Dr. Robin Stanley

Behind the Mask

November 9, 2020

Gabrielle Barr: Good afternoon. Today is November 19, 2020 and I have the pleasure of speaking with Dr Robin Stanley. Dr Stanley is an Earl Stadtman tenure track investigator and she's the head of the Nucleolar Integrity Group, within the National Institute of Environmental Health Sciences Transduction Laboratories (quite a mouthful). Thank you very much for being with us and speaking a little bit about your COVID-19 research.

Recently you received a grant to look at the structural and functional characterization of SARS-CoV-2 Nsp [non-structural protein] 15. In layman terms can you describe your research starting with what is the non-structural protein 15 and why is it so critical to understanding the replication of COVID-19?

Stanley: Sure. So, Nsp stands for non-structural protein and within all coronavirus genomes encode for a number of non-structural proteins. The non-structural means that this protein does not become part of the mature virus, but it's expressed in the host and all these proteins are really important for viral replication and pathogenesis. Nsp15, in particular, is something called an RNA processing factor, so Nsp15 plays a role in processing the viral RNA. Now exactly what Nsp15 is doing to the viral RNA is kind of still a mystery, but what we know is that if you don't have Nsp15, the virus actually becomes susceptible to host immune responses. So, somehow, Nsp15 is playing this role in masking the viral RNA so that it evades detection by host immune sensors.

Barr: Interesting. Did you have a role in discovering the RNA target of Nsp15?

Stanley: We didn't, but there have been two critical papers that have been published in the last year that have identified a couple of targets of Nsp15. One paper suggests that Nsp15 is really important for targeting a stretch of polyuridines that are found at the end of the negative strand of the viral RNA, and another paper suggests that Nsp15 can actually cleave many different places within the viral RNA, but both of these cleavage targets are thought to be really important for helping Nsp15 hide that viral RNA so that the host can't detect it.

Barr: Interesting. How was the cryo-EM (I may not be saying that right) structure of Nsp15 to atomic resolution? How did you all determine that in the conformational dynamics within the nucleus domain is identified?

Stanley: It's a cryogen, so cryogenic electron microscopy. Essentially what cryo-EM allows you to do is it allows you to take molecular snapshots of something. In this case we were taking molecular snapshots of Nsp15 and I'll say just in general terms cryo-EM has been incredibly powerful in helping us understand SARS-CoV-2, especially the spike protein.

There have been numerous cryo-EM structures determined by the spike protein which have shed a lot of information about how the spike is recognized by the ACE2 receptor and so on and so forth.

Our work on Nsp15, what we did, was we made the protein. We used a recombinant protein expression system in bacteria to produce the protein, we purified the protein, and then we took our protein and we vitrified it in a very thin layer of ice and put it on these really tiny grids that then get inserted into the electron microscope. And then essentially what you do is you just take a bunch of pictures and then, hopefully, if you have enough pictures of your particle in enough different orientations, you can generate a 3D molecular image of your protein. In the case of Nsp15, there were already a number of crystal structures of that protein, so we already knew what it looked like, but one thing that was intriguing and what we learned from cryo-EM is that the protein was a lot more dynamic than we thought. Dynamic means it kind of wiggles and wobbles around and so it's not actually fixed into position and we think that that might be important for its regulation.

Barr: That's very interesting. You're also looking at computational dynamics? Can you talk a little bit about that?

Stanley: Yeah. Our cryo-EM of the one domain was essentially fuzzy. If you think about it, if you're ever trying to take a picture of something, like a picture of little kids, and they're moving around, they're going to be fuzzy because they're not fixed into position. It's the same thing in cryo-EM. we noticed that there was a domain that was fuzzy because it's not fixed and it's moving around, so to go along with that, we did some computational studies called molecular dynamics and the molecular dynamics really support what we see by cryo-EM which suggests that there is movement going on in this protein.

Barr: How do you do your molecular dynamics studies? What kind of equipment do you need? How do you go about doing it? Your methodology?

Stanley: Molecular dynamics is all computational based, and I am not an expert in that. We refer to a collaborator. We have a really great person on site here called Lalith Perera, and he uses a number of different types of software. I think the one he uses most often is a program called Amber. This uses an insane amount of computational power but by doing these simulations we can learn a lot of different things about the protein.

Barr: Very interesting. So, through this research, you said, in addition, you'd like to establish how known inhibitors block Nsp15 activity and how a variety of factors regulate Nsp15 functions. What are some of these other functions or other factors?

