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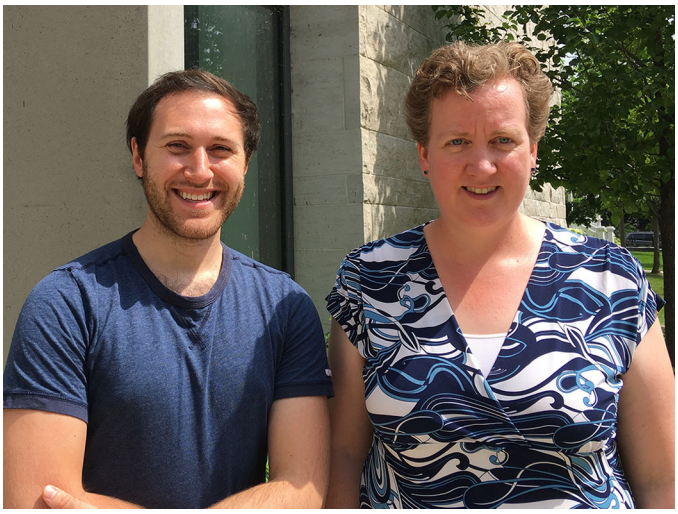
FIRST PERSON

First person – Brandy Hyndman and Mathieu Crupi

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. Brandy Hyndman and Mathieu Crupi are co-first authors on 'Differential recruitment of E3 ubiquitin ligase complexes regulates RET isoform internalization', published in Journal of Cell Science. Brandy is a Research Associate and Mathieu is a PhD student, both in the lab of Dr Lois Mulligan at the Queen's University Cancer Research Institute in Kingston, ON, Canada, investigating the ubiquitylation and trafficking of RET receptor tyrosine kinase isoforms.



Mathieu Crupi and Brandy Hyndman

How would you explain the main findings of your paper to non-scientific family and friends?

MC and BH: In our lab, we study a protein called RET that controls many cell processes, such as growth, survival and motility, as well as the development of the kidneys and nervous system of the gut. When RET is aberrantly expressed owing to a mutation in the protein or an over-abundance of RET on the cell surface, RET can continuously turn on signalling cascades that contribute to many types of cancer.

There are two main forms of the RET protein that are called RET9 and RET51, which differ in the number of amino acids that make up their tail ends. Although these forms are similar in some contexts, RET9 plays early and crucial roles in development and RET51 appears to contribute more to the invasiveness of cancer cells. Once activated, these different forms of RET can interact with ubiquitin ligases, which are factors that can alter their movement in the cell and ultimately lead to the shutdown of signaling pathways and breakdown of proteins.

Here, we show that RET9 can recruit the NEDD4 ubiquitin ligase, but RET51 interacts with a distinct ubiquitin ligase that is called CBL. We identified unique protein complexes that regulate levels of RET and are found at the cell surface by tagging RET with different ubiquitin signatures that target RET forms to enter the cell at different rates for signal attenuation and degradation. Understanding how RET forms are downregulated may ultimately lead to novel therapeutic approaches to treat cancer.

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

MC and BH: Generating cell lines with decreased CBL expression seemed challenging at first. We wanted to make sure that there were no residual levels of c-CBL in cells. We overcame this challenge by generating knockout cell lines using CRISPR. Briefly, we transfected cells with appropriate fluorescent CRISPR plasmids, flow-sorted individual colonies, and validated the knockout. These steps were simple but they required our patience as the cells had to grow to confluence.

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

MC: During my screen for different proteins which contain PDZ motifs for their interactions with RET, I decided to see if any of the candidate proteins enhanced interaction with NEDD4. I was pleasantly surprised when we saw that SHANK2 increased recruitment of NEDD4 to RET9. I was also very excited to share this finding with the lab, including Dr Mulligan and Dr Hyndman.

Have you had any significant mentors, and how have they helped you?

MC: My supervisor, Dr Lois Mulligan, has been a significant mentor throughout my PhD. She has provided me with the necessary guidance and support to pursue the questions that resonate with me in the lab. I have learned many laboratory techniques and skills from Dr Hyndman. In addition, Dr Antonescu has helped us tremendously with our analyses of TIRF microscopy data.

"I have helped with a number of cancer-related charities […], which constantly reminds me why cancer research is important"

What's the most important piece of advice you would give first-year PhD students?

MC: I would advise first-year PhD students to do more than they are expected to do, inside and outside of the lab. I have helped with a number of cancer-related charities, such as the Canadian Cancer Society and the Terry Fox Foundation, which constantly reminds me why cancer research is important. In addition, having a balanced lifestyle is essential since a PhD can take much time and energy to complete.

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“Increased opportunities to attend conferences and visit host laboratories through travelling fellowship support could improve the professional development of early-career scientists”

What changes do you think could improve the professional lives of early-career scientists?

MC: Increased opportunities to attend conferences and visit host laboratories through travelling fellowship support could improve the professional development of early-career scientists. Setting up new networks and collaborations is extremely rewarding for the groups involved.

What's next for you?

MC: I plan to continue my studies at the postdoctoral level. I am very excited for this next step in my career and hope to eventually become a professor.

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

MC: I love to travel. Whether I'm visiting a small or large city in Europe, the United States or Caribbean countries, I enjoy learning about different cultures, art and music, teaching myself how to speak other languages, and exploring historic sites.

Reference

Hyndman, B. D., Crupi, M. J. F., Peng, S., Bone, L. N., Rekab, A. N., Lian, E. Y., Wagner, S. M., Antonescu, C. N. and Mulligan, L. M. (2017). Differential recruitment of E3 ubiquitin ligase complexes regulates RET isoform internalization. *J. Cell Sci.* **130**, 3282-3296.

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