Mitochondria: An Overview

It's all about energy: having enough energy to power the thousands of things that a living cell needs to do just to make it through the day. This energy must be available to the cell on a moment's notice, must be present in constant and reliable quantities in each of the trillions of cells that make up the human body. Figuring out how this is accomplished has taken researchers over a century. We now have a clear understanding of the extremely complex processes that keep the power flowing in the cells--as well as what goes wrong when it doesn't.

ATP = Energy

The chemical reaction that supplies the cells with energy is clean and simple. It involves the binding of a molecule, known as adenosine, with three phosphate molecules to form adenosine triphosphate (ATP). Move outward from the adenosine and you'll encounter each phosphate in succession until you come to the third one. This terminal phosphate won't be there for long. It takes extra energy to bind it to the second phosphate and this is the power-source that the cell is after. With a precision that is breath-taking, the tiny amount of chemical-bond energy that held the terminal phosphate in place is removed and carefully transferred to other components of the cell. Left behind are adenosine diphosphate (ADP) and the third phosphate, now shorn from the main molecule. It won't remain separated, however, at least not in a normal cell. Energy derived from glucose, a sugar molecule that might have been in someone's breakfast cereal an hour before, will be quickly used to reattach the terminal phosphate.

This simple reaction, ATP yields ADP + P + energy, is so widespread and occurs so frequently in the human body that it would take a multi-volume book to describe its many uses. The manufacture of most large molecules in the cell is driven by energy from ATP. Myosin proteins in muscle are energized by ATP for their next round of contraction. Ion pumps in the cell membrane use ATP-derived energy to continually transport sodium out of cells, a process that is in turn linked to many important activities, including electrical signaling in the nervous system, shuttling nutrients into cells, and maintaining fluid balance in the body. Hormones such as insulin and adrenalin act by stimulating the production of messenger molecules inside target cells, yet another process that requires ATP.

Where Does ATP Come From?

It's interesting that such a vital molecule is not delivered directly to the cells. One might imagine that the blood is filled with ATP and that the cells pull it out whenever they need it. Instead, the digestive system has the job of breaking down the foods that we eat into the small molecules that are necessary for ATP production, molecules such as glucose and fatty acids. Once a cell obtains one of these fuels, it's ready to use them to produce its own supply of ATP. This scheme works well because almost all cells contain the enzymes required for extracting chemical-bond energy from glucose or other molecules and transferring it to ATP. The speed and efficiency of these enzymes are so great that it is not necessary for the cell to store large amounts of ATP: it can be made on

demand in a fraction of a second. Many of these enzymes are located in a cell organelle that has been known by a number of different names since it was first glimpsed through a microscope over 150 years ago. Today we call this energy-processing organelle the mitochondrion.

Mitochondria: The Energy Machines

Mitochondria are able to take the energy in foods and carefully transfer it in small successive steps to individual ATP molecules. We're familiar with the fact that each kind of food contains a specific number of calories, units that provide a measurement of the amount of energy available from the food. This energy could be gotten all at once by burning the food; the fire would immediately release all of the calories in the form of heat. But acquiring the energy this way would be of no use to living organisms. It is necessary to carefully transfer to ATP the chemical-bond energy present in the carbohydrates, fats, and proteins that we eat. ATP then readily donates this energy in amounts appropriate to the specific needs of individual cells.

Hold onto the idea of burning the food, because this is exactly what happens to nutrients as they are shuttled along the enzyme systems of the mitochondrion. The combustion of glucose in the cell does indeed release heat, but not enough to start a fire. The loss of heat means that only part of the available energy is transferred to ATP. Combustion, whether it occurs in a fireplace or in a cell, requires oxygen. Unlike burning a log, cellular combustion of glucose is a clean process that results in carbon dioxide and water as the sole waste products. The ATP-producing steps taking place inside the mitochondria are called "cellular respiration," reminding us of the continuous movement of oxygen into the organelles and the outward movement of carbon dioxide.

