Biochemistry 401 Lecture 22.

Today we're going to talk about ATP synthesis. We're then going to talk about the uncouplers of oxidative phosphorylation and the poisons that can interrupt the flow of electrons in the electron transport chain and ATP synthesis, so let's get started.

In the last lecture, we looked at the electron transport chain. During the electron transport chain energy from the passing of electrons from carrier to carrier was used to pump protons from the matrix into the inter-membrane space, thus creating a proton concentration gradient, and an electrical potential across the inner membrane. This is called chemiosmotic potential. There is both separation of charge, with more positive charges being in the inter-membrane space than there are in the matrix, and there's also a chemical gradient because there are so many more protons in the inter-membrane space. This electrical potential is similar to what we see in a battery. This chemiosmotic potential causes the protons to flow back into the mitochondrial matrix, and this turns a turbine-like molecular motor that's called ATP synthase. This synthase helps to synthesize ATP. In this lecture, we're going to look at this process very closely.

In this diagram, we see three different representations of ATP synthase. In all cases, the inter-membrane space is at the top of the slide, and the matrix is at the bottom of the slide. On the extreme left, we see a cartoon diagram of ATP synthase, and we can see that it is comprised of two main regions, F_0 and F_1, The F_0 region is a place into which protons will flow, whereas the F_1 region is a portion of the enzyme that actually synthesizes ATP. Now this enzyme uses proton flow to cause a portion of this enzyme to actually rotate, and this rotational energy helps ATP synthesis. Let's look for a minute at the diagram in the center, which is from your book. This shows that F_0 and F_1 are actually comprised of many subunits.

And so there are two different types of components in ATP synthase, those that rotate, and those that don't. The rotating components are the C-ring, and the gamma and epsilon region of the stalk, whereas the stationary components are the stator, this is comprised of a, beta-2 and delta subunits, and the alpha-three beta-3 hexameric ring, also is stationary, and this is because the stator is keeping it still. The stator is anchored in the inner mitochondrial membrane by the a-

region and the b2-region forms a link between the a-region and the delta-region. It is the delta-region that makes physical contact with the alpha-three beta-three hexamer ring, and keeps it still. Now the C-ring rotates within the plane of the membrane, and as it does, it causes the drive shaft, the delta-region to rotate as well, and it is protons that are moving from the inter-membrane space to the matrix that actually cause the C-ring to rotate. The protons do not go down through the central channel as one would expect, but rather the protons are transported across the inner mitochondrial membrane, by individual subunits of the C-ring. and we'll see that in a minute.

And so let's go over this really quickly. What's going to happen is this, protons are going to cause the C-ring to rotate. The rotation of the C-ring is going to cause central shaft to rotate as well. This rotation is going to cause a conformational change in the beta subunits of the alpha-3, beta-3 hexameric ring, and this conformational change is going to open and close regions in the beta subunits, allowing ADP and inorganic phosphate to enter to form ATP, and for ATP to exit. Now let's look at this little more closely,

Again, here we see the three-dimensional structure of ATP synthase. Now if you'll notice, the whole of the stator is not included, that's because it's a very difficult portion of the protein to actually crystallize, and so all we see is the a-region of the stator. Now, the gamma subunit acts as a central shaft and it rotates with in the hexameric ring, but you can see that this central shaft is not symmetric, and it is this asymmetry that causes conformational changes in the beta subunits. Here we see the central shaft as it lies in the middle of the hexameric ring. Portions of this ring have been stripped away so that you can see the gamma subunit in the center. As this gamma subunit rotates within the hexameric ring, it causes conformational changes to occur in the beta subunits.

And here again we see how the alpha-3, beta-3 hexameric ring surrounds the gamma subunit that comprises the central shaft,

and if we rotate the hexameric ring 90°, to look at a cross-section of it, you can see the central channel into which the gamma subunit extends.

And so we said that rotation of the C-ring causes the gamma subunit to rotate as well, and this causes a conformational change to occur in the beta subunits that helps to power ATP synthesis. But what causes this C-ring to rotate, and how does the protons go across the inner mitochondrial membrane? Well, this happens though the action of the subunit, a. This contains two half-channels, one that has direct contact with the cytoplasm, and one that has direct contact with the matrix, however neither these channels spans the membrane, but instead will abut the C unit this means that it has direct contact with the subunits of the C-ring. Now each C-subunit contains a critical aspartic acid that is important for C-ring rotation. Now we know that aspartic acid can act like an acid to lose a proton, and this residue has a key role in delivering protons from the inter-membrane space to the matrix, and also allowing the C-ring to rotate. Let's see how that works.

And so remember the inter-membrane space has a high concentration of protons, therefore the pH is low compared to that in the matrix. In the matrix, there is a low concentration of protons, and so the protons move down their concentration gradient, and this powers ATP synthase.

Here we see a cross-sectional representation of the C-ring, in which the protein backbone of each subunit is shown. We can get a good appreciation for the individual subunits this way and we can see quite easily the aspartate residue that's indicated by the arrow. This aspartate residue is going to pick up a proton from the inter-membrane space.

Protons will flow in to the cytoplasmic half-channel in subunit a, and once there, they interact with the aspartate residue in the C- subunit. Now aspartate in a proton-poor environment will be deprotonated to form aspartate. This is a negatively charged amino acid. However, in a proton-rich environment, at a pH much below its pKa, this residue will be protonated to form aspartic acid. Now aspartic acid is neutral, and so it can enter a hydrophobic membrane quite easily, whereas the aspartate cannot. The presence of aspartate in the C-unit, rather than aspartic acid, will prevent the ring's rotation. However once aspartate is protonated to form aspartic acid, that C-subunit can freely rotate.

And so here we see a schematic that represents the a-subunit as it interacts with the C-ring. We can see the half-channels illustrated quite nicely in the front