Speaking of Mol Bio Podcast Episode 7

Transcript

Steve Lewis 00:09
Welcome to Speaking of Mol Bio, a new podcast series about molecular biology and its trending applications in life sciences. I'm Steve Lewis,

Dr. Gabriel Alves 00:19
and I am Dr. Gabriel Alves.

Steve Lewis 00:22
So far in our first season of Speaking of Mol Bio, we've touched on three out of four exciting application areas: CRISPR cell engineering, multiomics, and exosomes. Today, we visit our fourth, single-cell engineering, with Dr. Stan Roux.

Dr. Gabriel Alves 00:39
Stan is a professor of Molecular Biosciences at the University of Texas at Austin. He studies signal transduction in plant cells and how environmental stimuli can change plant growth behavior. His research has even been used by NASA to help prepare astronauts to deal with microgravity and plant growth in space. We hope you enjoy our conversation.

Dr. Stan Roux 01:07
My early career was in electron microscopy, actually, I did my master's degree in electron microscopy. And then when I went for my PhD, I continued electron microscopy. But then I went in the summer for a research experience at Woods Hole in Massachusetts. And there I did research in biochemistry, actually, protein chemistry. And I became so fascinated with that, that I decided for my PhD instead of doing electron microscopy, I would do biochemistry, and specifically, biochemistry on a light-regulated protein called phytochrome, which regulates plant growth and development. And all of my early career then, from that point on, was in trying to understand the signal transduction events that converted the activation of this protein called phytochrome into changes in growth and development of the plant. That continued until I came across an enzyme in plants that was in the nucleus and was regulated by calcium. And this was very interesting to me, because in my study of how phytochrome converted light signals into growth and development changes, I found that one of the early steps was an increase in cytosolic calcium, which activated calcium-dependent signal transduction events. And one of the main converters of the calcium signal is a protein called calmodulin. And so I was very interested to see whether in the nucleus, since phytochrome regulated gene expression, and phytochrome turned on calcium events, I was very interested in trying to find a protein in the nucleus that was regulated by phytochrome and by calcium. And, in fact, I found one. This enzyme we call apyrase, and I'll refer to it as apyrase from here on out, was present in the nucleus, was regulated by phytochrome, and was dependent on calcium and calmodulin for its activation. So I thought, Wow, this is the perfect connection that connects my interest in calcium, and phytochrome, and signal transduction—all of that and gene expression was, all that was there. Well, when we published our early work on the regulation of this enzyme by phytochrome and by calcium, that work was read by a group at Harvard that were studying this same enzyme, called NTPDase or apyrase. And they noted that it was curious that my study on the nuclear apyrase was the only one that focused on the nuclear function of apyrase, not on its function in regulating the concentration of extracellular ATP. And I said, “What?? Extracellular ATP? I never heard of that.” There is no extracellular ATP. ATP is in the cell. It's not outside the

cell. So I became interested in that phenomenon. And I found out there was a huge literature in the animal literature publications on extracellular ATP being a regulator of physiology in human and all animal cells. They knew the receptor for it, and they knew that apyrases were the key enzyme that limited the concentration of extracellular ATP. And I said, “Wow, this is really novel, I’ve never heard of extracellular ATP.” And I couldn't imagine that apyrase would play a role outside the cell. But then when I found out when I studied some of our own research a little bit more, I found out that this enzyme that I was studying, this apyrase enzyme that was in the nucleus, also could be present on the plasma membrane with its active site facing to the outside, where it could regulate the concentration of extracellular ATP. And in the process of doing all of these studies, we also did transgenic experiments in which we overexpressed apyrase to see that effect on growth. And we also knocked out apyrase to see what that effect on growth would be. And there was a perfect correlation: if you suppress apyrase expression, you suppress growth. In fact, if you knock out apyrase, pollen doesn't germinate, and so it's male-lethal. We really have been focused more recently on the role of apyrase, both on the plasma membrane and regulating extracellular ATP, and on its role in the nucleus, where it regulates gene expression. That's kind of a thumbnail sketch.

Dr. Gabriel Alves 06:22
Very impressive. I have a couple of questions. I'm curious—because the apyrase has two different locations, I would imagine it has also two different amino acid conformations, that probably comes from the same gene and has different mRNA splicing and you'll get the same protein but with a few modifications in its amino acids. Is that correct?