Energy transfer in the mitochondrion involves a series of *oxidation-reduction* reactions. If we think about what happens to burning wood or even rusting iron as they are exposed to oxygen, it will help us remember that oxidation always involves taking something away. The role of oxygen in ATP synthesis is to accept two hydrogens and their electrons from an enzyme system known as cytochrome c oxidase, forming water. This is the last step in a series of reactions where electrons are sequentially donated (reduction) and removed (oxidation) from transport molecules. The overall process can be referred to as oxidative phosphorylation, sometimes inelegantly abbreviated as OXPHOS, because a molecule of ADP is *phosphorylated* to ATP in the presence of oxygen.

Building Up and Breaking Down

Before addressing the details of the complex sequence of events that occurs in a normal mitochondrion, let's think about the overall processing of energy in the human body. *Metabolism* refers to the sum of all chemical reactions that happen in cells. Some of these reactions are *anabolic*, i.e. energy-requiring, while others are *catabolic*, i.e. energy-producing. ATP is the link between these two types of reactions, with catabolism breaking down molecules to provide the energy necessary for anabolism, which is the building of larger molecules from smaller ones. We can illustrate this process by following the metabolism of a piece of bread after it's eaten. The primary component of

bread is starch, a polysaccharide that is readily broken down by the digestive system into its component monosaccharide, glucose. The resulting glucose molecules can be oxidized to produce ATP (catabolism) or they can be bound together to make another polysaccharide, glycogen (anabolism). Glycogen is stored in the liver and skeletal muscle cells as a ready source of glucose. The same story could be told for proteins and their building blocks, amino acids, or for fats and fatty acids. Every time a large molecule is synthesized from a smaller one, it uses up the energy from another ATP. So we eat food to acquire ATP from catabolism, but since we don't eat constantly, we need to synthesize and store glycogen and fat, anabolic processes that require ATP. These stored molecules can then be broken down later for oxidation to ATP. The interconnected processes of catabolism and anabolism continue for a lifetime.

Energy Production Outside and Inside the Mitochondria

Mitochondria that function normally are vital to maintaining the ongoing linkage between catabolism and anabolism, because it is inside these organelles that most of the body's ATP is generated. We say "most" for a small percentage (about 6%) of the total ATP derived from catabolism of glucose is gained outside of the mitochondria, through a process known as *glycolysis*. While the cell does collect two molecules of ATP from this reaction, it gains something much more important: a molecule called pyruvate. Glucose has six carbons and, through a multi-step procedure in the cell's cytoplasm, it is divided into two pyruvates, which are three-carbon compounds. Glycolysis means "to split glucose," which is just what happens. The significance of this is that, rather than having glucose entering the mitochondrion to undergo oxidation, it is the smaller pyruvate molecules that enter.

Perhaps this is as good a place as any to make a minor point about terminology. Many of the intermediates that are involved in energy metabolism can exist in two forms: acidic and basic. If the compound is in its basic from, then its name will end with the suffix "-ate;" if it is in its acidic form, then its name ends in "-ic acid." Thus the names pyruvate and pyruvic acid designate the same molecule as do lactate and lactic acid, to be discussed next. Suffice it to say that the chemical conditions of the human body are such that these compounds are present primarily in their basic forms.

The entrance of pyruvate into the mitochondrion is the important first step in producing the other 94% of the ATPs that are available beyond glycolysis. This mitochondrial ATP production is *aerobic*, i.e. it depends upon oxygen, while glycolysis is *anaerobic*, a distinction that becomes significant when a cell experiences low oxygen conditions. When oxygen is scarce, those two ATPs from glycolysis suddenly take on great importance. They allow the cell to still derive some energy from glucose even though many of its mitochondria are temporarily shut down due to lack of oxygen. As each glucose molecule continues to split into two pyruvates, these three-carbon compounds start to build up in the cell. For reasons that we will not go into here, continued movement of glucose through the glycolytic pathway requires the conversion of pyruvate to lactate. As long ago as the 19th century, biologists knew that mammalian tissues incubated under low oxygen levels accumulated lactate, while it was not formed when adequate oxygen was available. It's helpful to remember that a high level of lactate in the blood indicates that significant amounts of pyruvate are not currently being used

for ATP production in the mitochondria. This happens normally during exercise in anyone, but may also occur routinely in patients with mitochondrial disease. What we're interested in right now is what happens to the pyruvate when, instead of being converted into lactate outside of the mitochondrion, it actually makes it inside. Describing this will require the introduction of a class of very important molecules: the reducing coenzymes.

