction to the right, creating creatine phosphate. When muscular contraction commences, ATP levels fall and ADP levels climb. The above reaction then reverses and proceeds to synthesize ATP immediately. Thus creatine phosphate acts like a battery, storing energy when ATP levels are high and releasing it almost instantaneously to create ATP when its levels fall.

Summary

In summary, energy is needed for cells to perform the functions that they must carry out in order to stay alive. At its most basic level, this means fighting a continual battle with entropy, but it is not the only need for energy that cells have.

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