Stanley: In Nsp15, the 15 means it's the 15th non-structural protein. There are, I think, actually 16 of them in total, and many of these Nsp proteins are involved in RNA processing. There is some evidence out there from other coronaviruses that actually Nsp15 may not be working alone and that it may actually be working in concert with some of these other Nsp proteins, in particular I think it's Nsp7 and Nsp8. We are really interested to understand if we can actually form a larger complex with these other viral proteins and see how they kind of coordinate their activities together.

Barr: Is that the next step of your research to look at these other non-structural proteins?

Stanley: It's one of many. We have several, I think. I think our work thus far is kind of giving just a nice general understanding of how the protein works and now we're ready to answer some of these detailed questions about how other factors influence it and also some detailed questions about how it recognizes and processes RNA.

Barr: Can you speak more about the latter? The part that you said how it recognizes and processes RNA?

Stanley: Sure, Nsp15 is a nuclease and nucleases are like a pair of molecular scissors, so they literally come along and chop RNA in half essentially. Some nucleases are specific and they are searching for a certain sequence and that's what they recognize and cut, and other nucleuses are non-specific—they will just cut anything. We know that Nsp15 is very specific for one of the four RNA bases, for uridine, so it somehow specifically recognizes uridine and it cuts right after uridine. We would really like to understand why that is. From one of the structures we have, we actually have a uridine bound in the active site and that is allowing us to begin to understand what features of Nsp15 are involved in recognizing that specific base.

Barr: What is the ultimate goal of your study?

Stanley: I think the ultimate goal is that, hopefully, we're going to provide some sort of fundamental mechanisms and understanding how Nsp15 works and that by providing this information that's going to help us understand better how we might be able to inhibit this protein right, because if you inhibit the protein, that might be a great therapeutic target for coronavirus.

Barr: Yeah. Definitely. We hope so. What have been some challenges that you have experienced to date with your research? And also, on the optimistic side, were there any surprises that you have experienced?

Stanley: I can think of two big challenges. The first was doing science during a pandemic. At the beginning when we started working on this project, we were very limited as to who we could have on campus and in the lab and I think we were actually only allowed one person at a time and so we had to come up with this crazy schedule of, okay this person is going to come in these set hours and they're going to hand it off to this person and so on and so forth. That was a challenge, just took a lot of coordination. The other big challenge was that I thought it was going to be very easy to make this protein and I was totally wrong. We make the protein in a recombinant system and Nsp15…

Barr: What is a recombinant system? Sorry, that just shows my ignorance, but if you could explain that to those who [do not have a scientific background]?

Stanley: We sort of hijack bacteria because they're really inexpensive to grow and maintain, so we just sort of hijack bacteria and sort of get the bacteria to make the protein we want. We use a bacterial system and what we found is that actually when we made the wild-type protein the bacteria were totally unhappy and that makes sense because, actually, what Nsp15 is probably doing is cutting the bacterial RNA and it probably really doesn't like that. It took a lot of work to figure out how we could actually make the protein because the bacteria didn't like it but it took us about a month and we finally figured out a system that works.

Barr: That's great.

Stanley: And I would say a positive that has come out of this is, typically, in my lab, people have their own projects and it is very kind of individual, and we took on this project as a group project and so, everyone in my lab has worked on this project. I think that's been a huge positive because it's taught me, really, how great team science can be, and we really had so many wonderful group discussions where we are all working together on a common goal so that that was a huge positive.

Barr: That's very nice. One of the questions [is]: How has your educational and professional background prepared you to answer questions related to COVID-19, and have you worked on similar questions with other diseases?

Stanley: I am not a virologist at all. In fact, my knowledge of virology is very small although I have learned a lot in the last six months. My background is in RNA processing and so historically, in my lab, we

studied RNA processing enzymes and, you know, when the lab first got shut down, I started to read about SARS-CoV-2 and all the viral proteins and I realized that actually Nsp15 shares a lot of characteristics with some other nucleases that we were already studying in my lab. I thought that might be a really natural target for us to take what we already know about these other nucleases and apply that to the study of Nsp15.

Barr: Very interesting. We are going to transition now from your research more to your role as a scientist. What has been your role in this study?

Stanley: I have worn a couple different hats: one was just to get [it done]. I came up with the idea, and I put it forth to my lab and I said, you know, we have to all be working on this as a voluntary project. First step was to get my lab on board to volunteer to work on this project during the pandemic and 100% everyone was gung-ho. They wanted to do it. I have really let my lab do most of the hands-on lab work. I've done a little bit by myself, but most of the work has been done by postdocs and post bacs in the lab, and I have just been working with helping everybody coordinate what's going on. We've had to do things differently than we do in normal times, and so we had to come up with a virtual lab notebook that everyone could sort of see and follow along. We had to come up with a crazy excel spreadsheet for scheduling.