The Reducing Coenzymes

Most people are familiar with the USDA food guide pyramid, an attempt to let the public know what a healthy diet should look like. There are a variety of reasons for trying to follow these guidelines. One is that doing so ensures an adequate supply of the vitamins needed to make coenzymes, a class of functional molecules that work with cellular enzymes to speed up chemical reactions. Two of these vitamins are niacin and riboflavin, compounds that cells use to manufacture the reducing coenzymes NAD and FAD, respectively. Earlier, we referred to the oxidation of glucose as the means of transferring its chemical bond energy to ATP. We also mentioned that it involved sequential steps through which tiny amounts of energy were acquired, unlike with fire where all of the energy would be released at once. The removal of electrons from a molecule (oxidation) requires the presence of electron acceptors that will transport the electrons elsewhere and then donate them to other molecules (reduction). This is the job of NAD, FAD, and a third reducing coenzyme called ubiquinone or coenzyme Q. At this point, the important thing to remember about these coenzymes is that the continuous flow of electrons that they accomplish is directly linked to the formation of ATP in the mitochondrion. How this occurs will take some telling.

How the Mitochondria Make ATP, Part One: The Citric Acid Cycle

As soon as it enters the mitochondrion, pyruvate is attacked by an enzyme that radically changes its structure by doing two things: it removes electrons and donates them to NAD and also removes one of the carbons to make carbon dioxide. Every time you breathe out, you are exhaling carbon dioxide gas that was once part of a pyruvate molecule. The remaining two carbons are transferred to yet another coenzyme, this one called coenzyme A, which is made from the pantothenic acid contained in your daily multi-vitamin. This coenzyme will work with its associated enzyme to combine the two carbons (called an acetyl group) with a four-carbon compound called oxaloacetate to form citrate. Ironically, a complex sequence of events that began with one six-carbon molecule, glucose, has now resulted in the formation of another one: citrate. This is the beginning of what is called the citric acid cycle, also called the tricarboxylic acid cycle (for the type of chemical of which citrate is an example) or the Krebs cycle, after Hans Krebs, who first discerned its cyclic nature in the 1930's. What ever this complex cycle of chemical reactions is to be called, it has two straightforward outcomes. One is to continue removing electrons from its reactants and donating them to the three reducing coenzymes: NAD, FAD, or coenzyme Q. The other outcome is the formation of one more ATP molecule for each pyruvate that entered the mitochondrion. This makes a total, so far, of four ATPs: two from glycolysis and two from the citric acid cycle. This number is still far short of 32, which is the theoretical maximum number of ATPs that can be

generated from a single glucose molecule in humans. How are those 28 other ATPs obtained? The answer is found by following the reducing coenzymes to their final destination in another region of the mitochondrion: the electron transport chain.

How the Mitochondria Make ATP, Part Two: The Electron Transport Chain

By now we've encountered a wide variety of chemical reactions that require the presence of enzymes, specialized proteins that keep the reactions occurring at a rapid pace. These reactions would occur much too slowly to be effective if they were not pepped up by specific enzymes. The enzymes that catalyze glycolysis are found in the fluid component of the cell, the "cytosol," while all but one of the enzymes that catalyze the citric acid cycle are found in the fluid component of the mitochondrion, the "matrix." A third set of functional proteins, some of which are enzymes, are embedded in the mitochondrion's inner membrane and are called collectively the electron transport chain. The name denotes the shuttling of electrons along a chain of molecules, with their ultimate donation to oxygen to form water. The process is begun by one of the reducing coenzymes that resulted from the citric acid cycle, NAD. In its reduced form, this coenzyme is referred to as NADH.

