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FIRST PERSON

First person – Sudeshna Nag

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. Sudeshna Nag is the first author on 'Rab4A organizes endosomal domains for sorting cargo to lysosome-related organelles', published in Journal of Cell Science. Sudeshna is a PhD student in the lab of Professor Subba Rao Gangi Setty at the Indian Institute of Science, Bangalore, India, investigating the mechanisms of protein sorting and trafficking.

Sudeshna Nag

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

Proteins inside the cell are correctly trafficked to their functional places with the help of a complex machinery. This trafficking is similar to the traffic rules that we follow in our daily life. Any defect in this process affects timely delivery of proteins or induces their mis-delivery, which eventually shows up as mild symptoms or disease. Secretory and/or transmembrane proteins use vesicles as the transport carriers to reach their target locations. However, sorting of cargo into transport vesicles, especially within the endosomal network, is poorly understood. In our study, we have identified a novel mechanism of cargo segregation mediated by a small GTPase Rab4A at sorting endosomes. During this process, the active form of Rab4A localizes to endosomal membranes and interacts with the cargo sorting adaptor AP-3, which binds to an amino acid sequence at cargo tails. Furthermore, Rab4A associates with different combinations of Rab effectors, as well as with a kinesin motor, to create cargo-sorting domains on sorting endosomes. This transient segregation by Rab4A avoids the mixing of cargoes destined for different routes. Perturbation or dysfunction of these components results in organelle malfunction and/or biogenesis defects, such as melanosomes (in melanocytes) and unwanted protein secretion. Thus, cells utilize a basic mechanism of cargo segregation for organelles and cell function.

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

We initially thought that elucidating Rab4A function in cargo sorting processes would be straightforward like that of other Rab GTPases. But demonstrating its critical function proved challenging as Rab4A showed multiple interactions with a variety of proteins. We observed a large number of cellular cargo transport processes that were affected by Rab4A depletion, but it was difficult to probe their mislocalization routes. We predicted that a little of the cargo might be misrouted through intra-luminal vesicles called exosomes. To prove this hypothesis, we carried out several standardization experiments, which was the biggest task during the project. Moreover, visualization of cargo transport defects and their associated organelle defects was easy in melanocytes, but difficult in non-melanocytic cells due to the lack of reagents. It is still a challenge to show defective cargo sorting routes at sorting endosomes in cell types such as fibroblasts. On the other hand, studying *in vivo* protein-protein interaction in melanocytes is very difficult due to low DNA transfection efficiency. Nonetheless, we overcame the majority of these technical challenges and succeeded in identifying a novel cargo segregation mechanism on sorting endosomes.

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

Rab4A shares multiple effector proteins with another early endosomal Rab GTPase, Rab5. These effectors, namely Rabenosyn-5, Rabaptin-5 and Rabip4, all are important for the maturation of endosomes and receptor recycling, but not for cargo segregation. The 'eureka' moment came when we found that one of these effectors, Rabenosyn-5, forms a complex with Rab4A-AP3-KIF3. But, assigning the cargo segregation function to this complex was the biggest task, which we achieved by performing several biochemical, cell biological and live imaging experiments. Overall, these results helped us to demonstrate the function of Rab4A in possibly organizing cargo into multiple endosomal domains.

"The 'eureka' moment came when we found that […] Rabenosyn-5 forms a complex with Rab4A-AP3-KIF3."

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

Journal of Cell Science publishes a unique and broad range of cellular mechanisms relevant to basic cell biology. I have read several articles in this journal that are directly related to my work

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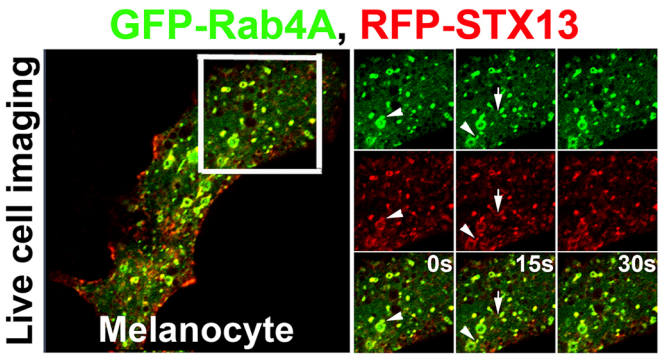

Rab4A localizes to sorting endosomes and partly to early/recycling endosomes. Super-resolution live cell imaging of GFP-Rab4A and RFP-STX13 in wild-type melanocytes. Arrows and arrowheads point to the localization of proteins at recycling endosomes and vesicles arising from vacuolar/sorting endosomes, respectively. The insets are a magnified view of the white boxed area at indicated time points.

and the journal also has a great reputation. Thus, we considered the submission of our article to this journal, so our work will reach a broad scientific community.

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

My first mentor is Professor Subba Rao Gangi Setty. I would like to thank him for his continuous guidance and support, which helped me in continuing a scientific career. Some aspects of this project were challenging and it was difficult to interpret the results. However, his constant scientific enthusiasm and support helped me overcome my hard times during the study. Moreover, scientific discussions with him enriched my knowledge and helped my scientific quest.

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?

I always try to seek answers to understand existing biological problems. The excitement in identifying clues to answer these problems maintains my love for science. I think each cellular phenotype or result is a kind of 'prism': the more we analyze, the more we will gain a different perspective. My father has type-2 diabetes and I started reading about this disease as a teenager. This made me curious about several cellular pathways, such as protein folding, protein trafficking and autophagy.

Who are your role models in science? Why?

As a female scientist, I believe in maintaining a balance between career and family life. I am inspired by the many scientists who have achieved success in their scientific careers without sacrificing a family life. One of my role models is Professor Graca Raposo at Institute Curie, Paris. The way she explains the most difficult techniques and hardest experiments in a simple way, is something I and many other students admire.

> **"As a female scientist, I believe in maintaining a balance between career and family life."**

What's next for you?

I would love to be actively associated with science; but I have not decided how yet! Right now, both academia and industry are doing exciting research. In the future, I want to be part of some interesting project, where I can discover or develop a unique protein and/or molecule for therapeutic application.

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

I love to write short stories, which I share on social networks or in magazines. My other stress busters are cooking and painting.

Reference

Nag, S., Rani, S., Mahanty, S., Bissig, C., Arora, P., Azevedo, C., Saiardi, A., van der Sluijs, P., Delevoye, C., van Niel, G. et al. (2018). Rab4A organizes endosomal domains for sorting cargo to lysosome-related organelles. *J. Cell Sci.* **131**, jcs216226.