University of Victoria | Graduate Studies

Notice of the Final Oral Examination
for the Degree of Doctor of Philosophy

of

ANDREW BOYCE

BSc (University of Victoria, 2012)

"Regulation of Pannexin 1 Trafficking by Adenosine Triphosphate"

Division of Medical Sciences

Tuesday, June 20, 2017
10:00 A.M.
Human and Social Development Building
Room A264

Supervisory Committee:
Dr. Leigh Anne Swayne, Division of Medical Sciences, University of Victoria (Supervisor)
Dr. Bob Chow, Department of Biology, UVic (Member)
Dr. Chris Nelson, Department of Biochemistry and Microbiology, Uvic (Outside Member)
Dr. Christian Naus, Department of Cellular & Physiological Sciences, UBC (Outside Member)

External Examiner:
Dr. Ian Winship, Department of Psychiatry, University of Alberta

Chair of Oral Examination:
Dr. Henning Struchtrup, Department of Mechanical Engineering, UVic

Dr. David Capson, Dean, Faculty of Graduate Studies

Abstract

The ubiquitously expressed pannexin 1 (Panx1) ion- and metabolite-permeable channel is capable of mediating ATP release in a multitude of cells and tissues. This leads to activation of nearby purinergic (P2X/P2Y) receptors in an autocrine/paracrine manner. Stimulation of P2 receptors also triggers Panx1 activation, leading to the formation of a positive feedback loop in cells where these proteins are co-expressed. Although the focus of Panx1 research has been on its expression at the cell surface, there is robust and stable expression of Panx1 on intracellular membranes. Whether intracellular Panx1 was the consequence of direct diversion from the secretory pathway or internalization from the cell surface was unknown at the onset of my studies. I postulated that Panx1 internalization to these membranes would require either constitutive endocytosis or an episodically released and ubiquitous stimulus to allow stable intracellular expression. ATP, a potent signalling molecule released via constitutive or regulated exocytosis, large pore channels, or cell lysis, fit this criterion. *My hypothesis was that ATP triggered Panx1 internalization to intracellular compartments*. Upon elevation of extracellular ATP, I observed P2X7R-mediated non-canonical internalization of Panx1 via macropinocytosis. This involved upstream cholesterol-dependent P2X7R-Panx1 clustering via a physical interaction between P2X7R-Panx1 ectodomains and possible contribution of phospholipid (PA, PIP, PIP2) interactions localized to the Panx1 Cterminus. P2X7R activation was directly linked (via phospholipase activation) to the production of these same lipids; consequently, physical P2X7R-Panx1 interaction may promote Panx1 association with actively endocytosing regions of the membrane. Internalized Panx1 was targeted to slow recycling Rab14/Rab11-positive endosomes in an Arf6-dependent mechanism. The data I presented here provides an additional negative feedback layer to P2X7R-Panx1 crosstalk in the many cell types where they are coexpressed. Further, this is the first evidence demonstrating that Panx1 surface expression is labile to changes in the cellular environment, which contributes to the understanding of the regulation of Panx1 and associated behaviours through trafficking mechanisms.