© 2019. Published by The Company of Biologists Ltd | Journal of Cell Science (2019) 132, jcs234245. doi:10.1242/jcs.234245

FIRST PERSON

First person – Mayank Sharma

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. Mayank Sharma is first author on 'Targeting specificity of nuclear-encoded organelle proteins with a self-assembling split-fluorescent protein toolkit', published in JCS. Mayank is a PhD student in the lab of Prof. Ralf Bernd Klösgen at Martin Luther University Halle-Wittenberg, Germany, investigating targeting specificity of nuclear-encoded proteins into endosymbiotic organelles in plant cells.

Mayank Sharma

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

Plant cells possess multiple sub-cellular compartments (or organelles) and each organelle performs a specific function in the cell with a specific set of proteins. Most of the organelle proteins are encoded by the cell nucleus, synthesized in the cytosol (the cellular fluid) and need to be transported into these organelles. The specificity of protein transport into the organelles is determined by the interaction between transport machineries at the organelle surface and the transport signals in the proteins. In most instances, the proteins are monospecific, i.e. they are targeted to a single organelle, while a number of proteins possess dual-targeting specificity, i.e. they can be targeted to two organelles simultaneously. This dual protein targeting is frequently observed for chloroplasts and mitochondria within the plant cell. These organelles are particularly interesting due to their common evolutionary lineage, i.e. they both are evolved from engulfment of bacterial ancestors by a progenitor cell. In this study, we developed a set of DNA vectors that can be utilized to transform living plant cells and to visualize where a given candidate protein is situated in the cell. These DNA vectors encode a modified version of green fluorescent protein (GFP), known as self-assembling split-GFP (*sa*split-GFP). With this *sa*split-GFP system, we were able to determine dual-targeting specificity of several candidate proteins, which has not been possible before with available methods.

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

The main challenge while working with dual-targeted proteins was the variability of results. We were constantly looking for an experimental system which can complement the available approaches to precisely determine protein targeting specificity. After a literature search, we came up with the idea of using the self-assembling split-GFP system for this purpose. I performed the first experiment with *sa*split-GFP constructs and was highly disappointed with the weak fluorescence signals obtained in transformed plant cells. However, I was able to find ways to overcome this problem and figured out that an efficient vector system could be a solution. Without any delay, we decided to collaborate with an institute colleague, Dr Johannes Stuttmann, who is an expert in plant expression vector construction. Indeed, the new vector system, which we named PlaMiNGo, proved to be more sensitive and efficient to determine dual targeting of several 'presumed' monospecific proteins.

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

It was unbelievable at first that the transport signals of two widely utilized, 'presumed' monospecific candidate proteins, namely mitochondrial CoxIV and chloroplastic small subunit of Rubisco, showed dual targeting to both endosymbiotic organelles with the system developed here. I still remember the moment when I first observed the intense fluorescence signals in plastids with CoxIV transit peptide.

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

Journal of Cell Science is a reputable journal covering all aspects of cell biology. However, I felt that plant cell science is under-represented in the journal. Recently, there was a special issue in JCS focusing on plant cell biology and it was inspiring to see the efforts made by the editors and authors to highlight plant cell science at the forefront of the journal. This was a hint for us that this journal could be the best fit for our paper. Besides this, the fast editorial process (e.g. the average submission to first decision time is 30 days) was another reason why we decided to submit our manuscript to JCS.

Have you had any significant mentors who have helped you beyond supervision in the lab? How was their guidance special?

My PhD supervisor, Prof. Ralf Bernd Klösgen, will always be a significant mentor in my scientific career. Unlike the typical

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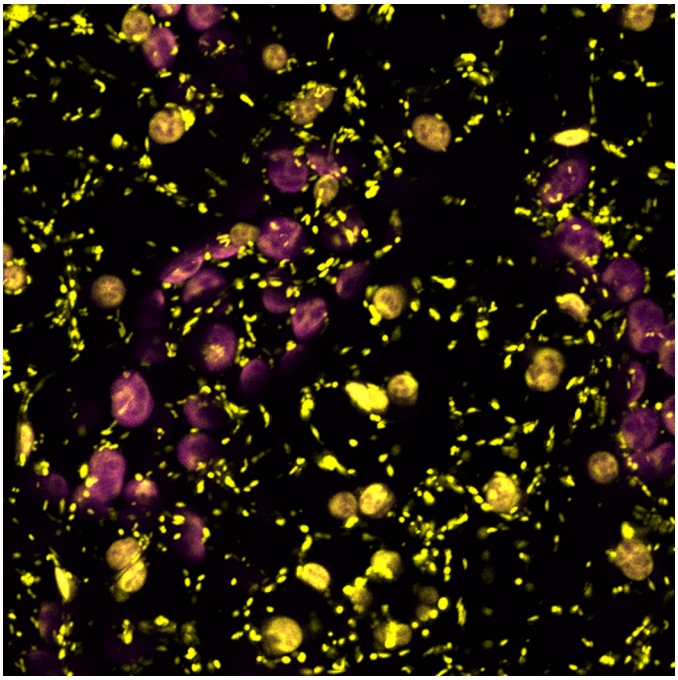
Journal of Cell Science (2019) 132, jcs234245. doi:10.1242/jcs.234245



Leaf epidermal cells of transgenic *Arabidopsis thaliana* plant expressing a dual-targeted protein, $TyrRS_{1\text{-}91}$, fused to enhanced yellow fluorescent protein (yellow). The chlorophyll autofluorescence coming from chloroplasts is highlighted in magenta.

doctoral supervisors, Prof. Klösgen lets his students explore their own ways to develop and execute a scientific project. This could also possibly explain why, despite being a PhD student in his lab, I am the first and the corresponding author of this paper.

“It was mesmerizing to see the rapid movement, fission and fusion of fluorescently labeled mitochondria”

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?

During senior high school, science textbooks played a major role in motivating me to pursue a career in science. After finishing my master’s degree in plant molecular biology, I joined a seed company to gain some industrial experience. A couple of years later, I realized that my scientific freedom was limited in the industrial set-up. I started looking for doctoral opportunities worldwide and got selected for a fellowship offered by the Erasmus Mundus Action 2 program to pursue doctoral studies in Germany. This brought a major change in my scientific career and interests. During my doctoral studies, I was introduced to fluorescence microscopy and to the amazing world of the plant cell. It was mesmerizing to see the rapid movement, fission and fusion of fluorescently labeled mitochondria, which look quite different under the microscope compared to what we see in textbooks. The four years of the doctoral work were enough to generate a budding interest in cell biology.

What’s next for you?

I have recently submitted my dissertation and hopefully will defend my PhD work very soon. I have chosen the traditional academic path and already accepted a postdoctoral position at ETH Zürich. I will continue working in the field of plant cell biology.

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

Well, I must admit that it’s a difficult question for an Indian kid raised in the 90s. I have been trying to dance Salsa for a couple of years now. Besides this, I spend most of my free time watching YouTube, although I am trying hard to avoid it.

Reference

Sharma, M., Kretschmer, C., Lampe, C., Stuttmann, J. and Klösgen, R. B. (2019). Targeting specificity of nuclear-encoded organelle proteins with a self-assembling split-fluorescent protein toolkit. *J. Cell Sci.* **132**, 230839. doi:10.1242/jcs.230839