First person – Cameron MacQuarrie

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

Cells require deformation of their external membrane to perform several processes, including the internalization of outside materials in a process known as endocytosis. Several proteins involved in generating the force needed for this process are expressed in both mammalian and yeast cells; however, how these proteins are regulated to ensure sufficient force is produced remains an open question. In yeast, the strongest activator of this process, Wsp1, eventually separates from the membrane, while a weaker activator, Myo1, remains. We discovered that the Wsp1-interacting protein Vrp1 acts as a bridge between Wsp1 and Myo1, increasing the time Wsp1 spends at the membrane, thereby enhancing the amount of force generated. This bridge is disrupted by the protein Bbc1, releasing Vrp1–Wsp1 from Myo1 and preventing excessive force generation at the membrane.

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

A big question we hoped to answer was whether or not the proteins Vrp1 and Bbc1 bind the Myo1 SH3 domain in fission yeast. Given the difficulty of purifying the 170.5 kDa Bbc1 protein, we opted to design an in vivo binding assay where fragments of the Myo1 tail were fused to a spindle pole body-localizing sequence and fluorescent mCherry. These fusion proteins were expressed in cells with mGFP-tagged proteins of interest, allowing us to observe relocalization of mGFP-tagged proteins to the mCherry-tagged Myo1 tail in a distinct section of the cell. Our lab has an extensive collection of yeast strains expressing different endocytic proteins tagged with GFP, giving us a unique opportunity to easily express these constructs in a variety of cells to determine whether a protein is able to sufficiently interact with the Myo1 tail in its native state. Using this assay, we were able to show that Vrp1 and Bbc1 are able to interact with Myo1 in vivo.

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

In wild-type cells, Myo1 always remains at the membrane and never internalizes with the endocytic vesicle. One morning I was imaging cells expressing mGFP–Myo1 in different genetic backgrounds to determine how certain genes impact the levels of Myo1 patch accumulation. However, while imaging cells in a sla1Δ background, I observed some Myo1 puncta internalizing to roughly the same distance that Sla1–mGFP does in wild-type cells. This was a very exciting result because it suggested that Sla1 has a novel function in regulating Myo1 positioning. Further, in a sla1Δ bbc1Δ double-deletion background, these puncta seemed to be internalizing nearly twice as far! This suggested that Bbc1 suppresses the force that causes vesicle internalization. We designed further experiments to probe for the distance travelled by endocytic structures and observed that our hypothesis was correct!

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

Journal of Cell Science is a well-respected journal with a broad audience of cell biologists. We wanted to publish in a journal that would allow this project to have the greatest reach. We are very excited to have our manuscript published by this quality journal.

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?

A career in science is very rewarding. Every day there is something new and exciting and each question is a puzzle with a unique solution. Like many, I started my undergraduate education as a pre-med student. I spent the summer before my junior year working in a C. elegans lab, hoping to boost my CV for med school applications; however, after being exposed to the freedom and excitement of working independently in a lab setting, I decided to switch routes and pursue a career in research.

Who are your role models in science? Why?

I was raised in a family of scientists. My father and oldest brother are both in medicine, my mother is a middle school science teacher, and my middle brother is a physicist. They have all provided a lot of support and I look up to them greatly.
What's next for you?
I am currently searching for postdoc positions and plan to stay in academia.

Tell us something interesting about yourself that wouldn't be on your CV
I enjoy hiking with my dog, cooking and playing board games.

Reference

Live fission yeast cells expressing mGFP–Wsp1 in various genetic backgrounds. In wild-type and *sla1Δ* cells, Wsp1 puncta form, internalize and dissipate. However, in *bbc1Δ* and *bbc1Δ sla1Δ* cells, Wsp1 splits in two, with a fraction remaining at the membrane (arrowheads) before dissipating.