The electron transport chain is envisioned as a sequence of respiratory complexes, numbered I through IV. The "chain" designation does not mean that the complexes are rigidly linked together; they actually move freely and randomly inside the inner membrane. Nevertheless, the reactions that they carry out do occur sequentially: NADH donates electrons to Complex I (the first step) and Complex IV donates electrons to oxygen to make water (the last step). Providing a detailed description of what happens in between, as well as how passing electrons between these complexes is linked to the production of ATP, is beyond the scope of this article. Nevertheless, because certain mitochondrial diseases result from malfunctions of specific respiratory complexes, it might be useful to identify these complexes by their descriptive names.

Complex I is called "NADH-coenzyme Q oxidoreductase." This mouthful of a name is actually helpful, because it describes what the complex does: it oxidizes a molecule of NADH to NAD and reduces coenzyme Q to $CoQH_2$.

Complex II, "Succinate-coenzyme Q oxidoreductase" is linked to the citric acid cycle intermediate succinate. The enzyme that catalyzes the conversion of succinate to fumarate, succinate dehydrogenase, is the only citric acid cycle enzyme that is not in solution in the mitochondrial matrix. Rather, like the electron transport chain proteins, it is embedded in the inner membrane. This seemingly obscure point is noteworthy because it involves the coordinated action of two reducing coenzymes, FAD, which is reduced to $FADH_2$ by succinate dehydrogenase, and coenzyme Q, which is reduced by $FADH_2$.

Complex III is called "Coenzyme Q-cytochrome c oxidoreductase," Perhaps a pattern in the naming scheme is now becoming clear. The "oxido-" refers to the oxidation of the first molecule, coenzyme Q, and the "-reductase" designates the reduction of another molecule called cytochrome c.

Complex IV, "Cytochrome c oxidase," transfers the electrons from cytochrome c to other molecules within the complex and ultimately to oxygen.

The origin of coenzyme Q and its role in the electron transport process is worth expanding upon. Terminology is a funny thing: this vital molecule is more properly

known as ubiquinone, because it has a wide or ubiquitous distribution throughout the body. It is located in the inner membrane of all mitochondria and is an important electron shuttle. Although ubiquinone is manufactured in the body, studies have suggested that it may be appropriate for some individuals to take it in supplementary form. These include victims of mitochondrial diseases and also patients who are taking any of the cholesterol-lowering "statin" drugs. In the latter case, taking oral ubiquinone may be helpful because, interestingly, cholesterol and ubiquinone share an early step in their manufacture. This step is inhibited by the statin drugs, with the result that slowing down cholesterol production can also slow down production of ubiquinone. In contrast, a mitochondrial disease patient may have normal levels of ubiquinone but, because ATP production occurs at a slower rate in these patients, providing their mitochondria with above normal amounts of the electron shuttling molecule through dietary supplements may be beneficial.

What Does It All Mean?

Everyone approaches a discussion like the preceding one from different perspectives. It's not surprising that someone who lacks a background in biochemistry will come away with a head full of terms that are only vaguely connected to each other. The major point for those who want to begin to understand the significance of mitochondrial disease is to perceive, if only dimly, the vital role played by mitochondria in the energy-requiring phenomenon that we call life. Something that most people can take for granted, that they will reliably and efficiently extract from their meals all the energy that they will ever need, is not a given for the mitochondrial disease patient. Theirs is an existence defined by mitochondria that struggle to make enough ATP. They live with the consequences of missing one or more normally functioning components from the cell machinery that we've described. The bewildering array of symptoms that these patients can have is explained by the observation that a complex machine can malfunction in a number of different ways, depending on which one of many parts is damaged. Further variation in symptoms occurs because some organs will have defective mitochondria while those in other organs may be normal.

And so we come back to where we began: energy. Most of us will never need to ask, "How will I get enough energy to make it through another day?" But researchers funded by the UMDF are seeking answers on behalf of those for whom it is not a trivial question.

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