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*

LINA ABOU ZEID

on Wednesday, May 26, 2021 at 9:30 a.m. (online)

Thesis Title: **Investigating the effect of hyperthermia and Hsp70 overexpression on microRNA biogenesis**

Examination Committee:

Dr. Ray Lu, Molecular and Cellular Biology (Exam Chair)
Dr. Terry Van Raay, Molecular and Cellular Biology
Dr. Jonathan LaMarre, Biomedical Sciences
Dr. Jim Uniacke, Molecular and Cellular Biology
Dr. Katey Rayner, Biochemistry, Microbiology, Immunology, University of Ottawa Heart Institute (External Examiner)

Advisory Committee:
Dr. Richard Mosser (Co-Advisor)
Dr. Terry Van Raay (Co-Advisor)
Dr. John Vessey
Dr. Jonathan LaMarre

Abstract: Heat shock, a form of severe proteotoxic stress, triggers apoptosis. A subset of molecular chaperones, heat shock proteins (HSPs), maintains proteostasis by assisting in the folding of nascent proteins and the refolding of misfolded proteins. HSPs, such as HSP70 provide a mechanism for survival following stress by limiting proteotoxic damage and suppressing apoptosis. Cellular stress also influences gene expression through altering microRNA (miRNA) levels and expression patterns. Mild hyperthermia has been shown to increase the abundance of some miRNAs and decrease the expression of others. Whether these changes in the abundance of particular miRNAs play a protective role is unknown. What is also unclear is the effect of severe hyperthermic stress on miRNA maturation and whether HSP70 plays a protective role in miRNA biogenesis under stress. MiRNAs are generated through a series of processing steps. The primary miRNA transcript (pri-miRNA), is cleaved by the Microprocessor Complex, composed of the ribonuclease Drosha and its partner protein DGCR8, into a shorter precursor miRNA (pre-miRNA). The pre-miRNA is converted to mature miRNA by the ribonuclease Dicer and its binding protein, TRBP. The mature miRNA associates with an Argonaute protein forming the RNA-induced silencing complex (RISC), through which the miRNA can interact with its target mRNAs and silence gene expression. We examined the fate of the miRNA processing proteins in a human acute lymphoblastic T cell line with tetracycline-regulated expression of HSP70.

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We were able to demonstrate a disruptive effect of heat stress on miRNA processing as it resulted in the caspase-mediated cleavage and degradation of Drosha and TRBP. We also observed a protective effect of HSP70 on miRNA processing under proteotoxic stress, as it prevents the cleavage of the miRNA processing proteins. We successfully mapped out a caspase cleavage site on TRBP, demonstrating that TRBP is cleaved between its second double-stranded RNA binding domains and its protein-binding domain. These findings suggest an imperative role in preventing miRNA biogenesis in apoptotic cells as four of the key miRNA processing proteins are inactivated by caspases. They also suggest an important role of HSP70 in maintaining miRNA biogenesis in stressed cells.

Curriculum Vitae: Lina obtained her Bachelor of Science (Honours), Cell Biology and Genetics, at the University of British Columbia in 2013. She began her M.Sc. in Dr. Richard Mosser's lab in the fall of 2015, and later transferred directly into the Ph.D. program.