Dr. Stan Roux 06:44
You raise a very good point. So what we found was that there is a nuclear localization signal in apyrase, and apyrase actually will bind to DNA. So it does have a DNA-binding domain, and it has a nuclear localization signal that could make it go to the nucleus. It also has a signal peptide, which would allow it to go to the plasma membrane. So then the question is, which of these addresses—apyrase has two different addresses, one says go to the nucleus, and the other one says go to the plasma membrane—so which one is dominant is the question, and how does the environment, like the light environment, for example, or the stress environment, alter which of these two addresses dominates which way apyrase goes? And actually, which address is more important for what apyrase does when it's regulating growth? So yeah, we haven't solved that problem yet. We know it has two different addresses, and we know that both of them work. We can prove and show that apyrase does go to the ECM, does go to the wall. And we can show that it does go to the nucleus, it does bind to DNA. And in fact, it's a chromatin-associated protein when it goes to the nucleus. But we haven't solved yet which address dominates under which set of circumstances.

Steve Lewis 08:24
I'm curious learning a little bit about what were the lab techniques that have changed over the years as you've progressed in your career?

Dr. Stan Roux 08:35
Yeah, I think there's no doubt in my mind that transcriptomics played a major, major role in my progress and understanding how apyrase worked. We did a study in 2014, in which we did a transcriptomic comparison between the genes that are normally expressed in a wild-type plant, versus the genes that are expressed when you suppress apyrase expression. The transcriptomic analysis which documented which genes were upregulated, which genes were downregulated, as you change the expression of apyrase, became, was really a great insight, and helped me understand that one of the things that apyrase does is it regulates gene

expression, and that specific gene expression changes that it alters are really important for growth.

Steve Lewis 09:37
And just to expand on the tools available at the time, what did transcriptomics look like when you were utilizing it? That breakthrough just...

Dr. Stan Roux 09:49
When I started it was microarray. You remember way back in those days? You could purchase a microarray kit, and they would document which genes were upregulated, and which genes are downregulated. But now we use RNA-Seq. Wow, what tremendous advances in that. It's become so much less expensive; you can do replications to do your statistics; in other words, you can do three independent RNA-Seq experiments and do the—or four or five—without much money, and do really rigorous statistics to say yes, this gene is definitely upregulated, this gene is definitely downregulated, statistically solid.

Dr. Gabriel Alves 10:37
Yes. And still staying on the field of multiomics. How do you see the integration of the multiomics field with other technologies such as single-cell analysis, for example, in improving our understanding of plant physiology?

Dr. Stan Roux 10:54
Breakthroughs in single-cell transcriptomics and proteomics have been phenomenal. We have not yet applied that technology to our questions, but I can easily see where it would help us understand apyrase better. So let me give you a specific example. We know that when you suppress apyrase expression in *Arabidopsis*, the growth of the root is severely inhibited. And when you look at it with the electron microscope, or scanning electron microscope, what you find is that when you suppress apyrase, the zone in the root that's called the elongation zone, which is the zone between the meristematic zone, where the cells are dividing, the root apical meristem, and the differentiation zone, where the root hairs come out,—there's this intermediate zone called the elongation zone. So between mitosis and differentiation, there's elongation, there's the zone of elongation in the root. That zone, when you knock out apyrase or when you suppress apyrase, that zone, the elongation zone, disappears. So, if you wanted to understand what was going on, if you did single-cell transcriptomics, you could compare the transcriptomic signature of the different specialized cell types in the root tip. What we don't know is if you suppress apyrase, how does that signature change in these different individual cell types? Does it change only in the fact that the elongation zone disappears? Or does it change also in other expanding regions of the root, like the root hairs, which grow out in the differentiation zone? The beauty of single-cell transcriptomics and proteomics is that you can now look at a root tip, where all these changes are taking place when you suppress apyrase, and find out what are the transcription changes that are taking place in each individual of these multiple different cell types and document that, and that would just provide tremendous insight into what apyrase is doing in different microenvironments in different cellular environments.

Steve Lewis 13:38
We hope you're enjoying this episode of Speaking of Mol Bio. We wanted to take a quick moment to tell you about the Invitrogen School of Molecular Biology. It's a great educational hub for molecular biology, with rich and reliable technical content designed for new and experienced molecular biologists alike. Check it out today at thermofisher.com/ismb. That's thermofisher.com/ismb. And now back to our conversation.

For some of our listeners out there, it would be a missed opportunity if we didn't ask you about some of your work with space and NASA as well, if you don't mind sharing.

