UNIVERSITY of GUELPH

COLLEGE of BIOLOGICAL SCIENCE

DEPARTMENT OF MOLECULAR AND CELLULAR BIOLOGY

Announcement:

All interested members of the university community are invited to attend the Final Oral Examination for the degree of ***Doctor of Philosophy*** *of*

ALICYIA WALCZYK-MOORADALLY

on Friday, November 18, 2022 at 1:30 p.m. (SSC 1511)

Thesis Title: **Transcriptional and post-translational regulation of Activity-regulated cytoskeleton-associated (Arc) protein**

Examination Committee:

Dr. Jaideep Mathur, Dept. of Molecular and Cellular Biology (Exam Chair)
Dr. Jasmin Lalonde, Dept. of Molecular and Cellular Biology
Dr. Giannina Descalzi, Dept. of Biomedical Sciences
Dr. Shaun Sanders, Dept. of Molecular and Cellular Biology
Dr. Erica Korb, Perelman School of Medicine, University of Pennsylvania (External Examiner)

Advisory Committee:
Dr. Jasmin Lalonde (Advisor)
Dr. Jim Uniacke
Dr. Giannina Descalzi

Abstract: The remarkable capacity of neurons to reorganize their structure, function, and connections in an activity-dependent manner is supported by an extensive network of molecules and effectors. Among those, Activity-regulated cytoskeleton-associated protein (Arc, also known as Arg3.1) is considered one of the central, most versatile, players. Arc is an immediate early gene product that support neuroplastic changes important for cognitive function and memory formation, while also oligomerizing to form capsids that can be released and can move independent of the synapse. Although a lot has been uncovered about the contributions of Arc to neuron biology and behavior, very little is known about how different functions of Arc are regulated both temporally and spatially in neurons. To answer to this question, the regulation of Arc expression must be monitored at various levels. This thesis analyzes how Arc can be transcriptionally and post-translationally regulated and how this confers neurobiological specificity. Being an immediate early gene, transcription of Arc needs to act rapidly in response to a neuronal stimulus, such as BDNF. The first aim of this thesis provides a model to describe how the Arc promoter can overcome topological constraints to facilitate rapid and persistent transcription, up to 6 hours post-BDNF exposure. Using chromatin analysis and biochemical techniques we have mapped regions of the Arc promoter that undergo double stranded DNA breaks to facilitate rapid transcription, and that BDNF-induced Arc non-coding RNA elements interact with DNA repair proteins such as Brd4 to effectively end transcription. The second aim of this thesis aims to determine how phosphorylation can spatiotemporally dictate the function of Arc. Mass spectrometry and sequence prediction strategies were used to map novel Arc phosphorylation sites. This approach led us to recognize S67 and T278 as residues that can be modified

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by TNIK, which is a kinase abundantly expressed in neurons and been implicated as a risk factor for psychiatric disorders. Characterization of TNIK-dependant phosphorylation at these two sites can strongly influence Arc's subcellular distribution and self-assembly as capsids. The multifaceted nature of Arc's neurobiology ranges from being a key component of long-term memory consolidation to participating in viral capsid intracellular communication. This thesis provides insight into Arc's moonlighting capabilities both at the transcription and post-translational levels.

Curriculum Vitae: Alicyia completed her Bachelor of Science (Hons.) in Biomedical Sciences at the University of Guelph in April 2018, working with Dr. Steffen Graether for an undergraduate thesis project. She then started her Master of Science in Molecular and Cellular Biology with a specialization in Neuroscience under the supervision of Dr. Jasmin Lalonde at the University of Guelph in September 2018. She transferred to the Doctor of Philosophy program in November 2019.

Awards: Graduate Tuition Scholarship from the University of Guelph (2018-2020); Rising Stars in Neuroscience from the University of Utah (2022)

Publications: Holborn, J.*, **Alicyia Walczyk-Mooradally***, Perrin, C., Alural, B., Aitchison, C., Slocum, S., Huang, XP., Roth, B. L., Khokar, J.Y., Akhtar, T.A., & Lalonde, J. Interference of Neuronal TrkB Signaling by Cannabis-Derived Flavonoids Cannflavins A and B.
Under revision at *Phytomedicine Plus*, August 2022.

Walczyk-Mooradally, A., Holborn, J., Singh, K., Tyler, M., Patnaik, D., Wesseling, H., Brandon N. J., Steen, J., Graether., S. P., Haggarty, S. J., & Lalonde, J. (2021). Phosphorylation-dependent control of Activity-regulated cytoskeleton-associated protein (Arc) protein by TNIK. *Journal of Neurochemistry*. 158, 1058–1073. https://doi.org/10.1111/jnc.15440

Sheppard, P.A.S., Asling, H.A., Armstrong, S.E., Elad, V.M., **Walczyk-Mooradally, A**., Lalonde, J., & Choleris, E (2021). Effects of dorsal hippocampal inhibition of actin polymerization or protein synthesis on rapid estrogen-facilitated social recognition, dendritic spines, and Arc protein expression in ovariectomized female mice. *Journal of Psychoneuroendocrinology*. 128, 105232. https://doi.org/10.1016/j.psyneuen.2021.105232

Ferreira, L.A., **Walczyk-Mooradally, A**., Zaslavsky, B.A., Uversky, V.N., and Graether. S.P. (2018). Effect of an Intrinsically Disordered Plant Stress Protein on the Properties of Water. *Biophysical Journal*. 115(9): 1-11. https://doi.org/10.1016/j.bpj.2018.09.014

Tilak, M., Lu., P., Alural, B., **Walczyk-Mooradally, A**., New, L., Lalonde, J., & Jones, N.
Characterizing the regulatory landscape of adaptor protein SchD.
In preparation to submit to *Journal of Biological Chemistry*, 2022.