



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 **Master of Science** of

MARIJKE MURRAY

on Wednesday, April 17th, 2024 at 9:30 a.m. (online)

Thesis Title: Exploring the Role of Pseudophosphorylation in the Nuclear Localization and Function of VrDHN1

Examination Committee:

Dr. Matthew Sorbara, Dept. of Molecular and Cellular Biology (Exam Chair)

Dr. Steffen Graether, Dept. of Molecular and Cellular Biology

Dr. Stephen Seah, Dept. of Molecular and Cellular Biology

Dr. Ian Tetlow, Dept. of Molecular and Cellular Biology

Advisory Committee:

Dr. Steffen Graether (Advisor)

Dr. Stephen Seah

Abstract: Dehydrins are group 2 late embryogenesis abundant proteins known for their ability to confer desiccation tolerance to plants. Genetic experiments have shown that dehydrins protect plants from cold, salinity and drought through a wide range of mechanisms; *in vitro* experiments have shown that dehydrins protect membranes, proteins, and DNA by binding to them. With the *Vitis riparia* dehydrin VrDHN1, I explore potential mechanisms for nuclear localization through phosphorylation of the S-segment and/or the proposed nuclear localization signal called the basic domain. Using pseudophosphorylated constructs, I show that the nuclear to cytoplasmic ratio did not increase proportionally to the number of modified serines in the S-segment, but that the S70E mutation caused a 27% increase in nuclear localization while modification of all five serines to glutamate caused a reduction in nuclear localization. Blocking phosphorylation and basic domain binding did not prevent nuclear localization, suggesting that phosphorylation may result in a passive to active switch in nuclear transport. Further experiments tested whether pseudophosphorylation modified the ability of VrDHN1 to bind membranes and to protect lactate dehydrogenase (LDH) during a cryoprotection assay. The results show that pseudophosphorylation increased the α -helicity in the presence of SDS micelles, suggesting stronger membrane binding. The cryoprotection assays showed a reduction in PD50, demonstrating an increase in LDH cryoprotection efficiency. The role of S-segment phosphorylation and nuclear localization signal binding in the active transport of dehydrins to the nucleus requires further research.

Curriculum Vitae: Marijke completed their BSc in Honours Specialization in Genetics and Biochemistry at Western University in 2021. They then began their M.Sc in Molecular and Cellular Biology in Winter 2022 under the supervision of Dr. Steffen Graether.

Publications: Murray, M.R., and Graether, S.P. (2022). Physiological, Structural, and Functional Insights into the Cryoprotection of Membranes by the Dehydrins. *Front. Plant Sci.* 13, 1–7.