Dr. Stan Roux 14:24
That's really exciting. Let me tell you a little bit about that system. It turns out that in gravity, I found a single-cell system that can both sense and respond to gravity. And that single cell is a spore cell of a fern called *Ceratopteris*. And what happens in this fern spore is that beautifully, one of the great aspects of it, is that you can put it in water, and it will remain dormant for a month, not do anything, unless you activate the phytochrome in the spore. Leave it in the dark for a month, and then give it red light, and bingo, immediately the clock starts ticking, and you can look at the developmental sequence that happens between time zero and when the cell responds to gravity. And the first response you can see in these cells—and you can actually see it—the first response of these cells to gravity happens at about 24 hours after you give the red light, and that is the nucleus migrates from the center of the cell to the bottom of the cell. So you have these cells on a plate, a petri dish, they're growing on a petri dish, and you can actually see them with a microscope. And you can look through the spore coat and see the nucleus, which conveniently enough is centered and actually in the middle of the cell. What happens is that at 24 hours, the nucleus begins to migrate down. At about 48 hours, after it's migrated down, it divides into two different cells, one of which begins to grow out as a root-like structure called a rhizoid, and the other, the other cell, develops into what's going to be the top of the developing gametophyte. We were studying gravity—the gravity response happens within 24 hours. And so anything that's important for the gravity response is happening before the cell divides, while it is a single cell. So this is a single cell that senses and responds to gravity, and in a very predictable way. So for the space experiment, we wanted to know whether we could watch the nucleus migrate in microgravity, and whether it would migrate down, or whether it would migrate at random, like if there was no gravity, or whether it wouldn't migrate at all. In other words, if you take away gravity, maybe the nucleus wouldn't migrate at all. So we worked with some engineers, and they set up a camera loaded onto the shuttle. And then, whenever the astronauts had a few minutes, all they had to do was push a button, and what that would do is turn on the light in this box with a camera. And then the camera was focused on the nucleus in the cell. And we could watch what was going on with the nucleus in space in microgravity. And what we found was that the nucleus does migrate in space. But instead of migrating down, it migrates randomly. I mean one of the alternative hypotheses was that gravity was needed for it to migrate, to move. But gravity wasn't necessary for it to move. All gravity did was direct which way it moved, and on Earth it moved down, in space it moved randomly. And then what happens after that, developmentally, is that whichever direction the nucleus migrated in space, that was the direction that the rhizoid ended up pointing when it emerged from the spore. And that was really cool.

Dr. Gabriel Alves 18:44
What are some benefits that the results of your research bring to the upcoming future?

Dr. Stan Roux 18:50
One of the generalities about the gravity response is that there are ion fluxes, especially calcium fluxes, that play a key role in orienting the growth response of a plant to gravity. So if you were concerned about whether plants would be able to orient themselves on the moon, without enough gravity they would, they wouldn't grow up, and the roots wouldn't grow down, and you would have—I mean, if roots aren't growing down and the seed-bearing part of the plant isn't growing up, you're not going to get much of a harvest. You know, the first colonies that we're going to do in space will probably be on the moon. I think the idea is that we set up colonies on the moon, and we solve the lack-of-gravity problems on the moon, and then we think about

going to Mars and solving the problems there. But if we don't solve the problems about lack of gravity on the moon, we ain't going anywhere. That's where it starts. So you've got to solve the problem there. And so one of the possibilities that could be done, and actually this has been demonstrated—you can take a plant that is growing sideways, and normally, let's say when a plant is growing horizontally, the shoots would grow up and the roots would grow down—what's been demonstrated is that you can make the shoot grow down and the root grow up, depending upon what electrical field you put across the plant. What I'm thinking is that some of the insights that we gain from understanding the molecular basis of orientation of plants—you know, the role of calcium, the role of electric fields, the role of electric currents, in orienting the gravity response—those insights can be valuable in helping us grow plants without gravity.

Steve Lewis 21:02
Did you ever expect that you would be working with NASA at some point in your career?

Dr. Stan Roux 21:10
No … I don't know if I should make a confession, but I was totally focused on phytochrome for the first part of my career—you know, light regulation, growth, and development. And I remember I was at a plant physiology meeting one year, and a friend of mine was in the line behind me, we were waiting to go to some cafeteria or something. And he said, "Hey, Stan, there's money available for research, from NASA." And I said, "Yeah, but what does that have to do with me?" And he said, "Well, doesn't light affect gravity responses in certain plants?" I said, "I don't know, maybe it does." So I looked it up, and it turns out that phytochrome regulates gravity responses in certain roots of certain plants. And he said, "Stan, if you can confirm that, then you could go to NASA and get money to look at what you love, is gravity as light effects, you can look at what light does to the gravity response." And I said, "Okay, I'll try that." And I did. And I was funded for 25 years by NASA and had the great joy of doing a space experiment, it was really very exciting. Very nerve-wracking, but very exciting.

Steve Lewis 22:39
It's amazing. It's a very short list of scientists who have that, and it's just very, very impressive, and very interesting.

Dr. Gabriel Alves 22:49
Staying on the field of career path, and your surprising story with your NASA experience, what do you have to say for the young ones that are listening to us right now, in terms of career? What are the greatest tips that you can give to the young ones listening to us, and how to be successful and succeed in their careers?

