

FIRST PERSON

First person – Poulomi Das

First Person is a series of interviews with the first authors of a selection of papers published in Journal of Cell Science, helping researchers promote themselves alongside their papers. Poulomi Das is first author on 'The Small Interactor of PKD2 protein promotes the assembly and ciliary entry of the *Chlamydomonas* PKD2–mastigoneme complexes', published in JCS. Poulomi is a PhD student in the lab of Dr Karl F. Lechtreck at the Department of Cellular Biology, University of Georgia, Athens, USA, where she investigates the molecular world of proteins using microscopy.

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

Channel proteins are the gates that help cells to communicate with their external environment. Polycystin 2 (PKD2) is one such channel protein, and variants in this protein lead to autosomal dominant polycystic kidney disease (ADPKD) in humans. PKD2 is a highly conserved protein, so it is expressed in a wide variety of species including our model organism *Chlamydomonas reinhardtii*, which is a freshwater alga with two thread-like appendages called flagella. Flagella mostly consist of the protein tubulin. In *Chlamydomonas*, PKD2 is expressed in the cell body and the flagella. For flagellar entry, PKD2 needs to undergo proteolytic processing in the cell body, where it gets cleaved into two fragments. In the flagella, PKD2 forms a proximal mobile zone and a distal stationary zone. In the distal zone, PKD2 interacts with hair-like mastigonemes, which are polymers of the glycoprotein MST1, and together they form two rows of 'hair' on the flagellar surface. The main goal of my project is to explore and understand the unique organization of PKD2–MST1 complexes inside flagella and to find the specific protein that leads to the docking of the PKD2–MST1 complexes in two hair-like rows. This in turn will help us to understand the internal asymmetry of motile flagella. In this study, immunoprecipitation of PKD2 complexes allowed us to identify Small Interactor of PKD2 (SIP), a single-pass transmembrane protein. SIP is involved in the proteolytic cleavage of PKD2, as cleaved PKD2 fragments are largely absent from the cell body of *sip* mutants. Thus, SIP plays an essential role in the flagellar entry of PKD2–MST1 complexes and also in the formation of the two hair-like rows.

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

There were two major challenges in my project. The first challenge was to maintain a stable liquid culture of the *sip* null mutant strain, because occasional re-expression of the protein was observed on western blots. This mutant was obtained from the *Chlamydomonas* CLiP library, which is a collection of 64,000 *Chlamydomonas* insertional mutants. The *sip* mutant strain has an insertion in the second intron of the *SIP* gene. A possible reason for the re-expression of the SIP protein is the ability of *Chlamydomonas* to



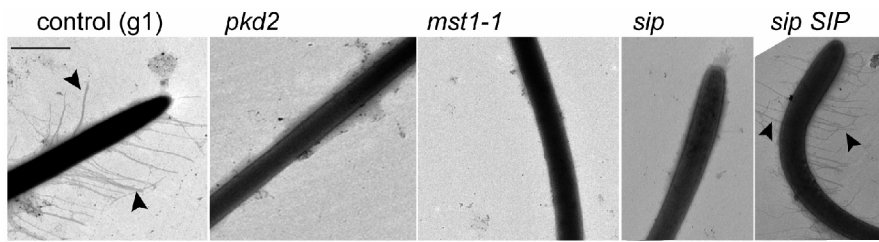
Poulomi Das

splice out the whole intron along with the insertion. To solve this problem, I maintained the *sip* culture on agar plates, and before every experiment, I prepared a fresh liquid culture from the plate and confirmed the absence of the protein using western blotting with an anti-SIP antibody. The second challenge was to generate a rescue strain of the *sip* mutant by expressing SIP protein with visual tags, including mNeonGreen, GFP and mCherry. Because SIP is a transmembrane protein with an N-terminal signal peptide, I tried to tag the C terminus and not the N terminus. Initially, we failed to generate a rescue strain expressing tagged SIP because of the orientation of its C terminus outside the cell, which leads to oxidation of the tag. To solve this problem, I expressed cDNA of the *SIP* gene without any tag and successfully generated a rescue strain, which was confirmed by PCR amplification as well as western blotting.

