Biochemistry 401 lecture 21
Today we're going to talk about the electron transport chain. We're going to begin with a discussion of how reducing equivalents get from the cytoplasm to the mitochondrial matrix, so that they can be used in electron transport chain. We're then going to talk about oxidative phosphorylation, and we'll start with an overview of this process. We'll talk specifically about the electron transport chain and about integral membrane electron carriers, and mobile electron carriers that make up this electron transport chain. We'll then follow electrons through the electron transport chain to see how this works

And so how do reducing equivalents get from the cytoplasm into the mitochondrial matrix so that they can be used in the electron transport chain? Well it's not actually the carrier that is transported, just the electrons, and so we're going to hand off electrons from one electron carrier to another. We'll start with an electron carrier that is in the cytoplasm that's reduced, and then we'll pass those electrons to a mitochondrial electron carrier and reduce that. So the electrons will be passed from the cytoplasm to the mitochondria by way of electron carriers. The electron carriers themselves don't cross the membrane, but the electrons do. There are two main ways that reducing equivalents get from the cytoplasm into the mitochondrial matrix. This is the glycerol three-phosphate shuttle, and the malate-aspartate shuttle. The glycerol three-phosphate shuttle uses cytoplasmic NADH and mitochondrial FAD. Now this mitochondrial FAD is shown here as e-FAD because this FAD is actually a prosthetic group that's bound into an enzyme, and we'll look at this in a little while. With the malate-aspartate shuttle, we begin with NADH carrying the electrons in the cytoplasm and we're going to pass those over to an NAD^+ molecule that's in the mitochondrion. We end up with NAD^+ in the cytoplasm and NADH in the mitochondria.

So now let's look at how these transporters work. This is the glycerol 3-phosphate shuttle, and it's known as the glycerol 3-phosphate shuttle because that is the molecule that's going to be oxidized to pass electrons to an electron carrier that has close links to the inner mitochondrion. This is an enzyme that is intimately

associated with the inner mitochondrial membrane. It sits on the cytoplasmic face of the inner mitochondrial membrane facing the inter-membrane space.

Now this process is going to happen in three steps. The first thing we're going to do is we're going to reduce dihydroxyacetone phosphate to yield glycerol 3-phosphate, and so the reaction is dihydroxyacetone phosphate plus NADH plus a proton yields NAD^+ plus glycerol 3-phosphate. Dihydroxyacetone phosphate is being reduced, and NADH is being oxidized to NAD^+. Now these electrons from glycerol 3-phosphate can be handed off to another electron carrier. This is bound into an enzyme, the mitochondrial glycerol 3-phosphate dehydrogenase. We're going to reduce FAD that's bound in this enzyme to yield $FADH_2$, and in the process, glycerol 3-phosphate will be oxidized to form dihydroxyacetone phosphate again. But this shuttle doesn't stop here. The electrons that are carried by $FADH_2$ can be passed off to a mobile inner-mitochondrial membrane electron carrier that is known as ubiquinone. Now ubiquinone is sometimes called Q10. This is a mobile carrier that is lipophilic, and shuttles back and forth in the inner mitochondrial membrane, carrying electrons from one electron carrier to another. Ubiquinone is often abbreviated Q, this is the oxidized form of the electron carrier whereas QH_2, ubiquinol, is the reduced form. We will look at this more closely in a little while.

This is the malate-aspartate shuttle. It is much more complex than the woman just looked at and we're going to follow this really slowly so that you can figure out what's going on. The enzymes that are involved in this are a malate dehydrogenase in the cytoplasm, an aspartate aminotransferase in the cytoplasm, a malate dehydrogenase in the mitochondrion and an aspartate aminotransferase in the mitochondrion. Now both of these mitochondrial enzymes are in the mitochondrial matrix.

And so we're going to start off with oxaloacetate in the cytoplasm, and we're going to reduce that to form malate. As we do this, the electrons from NADH will be transferred to oxaloacetate to yield malate and NAD^+. So oxaloacetate is reduced to malate and NADH is oxidized to NAD^+. Malate will leave the cytoplasm and go into the mitochondrial matrix. Once there, it will be oxidized again to form oxaloacetate, and reduce NAD^+ to NADH, and so now we have our electrons transferred from the cytoplasm into the mitochondrial matrix and that's what we want, however, what we don't want is we don't want a lot of oxaloacetate in the mitochondrial matrix, because this might slow up the TCA cycle, and so we're going to move this oxaloacetate out of the mitochondrial matrix. But wait a

minute. We can't do that, because we can't transport oxaloacetate directly out of the mitochondrial matrix. There's no transporter. So we're going to use another one of our tricks, and we're going to add an amino group to oxaloacetate. Now remember at the beginning of the semester, I said it's important to know which amino acids have four carbons, and which have five, and I said it's important to know which metabolites have four carbons and which have five, and this is why. Oxaloacetate has four carbons, so does aspartate, and so now what we're going to do is we're going to take an amino group from glutamate and transfer it to oxaloacetate. When this happens, oxaloacetate will be changed to aspartate. There are 4 carbons in oxaloacetate and 4 in aspartate. Aspartate can now leave the mitochondrial matrix by way of a transporter. But let's go back to the mitochondrial matrix for minute. We took the amino group off of glutamate, this is the alpha amino group, and when we do this, we change glutamate into alpha-ketoglutarate – 5 carbons in glutamate, 5 carbons in alpha-ketoglutarate. This is the same intermediate that we saw in the TCA cycle, so we don't want this to build up either, and what we're going to do is we're going to transport this out of mitochondrial matrix by way of a transporter. Once this alpha-ketoglutarate is in the cytoplasm, it can accept an amino group from aspartate to be turned back into glutamate to begin the cycle again, and aspartate, once it gives off that alpha-amino group, is changed from the 4-carbon aspartate into the 4-carbon ketoacid, oxaloacetate, and in this way this cycle is perpetuated.

Take a minute to look at the arrows and please note that when you see the transamination, what we're really doing is we're crisscrossing those arrows. So, an amino group is going from glutamate to oxaloacetate, and oxaloacetate is becoming aspartate, and glutamate is becoming alpha-ketoglutarate.

And just to make it a little more clear, let's look at the amino transfer that's happening in the matrix. We'll start off with oxaloacetate, it is going to pick up an amino group from glutamate to yield aspartate, so oxaloacetate plus an amino group yields aspartate. Glutamate minus an amino group is going to yield alpha-ketoglutarate, and you can see the crossing of the arrows to represent where glutamate is going and where oxaloacetate is going.

Aspartate aminotransferase works by way of an ordered biomolecular reaction and what this means is these reactants engage with the enzyme in an ordered manner. First aspartate is going to come in, and it's going to lose an amino