Dr. Stan Roux 23:17
For me, the key was, are you passionately interested in what you're doing? I can't emphasize enough that if you're excited by the idea of discovery, and you have a particular issue that you would like to know more about, and to be the first one in the history of the world to get an insight about how this works, then pursue that. And I know this sounds idealistic, but I think what hampers some people in research is that they get into it almost mechanically—mechanically do the experiments, and I'll probably get some answers and, you know, but at least they'll pay the bill and keep the lights on and so forth. I don't think that's the way you really become a very good scientist. I think the key is your passion for discovery, and your passion to try to understand how things work. I started off, strangely enough, as a philosophy and classics major. That was my major in college. And then while I was in college, I began growing tomato plants in,

this was in Lafayette, Louisiana, which has got a lot of rain and wonderful soil. And I grew plants from seed that got to be 10 feet high, and harvested an average of 10 pounds of tomatoes per plant. And I said, how can you go from a seed to a plant that's 10 feet high? I had to get on a ladder to harvest the top tomatoes. And that curiosity made me switch from classics and philosophy major to biology major. When you see that phenomenon, it kind of blows your mind.

Steve Lewis 25:20
I think your next TED Talk could be find your tomato plant, find your ten-foot tomato plant.

Dr. Gabriel Alves 25:30
Is there anything else that we didn't touch upon in the episode today, Steve and I, that you would like to talk about?

Dr. Stan Roux 25:41
Maybe one other small thing, we haven't covered the area of metabolomics. And I'm on the board of a group that's publishing a series of encyclopedia volumes, called The Useful Wild Plants of Texas. And what it does is it documents the value of wild plants that grow naturally in the area of Texas and the Southwest and Mexico. One of the goals of this encyclopedia series is to convince people of the value of plants. And one of the values of plants is the phenomenal chemistry that plants produce that you can only document with metabolomics. There are companies that will go in and tell you what are the 10,000 major organic compounds that this particular plant will make for you. Each one of which, each one of these compounds, is a potential cure of cancer, or help feed the world, or whatever. And it's an untapped treasure, it's an absolutely untapped treasure, the incredible metabolomic power of plants, that they can produce so many different compounds, and that we've only looked at a small percentage of those in terms of their value for humanity. I'm hoping that as the metabolomic field gets stronger and stronger, and the people, the specialists in that area, are able to document more and more really crazy, amazing compounds that plants make, that people will begin to say, “Well, maybe we shouldn't pave over the world with concrete, maybe we ought to let a few plants grow here and there.” Because they are treasures, they are treasures for humanity that we need to learn more about.

Steve Lewis 28:08
That's great. And one of the areas I think we would definitely want to touch on before we wrap up is also some of the molecular applications that you foresee, maybe on the horizon as it relates to metabolomics, or what you might be getting into, even today.

Dr. Stan Roux 28:29
Up until now all of my focus on what apyrase is doing has been on transcriptomics and proteomics. But what the company that's funding me is finding out is that in field trials, the overexpression or the transgenic expression of apyrase in soybeans and other crops can increase the root system architecture and the yield and the nutrient uptake of plants. So I would like to know a little bit more about the chemistry that happens—the chemical changes that happen, that allow for, for example, soybeans to have more seeds. And one of the possibilities that would be related to metabolomics would be, we found out from talking to growers, that most of the soybeans that form in a regular plant abort, and so all you really need to do is to reduce the rate of abortion of soybeans to increase the yield by a significant amount. And if the apyrase is playing a role in, for example, reducing the abortion of soybean seeds, there's got to be some really interesting chemistry that's going on in that change. And so maybe focusing on what are the changes in the transport of nutrients and other compounds into the developing seed pod. One of the transport changes is transport of auxin, the growth hormone. But that's just one

metabolite. What about salicylic acid? What about cytokinin? What about other chemical compounds that play huge, huge roles in regulating growth and development? How are those metabolic changes altered when apyrase is affecting plant growth and development? And we haven't even touched that yet. I mean, that's a whole area that, you know, I'm 80 years old, I'm not sure I'm going to be able to solve that problem anytime, but, you know, maybe somebody would want to look at that.

Dr. Gabriel Alves 31:10
And that was Dr. Stan Roux, Professor of Molecular Biosciences at the University of Texas at Austin. If you'd like to hear even more of today's conversation, you can view the extended video version of this interview by visiting thermofisher.com/molbiopodcast. And if you enjoyed our interview with Stan, consider leaving a positive review of the show, wherever you're listening. It helps more people find and enjoy our work. This episode was produced by Matt Ferris, Sarah Briganti, and Matthew Stock.