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Good morning. I'm Paul Taylor, and I am currently transitioning from a post-doctorate position with Kurt Fischbeck to my own laboratory at the University of Pennsylvania.

In preparing for this presentation, I anticipated that there might be some skepticism about the ability of studies in Drosophila, the fruit fly, to lend any, give us any, important insight into Kennedy's Disease. So, in preparing this presentation, I kept that in mind. What I'm going to talk about today, in what I promised would be a brief presentation, is why do I think studies of the fruit fly would, may, be a very powerful way to gain further understanding of Kennedy's Disease and, also, provide us an entrée to looking for therapeutics.

May I have the first slide please? So why use fruit flies? There's a very long and rich history of scientific research in Drosophila. In fact, the hundred-year anniversary is coming up in just five years. There was the first, this organism was first studied by Thomas Morgan at Columbia in 1908. And it's been a very fruitful, very fruitful organism which to study for these reasons. First is the simplicity; basically the tools that are used to study the fruit fly haven't changed in almost a hundred years. You basically just need bottles in order to keep the fruit flies, to do your matings, and you need some magnifying glass or microscope to look at the results of your matings.

The power comes from a couple other areas: conservation. And this is the concept that there's a lot of biological conservation between the fruit fly and the human. In fact, the human is a simpler system; humans have about 35 to 40 thousand genes, fruit flies only have about 12 to 14 thousand genes. It's a much simpler system; there's a much simpler brain, much simpler nervous system. But many of the principles that we find work in the fruit fly, apply directly to what's seen in humans.

A tremendous body of resources have developed over the last hundred years. They're doing research in the fruit fly, as evidenced by this cover of Science Magazine in 2000. The entire Drosophila genome has been sequenced. It's very well annotated, so we have a lot of information about almost all the genes in the fruit fly. And there are thousands upon thousands of mutant strains of fruit flies with defects or changes, or modifications of many of these different genes, that are freely exchanged around the world between fruit fly researchers that enable us to look at the impact of changing each of these individual changes on our particular interests.

And then the most powerful thing that fruit fly provides is the ability to do genetic screens. And this enables you to identify genes that you would never have anticipated play a role in the process you're interested in. Or to identify genes that were previously undiscovered and have no known function. So using the fruit fly, from the roots of the fruit fly I would say, the body of knowledge that we have today regarding genetics and development largely came from the system. In fact, the ability to identify the Kennedy's Disease gene by positional cloning has its origins and work done in the fruit fly. The concept of sex-linked inheritance was identified in the fruit fly. Can I have the next slide please?

I'm going to show you just a little bit of anatomy here. This is a magnification of the fruit fly head, and this is its neck and the beginning of its body. What you see is this enormous eye, beautiful structure, very highly organized, about 800 individual facets that you can see here otherwise known as omma-tidia. And this is a cross section through the Drosophila eye. You can see the surface here, you can see those individual facets, and beneath each one is an elongated tube. So this represents the Drosophila eye. The Drosophila retina is here - this is the very thick retinal layer that directly impinges on the

Drosophila brain. What's real nice about this is that each of these tubes, these omma-tidia, are made up largely of neurons. These are all photoreceptor neurons.

This is a schematic showing one cross section. So what you have here on the surface of the fly is a very rich resource with a very large structure that is made up largely of neurons, and it's on the external surface. So we can manipulate the fly, and we can look at our results very easily by just popping that fly right under the microscope and very quickly seeing what the consequences are of some treatment that we did, or some gene change that we made. Next slide please.

Now, the work done on the fruit fly has had tremendous indirect impact on biomedical research for many, many years. But a landmark paper was published in 1998 by a colleague at the University of Pennsylvania named Nancy Reed. And what she did here for the first time was, she applied fruit fly, the power of fruit fly, directly to studying human disease. And what she did, she expressed a polyglutamine-containing protein. In this case, it was a mutant protein that causes…(inaudible)…disease. Am I breaking up back there?

I'm not sure if it's me or…I'll try to stand still. What Nancy did was express a mutant form of the…(inaudible)…protein in the fruit fly eye. And what you see here – just focus on this panel – I'm not going to go through a lot of data, I'm just going to show you this panel here. This is the structure of the eye that you can see very clearly with the scanning electron microscope. But when the mutant disease protein was expressed, that eye undergoes degeneration. So this was a landmark study that opened the floodgates to a large number of researchers that then moved to this model system in order to study human nerve degenerative diseases. And an example of the power of this is shown in the next slide. This is another landmark study that came from the lab of Leslie Thompson at the University of California at Irvine. This is from Leslie Thompson at the University of California at Irvine. What they did is, they made a fly model of Huntington's Disease, and they used it to study a drug that is in the class called…(inaudible)…inhibitor, thus the title.

