[music]

Dr. Lightfoot: Please join me in welcoming Dr. Michael Roberts from Auburn University. [applause]

Dr. Roberts: Howdy.

[Audience]: Howdy.

Dr. Roberts: Can y'all hear me? Any war eagles out there [laughter]? Big football game this weekend. We're going to switch gears a little bit and talk about something that I believe is on the razor's edge of muscle biology, but before I get into that topic, I do want to talk about a researcher that has really inspired our laboratory to look at this in skeletal muscle and her name was Barbara McClintock. She was Cold Spring Harbor Laboratory. And in the early 1950s, Dr. McClintock identified what she or others have termed transposable elements. She did this in Corn, and she noticed different colors and color combinations in corn and attributed that to genes in the genome that we're able to literally transpose themselves and hop across chromosomes and reimplant themselves in other portions of the genome. And at the time she was a doing a lot of this work in the 1940s and 1950s and she met a lot of skepticism in the field but others after her doing clinical physiology also started noticing that these sorts of things happened in different types of cells and eventually her work was recognized and she received the Nobel Prize in 1983. The question I like to pose is kind of the whoa moment when we talk about this is, would you believe me if I told you that each one of your cells has jumping genes or genes that are able to move across the genome? And this is quite fascinating because I was taught at least in Biology 101 that the genome that we have is really stable and it doesn't change. You're able to express genes in the form of Messenger RNA, and that's very controllable depending upon what you do, whether it's exercise or eat certain types of foods, but the genome I've always learned is maintained in a sort of stable state.

What's interesting is that when you look at the composition of the genome, specifically the human genome, and we break it down into a pie chart, about 1.5% of our genome contains exons which are responsible for being transcribed into mRNA's and making enzymes and transporters and other types of proteins. About 5% of our genomes made up of entrons, which are spliced out of messenger RNAs. About 50% of our genome is made up of regulatory sequences or repetitive DNA elements. Non-coding RNA that scientists aren't quite sure what those pieces of DNA do. And then what's interesting is about 44% of our genome includes transposable element code, retro-transposons, and things that had been shown in other cell models capable of hopping across the genome. The LINE-1 sequence, the gene I'm going to talk about today, 17% of your genome is made up of this LINE-1 sequence. And that makes up about 500,000 copies. Again, that's 17% of the DNA. Now, when I say a retro-transposon, and LINE-1 being a retro-transposon, what it can do is it can copy itself and then reinsert itself somewhere else in your genome. But all 500,000 copies don't do that. A lot of them are mutated. However, there are a certain subset of LINE-1 elements per cell per nucleus that are able of self-expansion and geneticists have termed these are genetic parasites. What's interesting is that the cancer world has kind of taken off in terms of looking at LINE-1 and cancer and they say that the LINE-1 promoter and certain types of cancers you'll see the promoter existing in a hypomethylated state, which would indicate that LINE-1 mRNA expression could be up-regulated in certain types of cancers. And specifically, if LINE-1 mRNA expression is up, then it has the potential to reinsert itself somewhere else in the genome and cause mutations which could be causally linked to that sort of cancer.

Now, this is the structure of the LINE-1 gene I just want to discuss really quickly. It's bicistronic. So what that means is that it can encode two types of proteins from the messenger RNA, ORF1C sequence encodes an RNA binding protein, the ORF2 sequence encodes for a protein that has endonuclease and reverse transcriptase activity and methylation. If you methylate this 5' UTR those that study exercise genetics or genetics, then that's how you can shut down or reduce the expression of this gene at the mRNA level. So some details that are kind of important. Now, in terms of the mechanism how LINE-1 copies itself and then reinserts itself in the genome, I developed a little illustration here. So if we LINE-1 expression in the nucleus of one of our cells, being that blue circle that looks perforated, LINE-1 mRNA's going to find its way to the cytoplasm. It's isochronic, so it's going to be translated into the ORF1 and ORF2 proteins. And then those proteins preferentially reassociate with the LINE-1 messenger RNA. This forms the LINE-1 ribonucleoprotein complex, which then reenters the nucleus. It gets reverse transcribed, which then renters the nucleus. It gets reversed transcribed back into the genome and hence the copying and reinsertion of that new LINE-1 element. Okay? In terms of its prevalence in the scientific literature cancer researchers have really become interested in LINE-1 and cancer because again if you this random insertion of genetic elements into other portions of the genome all it takes is one cell where that can go rogue and you can insert it into some sort of tumor suppressor gene and then that cell becomes cancerous and can proliferate out of control.

