Ryerson University Faculty of Science

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Taking out the Rubbish: New Insights into Vacuolar Acidification

Lysosomes – also known as a vacuoles – are responsible for the same unglamorous but necessary task that sees me dragging giant plastic wheelie-bins to the curb once a week: we're all taking out the rubbish. While I'm doing it on a household level, those busy lysosomes are hard at work inside animal cells – and not just weekly, they are at it all the time. However, when the activity of these membrane-bound organelles (compartments within cells) is interrupted, harmful by-products accumulate. This can have a profound negative impact on cell survival and health, ultimately leading to disease.

Inside the "Degradation Hub"

PhD candidate Cheuk Y. (Shannon) Ho is the lead author on an article – recently published in the *Journal of Biological Chemistry* – that examines the function of the phosphatidylinositol 3,5-bisphosphate [PtdIns(3,5)P_2] in this process (PtdIns(3,5)P_2 accounts for 0.1% of total cellular phosphoinositides). Currently in the fourth year of her doctoral program in molecular science, Shannon carried out her research in The Botelho Lab for Organelle Identity and Function.

Under the supervision of Dr. Robert Botelho, Shannon (who also completed her MSc at Ryerson in 2011) examined the localization of PtdIns(3,5)P_2 on the late endolysosomes (or vacuoles) in yeast. Dysregulation of PtdIns(3,5)*P*2 is associated with diseases such as myotubular dystrophy, subtypes of Charcot-Marie-Tooth neuropathy type 4J and amyotrophic lateral sclerosis 11.

Calling them cells' "degradation hub," Shannon explains that lysosomes/vacuoles "contain an acidic lumen that is important for the optimal functioning of degradative enzymes." One of the main assays to check for the presence of vacuolar acidification in yeast is to use a fluorescent dye called quinacrine. Protonation (a type of chemical reaction) in the vacuole leads to the emission of green fluorescence, and this indicates an acidic environment.

Overturning Conventional Wisdom

Shannon conducted her investigations using yeast because of its many similarities to mammalian cells and the fact that it is easier to manipulate genetically for studying cell functions. Prior to Shannon's work, scientists believed that cells that are deficient in PtdIns(3,5)P_2 are unable to protonate and accumulate quinacrine in the vacuole. "As a result," Shannon says, "it was thought that PtdIns(3,5)P_2-deficient cells are unable to establish or maintain an acidic environment in the vacuole."

Shannon and her co-investigators found, however, that PtdIns(3,5)P_2-deficient cells "actually can establish and maintain proper acidification in both lysosomes and vacuoles, under basal conditions." They proved this by using two independent methods for both the yeast and the mammalian model.

"Contrary to what had been the accepted view, we now believe that we should not rely so heavily on quinacrine as an indicator of vacuolar acidification." Shannon continues, "We don't want to exclude quinacrine as an acidification

indicator, but we now know that more studies should be done to examine additional factors that could potentially affect quinacrine's accumulation and fluorescent ability."

The Ryerson Edge

The findings presented in the *Journal of Biological Chemistry* are part of Shannon's overarching doctoral program, which focuses on understanding how endosomal phosphoinositides are governed and how they function in cells.

"I am thrilled to conducting these investigations in the Botelho Lab for Organelle Identity and Function," says Shannon. "It's the first in the GTA – and possibly in Canada – to be equipped with an instrument for radiolabelling and detecting PtdIns*P*s. That's given us the ability to quantify the level of PtdIns*P*s in cells by radiolabelling them tritium (^{3}H)-conjugated inositol coupled to HPLC and flow scintillation, a really effective way of measuring the total level of individual PtdIns*P*s."

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