© 2018. Published by The Company of Biologists Ltd | Journal of Cell Science (2018) 131, jcs223636. doi:10.1242/jcs.223636

FIRST PERSON

First person – Varsha Singh

First Person is a series of interviews with the first authors of a selection of papers published in Journal of Cell Science, helping early-career researchers promote themselves alongside their papers. Varsha Singh is the first author on 'Cholera toxin inhibits SNX27-retromer-mediated delivery of cargo proteins to the plasma membrane', published in Journal of Cell Science. Varsha is an Instructor of Medicine in the lab of Mark Donowitz at Johns Hopkins University, Baltimore, USA, investigating the molecular regulation of the ion transport process in the human intestine, with a particular emphasis on membrane trafficking.

How would you explain the main findings of your paper in lay terms?

Diarrhea is a symptom of disease in which the process of absorption by the intestine is inhibited, resulting in watery stool, with an associated loss of water and electrolytes. This process is regulated by a protein called NHE3, located at the outer lining of the intestine, which is made up of cells called intestinal epithelial cells. A cell is like a machine, composed of different proteins performing specific functions. These proteins are dynamic, moving around inside the cell, and that is how their function is regulated. Inside the cell, there are assigned complexes that transport proteins to their specific location. These complexes are like a shuttle or train that picks up passengers (cargo proteins) and carries them to a specific location in a process called trafficking. Our study showed that in cholera toxin-induced diarrhea there is a defect in the trafficking of NHE3. This is because cholera toxin inactivates and destabilizes the complex that carries NHE3 to outer membrane of the intestine. Our study has shown that this defect in the trafficking of NHE3 can be reversed by using a small molecule (termed a pharmacological chaperone) to stabilize the protein complex that carries NHE3 to the outer membrane of the intestine, resulting in increased absorption by intestinal epithelial cells.

Were there any specific challenges associated with this project? If so, how did you overcome them?

The most challenging part was to prove that retromer stabilizer can increase the absorption of fluid by human intestinal epithelial cells. Fortunately, Hans Clever's lab had recently developed a 3D epithelial organoids model to measure fluid secretion by epithelial cells. This assay, called the forskolin-induced swelling assay, is a simple and relatively rapid assay for measuring CFTR-mediated fluid secretion and responses to CFTR modulators *in vitro*. The assay provides a cost-effective approach for the identification of a drug response in patients. By using an NHE3-specific inhibitor, we modified the assay to test the response of retromer stabilizer on NHE3-mediated fluid absorption by intestinal epithelial cells. The effect of the retromer stabilizer is encouraging, with potential to be developed as a drug for cholera diarrhea.

Varsha Singh's contact details: Division of Gastroenterology and Hepatology, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA.
E-mail: vsingh11@jhmi.edu

Varsha Singh

When doing the research, did you have a particular result or 'eureka' moment that has stuck with you?

Retromer SNX27 regulates trafficking of numerous cargo proteins, yet we don't know how this multiprotein complex is regulated. We thought about this for quite some time and hypothesized that there must be a switch to control cargo binding to SNX27. Since phosphorylation is the most important modification in physiology, I had a hypothesis that phosphorylation of a conserved serine residue adjacent to the PDZ motif regulates cargo binding and trafficking to the plasma membrane. I had done a lot of literature searches and read a great deal about retromer-mediated research, but when I started the mass spectrometry analysis to identify phosphorylation, I really had no idea whether there would be any differences in phosphorylation between control and cholera toxin-treated samples. We were so happy to find that there was a huge increase in phosphorylation of the serine residue in response to cholera toxin treatment. Moreover, when I first observed that a phosphorylation-mimicking mutant of SNX27 exhibited less binding with cargo proteins, that was my 'eureka' moment.

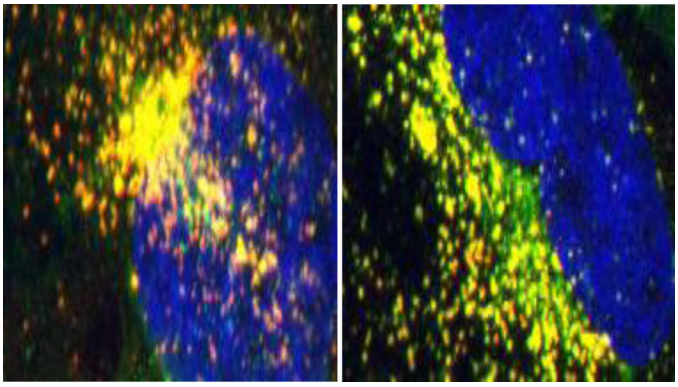
Why did you choose Journal of Cell Science for your paper?

We chose Journal of Cell Science because it has a strong reputation in the field of cell biology and we thought it would be suitable to share our findings with the field.

Have you had any significant mentors who have helped you beyond supervision in the lab?

My supervisor, Mark Donowitz. He has always trusted me enough to give me lots of space and opportunity to explore and work

Journal of Cell Science (2018) 131, jcs223636. doi:10.1242/jcs.223636



SNX27-knockdown (left) and SNX27-knockdown (right) cells expressing an SNX27-PDZ-S49D phosphomimetic mutant, showing co-localization of Glut-1, a ligand associated with the SNX27-retromer pathway (green), and the lysosomal marker LAMP1 (red).

independently, while at the same time knowing when to interrupt and give advice.

What motivated you to pursue a career in science, and what have been the most interesting moments on the path that led you to where you are now?

Curiosity. I have always been fascinated by human physiology and since childhood I have wanted to know how cells perform their functions, how they are regulated and what happens in disease. So here I am, doing what I have always wanted to do. Human physiology is still a big mystery and we have a lot of questions to answer. If my research can make even a small difference to help develop a treatment for even one disease, it will all be worthwhile.

Who are your role models in science? Why?

I have a lot of role models from past to present. I really admire the scientists of the past who made some fundamental discoveries with limited resources. In general, I respect scientists who are critical thinkers, show passion for their work and are never afraid to think outside the box. I admire my current supervisor, Mark Donowitz, who is so dedicated to science. I hope to acquire some of his scientific passion. I also respect scientists who are humble when teaching, and, most importantly, are open to collaborations.

> **“Read a lot of literature and give your brain some time to critically process the information before jumping into doing meaningless experiments.”**

What's the most important piece of advice you would give PhD students?

Doing a PhD is often a young researcher's first big exposure to science; enjoy it, don't be too hard on yourself. Read a lot of literature and give your brain some time to critically process the information before jumping into doing meaningless experiments. 'PhD' stands for 'Doctor of Philosophy', meaning you have to doctor your thinking and reasoning skills. So give yourself time to develop your critical thinking skills rather than trying to simply earn a degree. A good work–life balance is very important too. Take time away from the lab, enjoy being with friends and family as this promotes ideas and creativity.

What's next for you?

I am applying for independent funding and am looking forward to new challenges. I know it's going to be very challenging but I can't wait to establish my own research group.

Tell us something interesting about yourself that wouldn't be on your CV

When not in the lab and not writing at home, I love spending time with my family and friends. I also enjoy sketching, it's very relaxing.

Reference

Singh, V., Yang, J., Yin, J., Cole, R., Tse, M., Berman, D. E., Small, S. A., Petsko, G. and Donowitz, M. (2018). Cholera toxin inhibits SNX27-retromer-mediated delivery of cargo proteins to the plasma membrane. *J. Cell Sci.* **131**, jcs218610.