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PEOPLE & IDEAS

Yogesh Kulathu: Decoding complex intracellular messages

Lucia Morgado-Palacin

Yogesh Kulathu studies signaling mechanisms with a focus on ubiquitin and other post-translational modifications such as UFMylation.

"I would never have guessed that I could get my dream job while dropping off a bunch of samples," says Yogesh Kulathu. He was studying how ubiquitin-binding domains and the ovarian tumor (OTU) family of deubiquitinases can recognize different chains of polyubiquitin, which are just distinguishable by their linkage type. One afternoon, while heading to the reception at the MRC Laboratory of Molecular Biology to send his tubes for sequencing, Yogesh ran into Doreen Cantrell, who was waiting for her taxi to the airport. Yogesh knew Doreen from conferences and started chit-chatting with her. The conversation ended with an invitation to Yogesh to give a seminar in Dundee. Little did Yogesh know that Dario Alessi, who was going to be appointed the director of the MRC Protein Phosphorylation & Ubiquitylation Unit (PPU) in Dundee, UK, was in the audience and saw potential in Yogesh's research, so he interviewed him. A year later, in 2013, Yogesh opened his own lab at the MRC PPU. His team studies signaling mechanisms with a focus on ubiquitin and other post-translational modifications such as UFMylation. We contacted Yogesh to learn about his scientific journey and new projects.

Yogesh, did your love for signaling mechanisms start with serendipity or genuine interest?

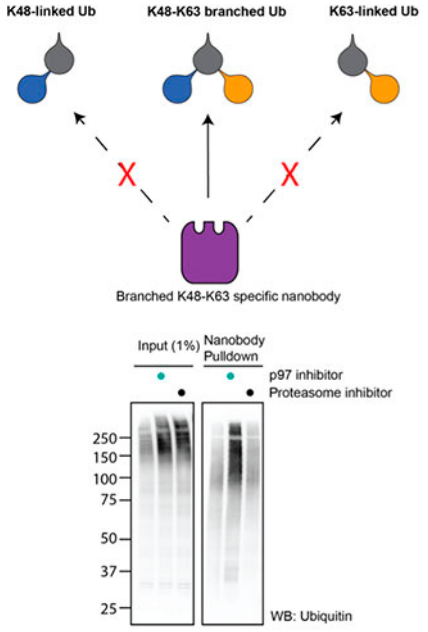
It was genuine interest, of course! Although the path that brought me there was relatively serendipitous. I'm a chemical engineer by training. I studied chemical engineering at the Birla Institute of Technology and Science (BITS) in Pilani, India. One day, by chance, I attended a lecture on recombinant DNA technology and heard about cloning and transgenesis. That was the first insight I got into biotechnology, and it made me want to learn more. So I signed up for an elective course on immunology, and even did a short summer stay in a lab. I fell in love with the immune system. I felt like I had found my calling in life, so I pursued a Master's in Biotechnology, also at BITS, and then I moved to Michael Reth's lab at the Max Planck Institute for Immunology in Freiburg, Germany, to do my PhD. Michael liked my chemical engineering background. He wanted me to reconstitute human B cell antigen receptor (BCR) signaling in evolutionary distant *Drosophila* S2 cells to understand how this immune signaling was initiated and amplified. Early events in BCR signaling involve phosphorylation and domains within signaling proteins that bind to these phosphorylated residues. I was amazed by how this cascade of information transfer occurs in a crowded cellular environment. If you think about it, the mechanistic precision that exists to transmit the right message with accuracy is just wow! This was what first got me interested in working on signaling mechanisms.

Yogesh Kulathu. Photo courtesy of Yogesh Kulathu.

Then, you kept working on signaling mechanisms for your postdoc, in particular ubiquitylation...

Yes, I went to the MRC Laboratory of Molecular Biology, Cambridge to do my postdoc in David Komander's lab on the structural basis driving linkage specificity in ubiquitin binding domains and in the OTU family of deubiquitinases. Compared to phosphorylation, where a phosphate is attached to proteins, during ubiquitylation, a 76 amino acid protein is attached to proteins which can in addition form polymers (polyubiquitin). I found it remarkable that proteins can tell the difference between these polymers which are assembled from the same building block, ubiquitin, but the only difference being the way they are

lmorgado@rockefeller.edu.



Nanobodies that bind specifically to branched ubiquitin chains and not to unbranched chains reveal that K48-K63 branched chains accumulate following inhibition of p97/VCP. Image courtesy of Matthew McFarland.

linked together. This, in turn, determines the fate of the ubiquitylated protein, for instance, if it's targeted for degradation.

Wow, you have touched multiple fields over these years: chemical engineering, immunology, and structural biology. What would you emphasize is the best of having had a such multidisciplinary background?

