© 2021. Published by The Company of Biologists Ltd | Journal of Cell Science (2021) 134, jcs258327. doi:10.1242/jcs.258327

FIRST PERSON

First person – Srija Bhagavatula

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. Srija Bhagavatula is first author on 'A putative stem-loop structure in *Drosophila crumbs* is required for mRNA localisation in epithelia and germline cells', published in JCS. Srija is a post-doc in the lab of Dr Elisabeth Knust at Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany, investigating the significance of mRNA localization in epithelia.

Srija Bhagavatula

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

Many cells are polarized. This requires a well-organized machinery to place molecules, for example, proteins at specific locations within the cell, where they have a functional role. Epithelial cells are clearly polarized; these cells are tightly connected and act as a barrier between the external and internal environment. Examples include the skin and the lining of our gut. The apical side of epithelial cells faces the outside and plays an important role in maintaining homeostasis in the face of various environmental stressors. Crumbs is a very important protein present in the apical membrane of epithelial cells. This protein is required to maintain epithelial integrity in many metazoans. Along with the protein, the mRNA, which contains information for the formation of the protein, is also localized to the apical cytoplasm of epithelial cells in the *Drosophila* embryo. Localized mRNAs often contain sequences [known as localization elements (LEs)] that are recognized by protein complexes and loaded onto cellular transport systems. These transport systems actively bring and localize the mRNA to its specific destination inside the cell, and this ensures that the protein is made in the same location as the mRNA. In our study, we narrowed down the LE of the *crumbs* mRNA of *Drosophila* to 47 nucleotides by injecting *in vitro* synthesized, fluorescently labelled RNA into early embryos (see figure overleaf). Similarly, deleting the LE from the *crumbs* gene abolishes apical localization of its mRNA in epithelia. Unexpectedly, this did not affect Crumbs protein localization, which was still present in the apical membrane.

mRNA found in the egg and early embryo is deposited by the mother. The *Drosophila* ovary contains a chain of egg chambers, each of which gives rise to one egg. Each egg chamber contains an oocyte and 15 nurse cells, which nurture the oocyte, and the follicular epithelium surrounding these. *crumbs* mRNA is normally deposited in the oocyte of the egg chambers. However, *crumbs* mutants lacking the LE fail to do so. Additionally, we found Crumbs protein in the nurse cells of mutant flies, where it normally cannot be detected. From this we concluded that *crumbs* mRNA is actively transported into the oocyte to prevent the presence of Crumbs protein in the nurse cells.

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

CRISPR was a new technique when I started my PhD. This technique is very powerful and allows one to edit and modify genes of interest precisely as per our requirement. It required a lot of standardization, and therefore it took a very long time to establish mutant fly lines. Having experienced colleagues around really made it easier. Another challenging aspect I particularly remember was during the standardization of mRNA injections. I had to inject fluorescently labelled mRNA into early *Drosophila* embryos. It took a lot of trial and error to standardize every step of the injection from embryo collection to image acquisition. I initially tried to inject all the embryos in one go and then image them. But then I missed the right developmental time points in some of them. Thankfully the institute has a spinning disc confocal system with an injection setup mounted on it. Then I realized that I must tag the mRNA with a different fluorophore to avoid background signal from the yolk. Through the entire standardization, I took the suggestions and help from our Microscopy facility and Dr Shovamayee Maharana, previously a post-doc at MPI. I must mention a former post-doc from our lab, Dr David Flores-Benitez, who helped me with microscopy and image analysis on multiple occasions. Whenever I had issues with any experiment, I often discussed them with my colleagues and also those from neighbouring labs. The scientific community at MPI-CBG is very

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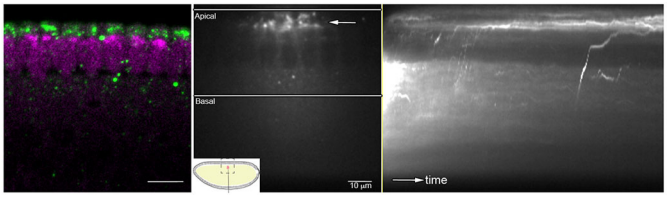