So what the back story here is, some research that came out of Kurt Fischbeck's lab suggested that this class of drugs might be beneficial for polyglutamine diseases, in particular for Kennedy's Disease. And this was based on studies done by a graduate student in cells, in cultured cells. So it's a lot, there's a large gap between finding a phenotype, some kind of defect in cells in culture, and then trying to repair that with a drug with a big jump to humans. So what the fly provides here is an intermediate step.

What Leslie did in this study was she made Drosophila have Huntington's Disease in their eye, and they have a progressive degeneration of their eye. She treated them with this drug and validated the results that Allison found in cells in culture. And this is just an example of how you can use the fly as an intermediate step. So what happened, what's happened subsequent to this, which is very exciting, is that this class of drugs, after this study came out, were then applied to two different mouse models of polyglutamine disease. And this result has now been validated in two different mouse models and is now being prepared for a clinical trial in humans. So this, the fly provides the opportunity to screen large numbers of candidate drugs, and you can pick the best ones to study by mouse model. And the reason is, it's very easy, and fast, and cheap. You can find these kinds of studies in the fruit fly, but it's very time consuming and expensive to do it in a mouse, so it will narrow, it'll sort of narrow or funnel.

Next slide please. Okay, so, as an example of the kinds of things that you can do in the fruit fly, this is also a study that came out of Kurt Fischbeck's lab. And what you see here in green is a polyglutamine inclusion of the androgen receptor inside a nucleus shown in blue. And, may I get the next slide please?

Lights out for a second. So what you can see here, in blue inside the nucleus, these are some normal cells. These are motor neurons in culture, and inside these motor neuron nuclei you see these red punctuate staining. That's normal. That's a protein called CBP or Kreb-binding protein. And it's normally located all throughout the nucleus like that in little speckles, and that's how it should be. But in the nucleus that has the polyglutamine inclusion, you can see that virtually all of that CBP got sucked right into the inclusion. So the question was, is, if this was there, a functional consequence to this? And does this relate to polyglutamine pathogenesis? Next slide please.

So this is a study, this is evidence actually of collaboration between the groups. This was done in Dr. Sobue's lab. This is a sample from a patient showing

these nuclear inclusions of the androgen receptor, and the same phenomenon is observed. That same protein, the Kreb-binding protein, is found sucked into those inclusions. So to follow up on that – next slide please – we will be moved to a fruit fly model.

So here you see a normal fruit fly eye, shown by light microscopy or scanning electronic microscopy or very high-powered scanning electron microscopy, and you can contrast that with the fly that's contrasting expressing the polyglutamine here, and you readily see the degeneration that's occurred. And the question we asked is, if we now manipulate the levels of this Kreb-binding protein either up or down, is there a consequence on degeneration? And there is when you over express that Kreb-binding protein. So this is a fly that's now been engineered to drive up the expression of that protein. You see a rescue of the degeneration, but if you drive that expression down, you get the opposite effect. So, this is an example where the fly provides a model where you can validate something also seen by yourselves, some culture.

Audience member:

Can I ask you a question about that? (Inaudible)

J. Paul Taylor, M.D., Ph.D.

That's a very good question. So, no, this is not the same plot, and we're going to get to that because in previous technology it was not possible to do the experiment that you're asking. So each of these is a different fly line that's been engineered to express a different constellation of genes. But that's changed because of the…next slide, please.

This is the third landmark study that I want to mention, and this came out from Dr. Cato's lab. And it came out in an issue of Neuron, a very prestigious journal, a year ago August, which was a very powerful issue to everybody here because two very important papers came out in that study, one from Dr. Cato's lab and one from Dr. Sobue's lab. And what they showed definitively was the reason that men get Kennedy's Disease and women do not is because the degeneration depends on the testosterone. The lygand that binds the androgen receptor makes it move as a nucleus and what that, affords us the Drosophilus in an inducible system.

So what you see here is a normal eye of a fly that's expressing a normal androgen receptor in the eye. To provide that fly with testosterone, nothing happens, it's fine. But in a fly that has an androgen receptor that's been mutated with expansion, once you add the testosterone, the eye degenerates, and that's shown a little bit clearer.