But in terms of skeletal muscle when you try to search this on PubMed or Google Scholar, you find one article and this was a very profound article because it really turned us on as a laboratory to studying LINE-1 in muscle that was done by John Sedivy group at Brown University. And what they noticed they took mice that were five months of age, so kind of young adults, they took mice that were 24 months of age, so a little bit older adults, and then they took mice that were 36 months of age, which is towards the end of life for a mouse, and they looked at LINE-1 RNA expression in liver and in skeletal muscle. And you can see in both tissues, especially with the 36-month-old mice, LINE-1 expression is significantly upregulated at the RNA level. So these active copies of LINE-1 become kind of rogue, and they start overexpressing LINE-1 RNA. And so their working model in explaining this in their scientific paper was if you have a young cell, whether it's a muscle cell or a liver cell, that these LINE-1 elements exist in these compact regions of the genome called heterochromatin. But as we age, the chromatin structures of a lot of our cells become loose. Thus, exposing these LINE-1 elements to things like RNA polymerase. And because of that, we can have this increased expression of LINE-1 and the formation of more L1-RNP and those eventually enter the cell and they create more genomic DNA copies of LINE-1. And in fact, they showed at the DNA level, there were a lot more copies when you looked at the livers and the muscles of these really old mice and it seems to increase in an age-dependent manner. Okay?

So me having an exercise physiology background, we decided to look at tissue we had in the bank. We had muscle biopsies from college-aged men, but I'll get into that detail a little bit later on. And this was really spurred by Matt Romero who's a PhD student in Petey Mumford as well as Paul Roberson in our lab. These were 13 untrained college-aged males. Again, this was just tissue lying around in our freezer. We took a pre-training muscle biopsy out of the vastus lateralis. They trained for 12 weeks. Resistance training with weights in the weight room Monday, Wednesday, Friday. So three days per week. And then we took a post-training biopsy. And we started saying, "Does resistance exercise do anything to this LINE-1 pathway in skeletal muscle?" And sure enough, we saw, I guess, a tendency for LINE-1 messenger RNA to become down-regulated from pre-exercise to post-training. We saw LINE-1

promoter methylation increase with training. And then we looked at reverse transcriptase activity because the way that LINE-1 operates in terms of reintroducing itself, a new copy into the genome, it through its reverse transcriptase activity, and that too trended downward with 12 weeks of resistance training. So our preliminary thoughts are, "Well, exercise down-regulates this genetic parasite that seems to proliferate with aging in skeletal muscle and this could be one of the great health benefits of exercise in terms of maintaining muscle health. But then we started saying, "Well, we only looked at college-aged males. We still don't know anything in terms of how exercise, maybe over a lifetime affects this pathway." And we really still don't know how LINE-1 mechanistically affects skeletal muscle. So John Sedivy shows that with aging, it coincidentally increases in terms of LINE-1 RNA as well as LINE-1 genomic DNA and they speculated this could be bad if this genetic parasite is creating more copies because it could lead to muscle mutations in certain critical genes but no one has ever really shown that in terms of proof of concept.

Now, in terms of the exercise in aging question, Matt Romero and Petey Mumford right now, we were able to highjack skeletal muscle from my postdoc mentor Dr. Frank Booth, who by the way this carpet is here because of Frank because he would wander off. He was here for the first discussion. And so he gave us muscle from animals that he had exposed to voluntary wheel running for five months and we also got muscle from animals that were just sedentary. So they were aged-matched controls. We got gastrocnemius muscles, which are hind limb muscles that are activated during voluntary wheel running. So the idea is we wanted to see in animals that ran for five months versus those that were sedentary what's going on with the LINE-1 pathway. And Matt has shown that in these exercised animals LINE-1 RNA is becoming downregulated and the LINE-1 promoter is upregulated. So this is definitely supporting our hypothesis and is a continuation of our data from humans. In terms of how LINE-1 mechanistically affects skeletal muscle, I'm flat out lucky because I found the perfect collaborator. His name is Dr. John McCarthy. And he is a guru in terms of breeding special mouse models. And what he's bred for us is a mouse; I won't go into the details because I still have trouble. He's such a brilliant scientist. But what we can do with this mouse is watch it grow normally until adulthood. We can put doxycycline in the water and then we can artificially through that doxycycline up-regulate LINE-1 only in skeletal muscle. So it's a [inaudible] muscle specific inducible mouse. And so with that experiment, we're going to do a lot of things. We're going to see just how overexpressing LINE-1 only in muscle does that lead to premature muscle aging. If it does, then the idea is we're going to get those animals and we're going to give them voluntary wheel running to see if that rescues the effect. So we're really excited there and hopefully, we'll have papers coming out soon on that.