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

I had three 'eureka' moments while working on this project, and those moments will always stay with me. The first moment happened while analysing several CLiP mutants, which were chosen after mass spectrometry analysis of the immunopurified PKD2 complexes. While performing whole-mount negative staining, I observed the absence of the typical mastigoneme rows from the flagellar surface of the *sip* mutant strain. This gave the first confirmation of the SIP protein being a potential hit as an interactor

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Mastigoneme rows are present on flagella of wild-type control *Chlamydomonas*; absent in *pkd2*, *mst1* and *sip* mutant strains; and rescued in a *sip* rescue strain.

of the PKD2–MST1 complex based on the mass spectrometry data. The second moment happened while analysing whole-cell samples of wild-type, *sip* mutant and *sip* rescue strains using western blotting. The anti-PKD2 antibody showed three bands of PKD2 (two proteolysed fragments and one full-length protein) in the wild-type strain and one band in the *sip* mutant strain. The two proteolysed PKD2 bands were restored in the *sip* rescue strain, giving three bands again. This indicated the involvement of SIP in proteolysis of PKD2 in the cell body. This was a surprising function of SIP, and discovering it made me super happy. Last, but not least, the final moment happened when I saw on the *Chlamydomonas* CLiP website that the uncharacterized protein (our potential hit) had been renamed as Small Interactor of PKD2 (SIP), as suggested by me. This made me ecstatic because I realized at that very moment that I had discovered and named a novel protein.

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

My work involved delving into the signalling pathways and localization of the PKD2 complex in *Chlamydomonas*. Journal of Cell Science provides me with the best platform to share my data with a large readership. I am an avid reader of papers from Journal of Cell Science, and JCS is a very reputed journal in the field of cell biology. I am super proud to publish my paper in JCS.

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

My PhD supervisor, Dr Karl F. Lechtreck, has been an inspiration in many ways. Besides motivating me to work in the lab with a positive outlook, he inspires me to maintain a healthy balance between work and my personal life. His supportive nature helped me to get over my fear of making mistakes and free my mind to explore and execute different ideas. I also learnt from him that research should be qualitative and not quantitative. My mother is always the most important mentor in my life. Whenever I was dealing with some issues in my experiments, she always encouraged me to ask for help. This helped me to learn that asking for help and discussing our ideas are two very important factors for progress in any field of life.

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?

When I was in fifth grade, one of my classmate's fathers, who was a scientist, visited our school a few times to show his video recordings of his fieldwork, as well as lab work, from Antarctica. His research was on the microbiome of environments with extreme weather conditions. I became fascinated with scientists and their lives, and I started focusing more on biology and mathematics, with the goal of being a part of the scientific community in the future. After

finishing school, I joined the National Institute of Science Education and Research (NISER) in Jatni, India to pursue my integrated master's degree in Biology and Chemistry. Every summer as a student at NISER, I got a scholarship to move to a new city and join a research institute for a 45-day summer project. The turning point in my research career happened in 2017, when I went to the National Chemical Laboratory (NCL) in Pune, India. There, I was given a project to immuno-purify a protein after incorporating a point mutation. This allowed me to perform molecular biology, microbiology and biochemistry very independently for the first time. The appreciation of my work at NCL inspired me a lot, gave me tremendous happiness and confidence, and also reassured me that I can think in a scientific way. There, I discovered my love for protein biochemistry, and I decided to pursue a PhD after my integrated master's degree.

Who are your role models in science? Why?

While growing up, my mother used to tell me a variety of stories. Those stories included the lives of various scientists. I became very motivated by the lives of Isaac Newton and Jagadish Chandra Bose, and by their discoveries and inventions. I was amazed by the discovery that plants also respond to stimuli. The capability of scientists to either discover or invent things acted as a catalyst for my desire to start my research journey.

What's next for you?

After graduating from my PhD, I am planning to join a research institute as an independent scientist and work in the fields of biochemistry and molecular biology. My focus would be on protein interactions and signalling pathways, because these areas intrigue me a lot. I will definitely be doing imaging, because I love to witness the molecular world of tagged proteins.

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

Apart from doing research, I love music and cooking. Most of my free time goes to listening to music. During my school days, I participated in different cultural festivals for dancing and singing. I also love to make friends and spend time with them. This allows me to share my life and to become a part of their lives, and in return I get emotional fulfillment. I experience an equivalent level of happiness from my work as I do from my leisure activities because both are interdependent on each other for me to function in my daily life with the utmost satisfaction.

Reference

Das, P., Mekonnen, B., Alkhofash, R., Ingle, A. V., Workman, E. B., Feather, A., Zhang, G., Chasen, N., Liu, P. and Lechtreck, K. F. (2024). The Small Interactor of PKD2 protein promotes the assembly and ciliary entry of the *Chlamydomonas* PKD2–mastigoneme complexes. *J. Cell Sci.* **137**, jcs261497. doi:10.1242/jcs.261497