Barr: How did you do your virtual lab notebook? Is there a certain program that you use?

Stanley: We just used like an online password-protected website that we could all get access to and that was really important because, again, we were passing things off from one person to the next and we needed to be able to follow along and see what everybody was doing, and I remember sometimes I would be sitting at home and I would have the notebook open and I could just say, "Look what just happened in the lab." It's been a learning experience as to how we do things this way.

Barr: Right. Do you feel like you've gotten better at keeping track of what everybody is doing more than sort of the old way of doing things?

Stanley: I have definitely been following along, I guess, more closely and that's just because, again, doing things remotely versus in person you just have to kind of adapt.

Barr: Right. Yes. Have you been on campus, at home, or a combination—and just so people know that you are not on the Bethesda campus, you are in North Carolina, so you've had a little bit of a different experience than many NIH people.

Stanley: Yeah, absolutely. NIEHS [is] down in the Research Triangle Park area. I have been a mix of all those. From March until the middle of July we were basically shut down except for COVID-19 research. I think that time I came on campus four or five times and then since July we've been allowed back on campus at a reduced capacity. We are all wearing our masks, having temperature checks at the gate, and all that, and we have a wonderful asymptomatic testing program. Now I'd say I am on campus more often than not, but again we're all doing our best to take all the proper precautions to stay six feet apart and all that good stuff.

Barr: Definitely. What has it been like to manage a team during this time? It must have some added difficulties.

Stanley: It's definitely been a learning experience. Again, because I am so used to being in the lab interacting with people face to face on a daily basis, I remember the first two weeks of the shutdown we tested out all the different video conferencing platforms. We tried Zoom and WebEx, and Microsoft Teams, and I was like, okay, we're just going to find the one that we like the best, just so that, you know, we have a system and it works. So we ended up all liking Zoom the best. So I feel like I have become a Zoom pro in the last few months just because that's been our mechanism of communication.

Barr: Yeah. Definitely. Have there been any personal opportunities for you at this time as well as any personal challenges due to the pandemic?

Stanley: I think that one of the reasons I was motivated to work on a SARS-CoV-2 project was because I saw it as a learning opportunity for my lab and I thought this would be a great time for us to all really push our priority and skills. And, actually, it worked out really well because we were no longer competing for a microscope with any other project. It was like we had all this access and so we were able to collect so much data in such a short period of time and that doesn't normally happen. That was a great benefit to everyone in the lab.

I think a challenging thing is, you know, I am a mom and I have an almost eight-year-old daughter and so of course, in March her school was closed and so suddenly I lost all childcare and I had to become a first-grade teacher. That was definitely a challenge that I never thought I was going to have, to be like, okay, let's do math now, let's do reading, but we've adapted and actually my daughter is now back at school and very happy to be back at school. So, thankfully, that's gotten a little bit easier recently.

Barr: That's good. That's definitely good. Just wondering, you know you're doing your SARS-CoV-2 research, but is your lab continuing with some of its research that you were doing prior to the pandemic?

Stanley: We are. In July, mid-July, we were able to get our other projects back up and running so yeah, we are slowly ramping back up on our other projects as well.

Barr: How are you juggling doing so many things out at once?

Stanley: Well, we initially started the SARS-Cov-2 project and it was a total everyone in the lab kind of participating in it. Now I have put one postdoc on the lead, so she has taken the lead on that project and that way people have more time to work on their individual projects as well.

Barr: That's really good to hear. Well, this is a fun question: Where have you turned for solace and inspiration during the pandemic?

Stanley: One thing I've been doing is I'm a very avid runner so I, typically, would run probably five days a week but I've never done a running streak. I decided that when we got sent home for quarantine, I was going to run every single day because that was just, I knew I could get outside and I could have some time to think and since, I think, March 15th, I have run every single day.

Barr: That's quite an accomplishment.

Stanley: Still going. We'll see how long I make it. I was joking with my husband the other day, I need to pick a target for like an end point and so I decided maybe the day I get the vaccine will be a good day to end my running streak.

Barr: That's definitely very noteworthy. Well, is there anything else you would like to share either as an NIH scientist or as just a person who is experiencing the pandemic?

Stanley: I'll just say this has been a very challenging time, but I've really been trying to do my best to keep the morale up with my lab and keep us safe but also keep us growing and learning as a scientist.

Barr: Well, thank you very much for giving us your perspective. I wish you the best with your research and I hope that you and your family continue to stay safe.

Stanley: Thank you