But while we're really early in the game on this topic we are hypothesizing that exercise decreases skeletal LINE-1 activity. Given that A is true, it's possible that lifelong exercise could prevent genomic LINE-1 DNA inserts in skeletal muscle and perhaps other tissues. And Paul Roberson's doing a nice human study right now where we're collecting other tissues to test that hypothesis. And if A and B are true, then is this the primary mechanism through which muscle health is realized through lifelong exercise? So with that, I do want to thank Dr. Lightfoot, Carlos, and the other [inaudible] organizers as well as members and collaborators and our funding sources. That's it. [applause]

Dr. Lightfoot: Thank you. Dr. Roberts. Great talk. I'm not sure that I ever thought I would hear the phrase genetic parasite and exercise in the same talk before [laughter]. Yeah. I'm not sure how many exercise physiology courses you see those phrases together. I have a couple of questions here. One from Maleah Holland at Augusta University. Does LINE-1 decrease in specific muscle that were resistance trained or does it decrease in all

muscles, even if only one specific muscle is trained?

Dr. Roberts: Great. And Maleah was a former doc student, so hello Maleah. So we don't know the answer to that because of the accessibility with human studies. VL is commonly biopsied since it's safe to do so. You can biopsy other muscles, but that gets a little bit more technical. We think it's whatever muscle you're training. That muscle probably is seeing this benefit. We haven't proven that yet. We've been looking at one muscle at a time in each model in humans and rats. What Matt is going to do is we're going to put some exercise mimetics like AICAR and caffeine on muscle cells in a petri dish to see if these exercise signals down-regulate LINE-1 MRA. So we're kind of getting there.

Dr. Lightfoot: Excellent. I think they're in the same room at Augusta University. Jennifer Dawn says, is there any research into using microRNA's to bind and down-regulate the expression of these jumping genes?

Dr. Roberts: Yes. I think they were HeLa cells. It was a 2015 paper because John McCarthy our collaborator is a big pioneer in the microRNA world. I think microRNA 128 and other cells have been shown to down-regulate LINE-1 by increasing a degradation pathway of LINE-1. So miR-128 is one, but I don't know its expression in muscle. If it's muscle specific or other tissues have it more enriched than skeletal muscle.

Dr. Lightfoot: Okay. This is a little bit of an alteration of this talk. This is a question from Cherie Queens at the University of Charlotte. He asked or she asked, do you believe that jumping gene is a cause of cancer? And I guess I'd like to extend that. We always know that exercise down-regulates or prevents some forms of cancer.

Dr. Roberts: Correct.

Dr. Lightfoot: Could this be a reason?

Dr. Roberts: Right. Yeah. So that's what we've been talking about. That's where we've adapted our thinking here. What if exercise is the polypill that prevents cancer? Which it does in certain types of cancers and Frank talks about that all the time. We don't know [laughter]. To answer that question, there are researchers thinking that LINE-1 does directly cause certain types of cancers through mutagenesis. Whether exercise can act in a preventative way for all cells, we don't know. Are myokines secreted, which then target cells to down-regulate things which are related to LINE-1? We don't know. Yeah.

Dr. Lightfoot: It's a complicated picture.

Dr. Roberts: Very.

Dr. Lightfoot: Absolutely. Dr. Roberts, thank you so much.

Dr. Roberts: Thank you, sir. Appreciate it.

Dr. Lightfoot: Please join me in thanking Dr. Roberts. [applause] [music]