I think having been exposed to such different fields has made me resilient and unafraid to pursue new areas of research in my lab. It means I can tackle signaling questions from different angles using structural biology to understand the molecular mechanisms, biochemistry, and cell biology to investigate cellular roles and in vivo mouse models to investigate at the organism level the role of ubiquitin and UBL signaling, particularly in lymphocyte function.

What are you currently working on?

One of the questions we started working on 5 yr ago was to understand how the poorly studied ubiquitin-like modifier UFM1 controls protein homeostasis at the ER. Compared to the ubiquitin system, where there are hundreds of enzymes to attach ubiquitin onto proteins and thousands of ubiquitylated sites, UFMylation is parsimonious with only a handful of enzymes and seemingly just one major target in cells. This target is a single lysine residue on RPL26/uL24, a 60S ribosomal subunit at the ER membrane. We set up an in vitro reconstitution to establish the minimal requirements and biochemical principles of UFMylation (1). This led us to discover that an unusual E3 ligase complex made up of three proteins catalyzes ribosome UFMylation. We are now working to determine cryo-EM structures of this ligase complex in the process of transferring UFM1 onto ribosomes. These structures will reveal how the ligase specifically recognizes and modifies ribosomes. Our unpublished work using mouse models to knock out UFM1 expression in lymphocytes suggests that UFMylation is important to support membrane and secretory protein biogenesis. The big questions now are to understand what specific signals trigger ribosome UFMylation, how exactly attachment of a small 83 aa protein to a ribosomal subunit is required for protein biogenesis at the ER, and why the loss of UFMylation results in ER stress. We are taking a multidisciplinary approach to answer these questions, which I think will reveal new concepts of how protein quality control and homeostasis are achieved at the ER.

Another major area of research in my lab is to understand how branched ubiquitin chains are formed. These are complex modifications formed when two ubiquitin molecules are attached to a single ubiquitin via different attachment points. Around 10–20% of all ubiquitin polymers in unperturbed cells exist as branched architectures, and it is thought that their abundance goes up under particular conditions. However, we lack the tools and methods to study these chains. We've been innovating new approaches to be able to investigate the functions of these modifications. Recently, we identified that specific "reader" modules exist in cells that can specifically bind to branched ubiquitin chains (2). This answers a long-standing question of whether specific reader modules even exist in cells to decode such complex architectures. We also used protein engineering approaches to develop nanobodies that bind to branched chains with very high affinity and specificity, which helped us identify that K48-K63 branched chains are a signal for p97/VCP mediated processes. We are now using these newly developed tools as sensors to monitor branched chain formation in living cells. Our working hypothesis is that branched chains of specific types are formed in response to cellular stress, and these branched chains function as specialized priority signals to trigger stress response pathways.

Which has been the most exciting development in your field in recent years?

Without a doubt, it has been the discoveries that ubiquitin can also be attached to proteins, lipids, and sugars through ester linkages, in addition to lysine residues. Some of these discoveries were made right here in Dundee! Another development that has reshaped our thinking of how ubiquitin signaling maintains cellular homeostasis is the identification that pathogens interfere with the ubiquitin system to hijack host defenses.

What has been the biggest accomplishment in your career so far?

I've been most excited about discovering two new families of deubiquitinases (DUBs) and identifying functions in previously unannotated proteins (3, 4). Getting to name them was fun, too.

What is the approach that you bring to your lab?

My PhD supervisor Michael Reth had a big effect on how I think and approach scientific problems. He taught me to "learn by playing," and I strive to bring this approach to work, where I give people in my lab the same freedom to be curious and explore. Making research fun is my top priority. My team has also continually had a big influence in setting our lab's culture, and we have a diverse environment with researchers from different parts of the world, making the lab a welcoming and inclusive environment where we all enjoy doing science.

So, your first cohort of lab members really set the mood. What is your advice for new PIs when recruiting their lab members?

One thing I did right when I became PI was being patient and not rushing to fill positions in my fledgling lab. The patience paid off and 6 mo after I started, my team of

The code breakers. Photo courtesy of Matthew Elliott.

amazing, motivated researchers was in place and set the foundations for all we have managed to do. I benefited hugely from attending EMBO's lab management courses, and I was also very lucky to have had Helen Walden as a mentor. She was in the office next to mine and was always there to help and provide support and advice when needed.

Do you have any wish for a change in academia?

My wish is for more blue skies research. We need to make it easier to get funding for ideas. Currently, there's a huge emphasis on funding medically relevant research or applied research, and I feel we are missing out on discovery.

On a final note, what has been your biggest accomplishment outside of the lab, and what are your hobbies?

To be the father of two wonderful children and the husband of an amazing scientist. Also, I've recently started learning Carnatic music, a form of classical music from South India, where I'm from. My mother-in-law insisted that it is never too late to start, and I have been singing Carnatic music for the past 2 yr!

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