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FIRST PERSON

First person – Bouchra Khalaf

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. Bouchra Khalaf is first author on 'Ankyrin-G induces nucleoporin Nup358 to associate with the axon initial segment of neurons', published in JCS. Bouchra conducted the research described in this article while a PhD student in Paolo Macchi's lab at the University of Trento, Italy. She is now a postdoc in the lab of Alain Dabdoub at Sunnybrook Research Institute, Toronto, Canada, investigating the molecular and cellular neurobiology of development and disease progression.

Bouchra Khalaf

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

One hallmark of eukaryotic cells is the presence of the nucleus that separates the genetic material from the cytoplasm. However, communication between the nucleus and the cytoplasm is required for cell survival and proper cellular function. This communication – represented by the transport of molecules between the nucleus and the cytoplasm – is regulated by a complex of proteins known as the nuclear pore complex (NPC) whose building blocks are known as nucleoporins or simply Nups. Though Nups, collectively, have a role in the overall function of the NPC, individual Nups can be present in other regions of the cell and contribute in other cellular processes. In this work, we characterize the multifunctional Nup358 in neurons. We show that Nup358 has a unique distribution profile, present at the nuclear rim associated with the NPC, in the soma having a punctate pattern, and at the axon initial segment (AIS). Since the AIS is a highly specialized region of the neuron, our result showing Nup358 localized here shaped the direction of our study, and we examined whether Nup358 depends on the master protein in the AIS, Ankyrin-G, for association with the AIS during neuronal development. This study, thus, sheds light on an unprecedented involvement of Nup358 in the AIS composition and provides a platform for future investigations on its function in neurons.

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

Just like in any work, whether it is research or another type of work or activity, there were some challenges to face (and overcome!). In this project, my biggest challenge was to transfect primary neurons with Nup358 constructs; and this was not an easy task to do for two reasons. First is because primary cells are generally more sensitive to transfection reagents than cell lines, which are routinely used in the lab. Second, Nup358 is, in fact, an enormous protein that is around 341 kDa in mice. So, transfecting primary neurons with a construct as big as 14 kb can give you a real headache before you could successfully manage to do so. After troubleshooting this specific experiment and changing many factors such as the transfection reagent, concentration of the construct, and the incubation period, success was worth the effort at the end of the day. And I was happy to spend some time at the microscope acquiring beautiful images of transfected neurons and add an important piece of data to the study.

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

In the very beginning of working on this project, we were quite intrigued by our finding that Nup358 has a non-classical localization in neurons. This finding would be the first evidence to show that Nup358 is present at the AIS of neurons. We then assumed that the principal AIS protein Ank-G might induce Nup358 to associate with the AIS. However, data supporting this hypothesis was missing at this point. To this end, I had to downregulate AnkG or Nup358 in neurons and examine the effect on the distribution pattern of the other protein. In AnkG-depleted neurons, Nup358 was present at the nuclear rim but absent from the AIS. While in Nup358-depleted neurons, where Nup358 was lacking from the nuclear rim and the AIS, AnkG expression was significantly reduced. This result was, indeed, fundamental to prove the interdependence of Nup358 and AnkG for expression at the AIS, and supported our data that Nup358 expression at the AIS is specific.

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

JCS has a prominent reputation in publishing cutting-edge studies that interest a broad readership of cell biologists. Since my project would be of interest to not only neurobiologists in particular but cell

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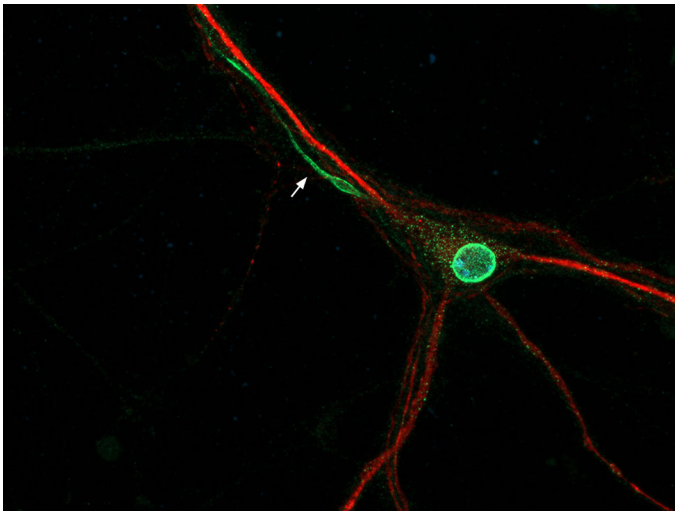

Nup358 decorates the nuclear rim and the axon initial segment (arrow) of mouse cortical neurons. Nup358 is shown in green and the dendritic marker, MAP2, in red.

biologists in general, we thought that the journal would be an excellent fit to reach a diverse scientific community.

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?

Biology has always been one of my favorite subjects to study, thanks to my biology teacher Ziad Turk who was passionate about teaching and very knowledgeable about various topics in life sciences. One important moment that I recall during my high school years was when I participated with a couple of my friends in a science fair and we presented the concept of *in vitro* fertilization. Though we did not win, that was an eye-opening moment for me and I realized how years of research can bring hope, and change the lives of people worldwide. At university, I decided to major in biochemistry and I learned the basics of conducting research in the lab of Prof. Julnar Usta at the American University of Beirut. I investigated the mechanism of action of an anti-cancer herbal component. The research project was exciting to me and I enjoyed spending time in the lab, doing experiments, reading papers and learning new techniques. For my PhD, I wasn't really sure of the topic I wanted to work on. Then, Prof. Paolo Macchi showed me some fluorescent images of immunostained neurons. I was fascinated by the unique morphology of neurons and decided to immerse myself in the world of neurobiology.

What's next for you?

Soon after finishing the Nup358 project, I came back to Lebanon to have some quality time with my family and get a good kick of energy to keep me going. Now I look forward to the next step in my career as a postdoc in the hearing regeneration lab in Sunnybrook Research Institute.

Reference

Khalaf, B., Roncador, A., Pischedda, F., Casini, A., Thomas, S., Piccoli, G., Kiebler, M. and Macchi, P. (2019). Ankyrin-G induces nucleoporin Nup358 to associate with the axon initial segment of neurons. *J. Cell Sci.* **132**, jcs222802. doi:10.1242/jcs.222802