47 nucleotides in *crumbs* 3′UTR are sufficient to mediate apical localization of mRNA. Left: apically localized *crumbs* mRNA in the early *Drosophila* embryo. Green, *crumbs* mRNA; Magenta, nuclei. Middle: a snapshot of an embryo injected with reporter mRNA tagged to the 47-nucleotide LE of *crumbs* mRNA. The reporter mRNA localizes apically, similar to *crumbs* mRNA. Cartoon in the inset shows injection of mRNA into the *Drosophila* embryo. Right: time-lapse image of the same embryo over 10 min.

enthusiastic, friendly and helpful. They would either offer me valuable input or direct me to an expert. The facilities at MPI were a huge help with technical aspects and allowed me to focus on the science.

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

It was the experiment to narrow down the precise sequence responsible for actively transporting *crumbs* mRNA. I was injecting mRNAs with the *crumbs* 3′UTR that had various deletions into the *Drosophila* embryos. The first time when an mRNA lacking the *crumbs* LE was unable to travel all the way up to the apical end of the cytoplasm, was the 'gotcha!' moment for me. I remember the entire scene, sitting in front of the spinning disc confocal system, in the rotating chair. I actually immediately called up my dad in India and said exactly that "I found it".

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

My research interests and my study deal with core cell biology concepts. They align perfectly well with the focus of JCS. I really like its collection of research topics and their presentation. The journal covers a broad spectrum within cell biology and reach a wide audience. I also like the fast review process and author-friendly working style. Alongside that, I particularly like The Company of Biologists. Through their publication policies and other initiatives (the Node), I think they do a great service to the scientific community and really bring scientists together.

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

There were many. I must thank my PI, Dr Elisabeth Knust. Eli has been very encouraging and gave me all the scientific freedom I needed. Although I had valuable input and suggestions from every lab mate here, I must particularly thank Dr David Flores-Benitez for his timely guidance and suggestions. I was lucky to have a perfect blend of critical lab meetings and friendly research guidance in this lab. I must also name Dr Helena Klara Jambor, a former post-doc at MPI-CBG from Pavel Tomancak's lab. I discussed my project extensively with her and she often suggested interesting experiments. I often discussed my work with Dr Shovamayee Maharana, another post-doc at MPI-CBG, and our discussions always gave me a fresh outlook.

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 was taught from childhood that it's never wrong to doubt and question anything in life, as long as it is done respectfully and peacefully. I never decided to do science. It just seemed the only possible path for me. I was initially very interested in physics (I still am). The biology in text books seemed too factual to enjoy. I was introduced to a very different perspective during my Bachelor's, at my *alma mater* (IISER, Pune). Our biology faculty there encouraged a research mindset and introduced us to tons of unanswered questions from the very beginning. I started to really enjoy cell biology and decided to major in life sciences.

Who are your role models in science? Why?

I would say all the women in science. I believe in finding role models right around us. My boss Eli, the late Dr Suzanne Eaton, my ex-lab mate Anna, Dr Richa Rikhy at IISER, Pune to name a few. I find their enthusiasm and passion for science very motivating. When you enjoy doing something, you do it well, and I find that especially true for science. It's easy to get exasperated when experiments don't work, and this happens often. While from some people, I learnt the importance of paying attention to detail, I learnt from others not to lose sight of the bigger picture. Never forget what got you excited in the first place and always enjoy the science behind the experiments.

What's next for you?

I really enjoy doing science. I would love to continue working at the bench and having many more such eureka moments. I enjoy basic cell biology and am particularly interested in working on the role of RNA in transcriptional regulation. I also like to write about science. I believe that lab science should reach the public and be intellectually accessible to a larger community. I would love to be in a research environment and write about the science happening all around me for the scientific community and beyond.

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

When not in the lab, I like to sing and crochet. I am training in Indian Carnatic classical music. A research career is not only mentally but also emotionally quite challenging. These hobbies helped me gain the required patience and persistence.

Reference

Bhagavatula, S. and Knust, E. (2021). A putative stem-loop structure in *Drosophila crumbs* is required for mRNA localisation in epithelia and germline cells. *J. Cell Sci.* **134**, jcs236497. doi:10.1242/jcs.236497