© 2018. Published by The Company of Biologists Ltd | Journal of Cell Science (2018) 131, jcs216820. doi:10.1242/jcs.216820

FIRST PERSON

First person – Anuttoma Ray

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. Anuttoma Ray is the first author on 'Dileucine-like motifs in the C-terminal tail of connexin32 control its endocytosis and assembly into gap junctions', published in Journal of Cell Science. Anuttoma is a PhD student in the lab of Dr Parmender P. Mehta at the University of Nebraska Medical Center, Omaha, USA, investigating the molecular mechanisms that regulate assembly of connexins into gap junctions.

Anuttoma Ray

How would you explain the main findings of your paper to non-scientific family and friends?

In the same way that communication between individuals is necessary in our daily lives and to maintain social harmony, the cells in our body also need to communicate with each other. Communication between cells involves the exchange of chemical and electrical signals, and is necessary for cells to function normally. Gap junctions are like conduits or pipes connecting the internal environment of one cell with its neighbouring cell, and are formed by an assembly of proteins called connexins at the cell surface. In some genetic diseases and cancers, problems with the assembly of gap junctions have been observed. As a potential means to prevent such diseases, it is necessary to understand the basic mechanisms that control the assembly of connexins into gap junctions and their disassembly from the cell surface. In this study, we focus on a particular connexin, connexin32, and show how certain stretches of amino acids (motifs) within the protein are responsible for its internalization and degradation from the cell surface. Future research targeting these motifs will thus be an important part of investigations into diseases where gap junction assembly is impaired.

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

We found it difficult to define the secretory itinerary of wild-type connexin32 and its dileucine mutant. We performed immunofluorescence using several different cell secretory markers, but failed to achieve discernible colocalization with any of these. Eventually, we used pharmacological inhibitors brefeldin and monensin, and after these treatments, we were able to achieve significant colocalisation with markers that allowed us to conclude that connexin32 and its dileucine mutant traffic normally along the secretory pathway.

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

One of the dileucine mutants of connexin32 had a very different phenotype to what was expected. We found that instead of forming gap junctions at the cell surface, the mutant protein was scattered throughout the cytoplasm. We struggled to reason out the cause of this phenotype, but as part of our investigations chose to do an experiment where we blocked endocytosis in our cells. We were very surprised to find that the treatment caused an almost complete rescue of this defective phenotype. The change in phenotype was robustly confirmed with immunofluorescence and biotinylation experiments where the mutant was expressed on the cell surface, suggesting that endocytosis before assembly was the reason behind its defective phenotype. This was a kind of 'eureka' moment for us, and it gave us confidence in our findings and paved the way for future experiments.

Have you had any significant mentors, and how have they helped you?

I would like to mention my PhD mentor, Dr Parmender P. Mehta. He has been an instrumental person in shaping my scientific career. He has honed my scientific thinking and writing skills, and has always motivated me to stay positive.

> **"You must prepare yourself to face failures, yet have the will and perseverance to get up and try again."**

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

A PhD is a long commitment of 5–6 years. You must prepare yourself to face failures, yet have the will and perseverance to get up and try again. Some things will take a really long time, but you should have patience and faith and not get demoralized. Also, you should do your experiments and interpret your results in a non-biased way, and should be open to any possibilities. It is important to have proper controls while designing experiments, which will

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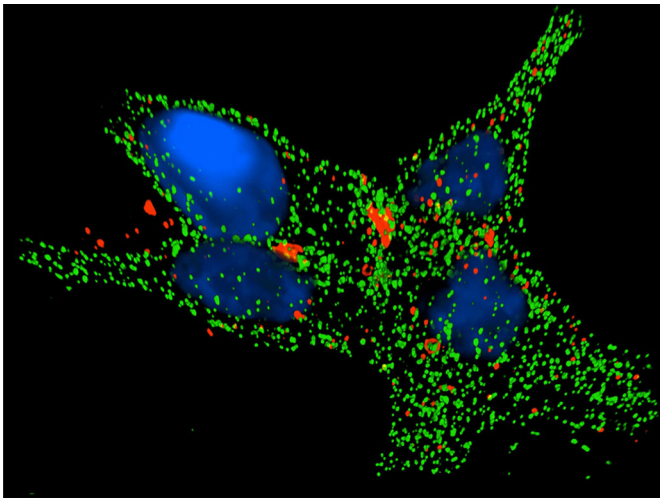
Journal of Cell Science (2018) 131, jcs216820. doi:10.1242/jcs.216820



3D image of LNCaP cells expressing connexin32 (red) and clathrin adaptor protein AP-2 (green), nuclei stained with DAPI (blue).

help you to correctly interpret your results and to rule out false positives. It is also very important to present your work at conferences and national meetings, as this will not only give you confidence in yourself and your work, but the exposure will also widen your horizons on the current state of scientific research in your field as well as other fields. This will help you in shaping your future research career.

What changes do you think could improve the professional lives of early-career scientists?

At present, I think funding is the main problem for early-career scientists. Adequate start-up grants must be made available to them, so that they do not always have to worry about acquiring enough funding, and instead can fully engage in doing cutting-edge scientific research.

What's next for you?

I am in the final year of my PhD and looking forward to finishing up soon. I have started looking for post doctoral positions and I am especially interested in doing my future research in the field of cell biology.

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

Apart from academia, I am very interested in sports. I used to participate in different sports tournaments at school and college level. I am a big animal lover and love spending time with pets. In my spare time, I like cooking new dishes.

Reference

Ray, A., Katoch, P., Jain, N. and Mehta, P. P. (2018). Dileucine-like motifs in the C-terminal tail of connexin32 control its endocytosis and assembly into gap junctions. *J. Cell Sci.* **131**, jcs207340.