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*

NATHAN DONER

on Friday, November 11, 2022 at 1:00 p.m. (SSC 1511)

Thesis Title: **Identification and characterization of new lipid droplet proteins in *Arabidopsis thaliana*: LIPID DROPLET PROTEIN OF SEEDS and EARLY RESPONSIVE TO DEHYDRATION 7**

Examination Committee:

Dr. Jaideep Mathur, Dept. of Molecular and Cellular Biology (Exam Chair)
Dr. Cezar Khursigara, Dept. of Molecular and Cellular Biology
Dr. Annette Nassuth, Dept. of Molecular and Cellular Biology
Dr. Yang Xu, Dept. of Molecular and Cellular Biology
Dr. Heather McFarlane, Dept. Cell and Systems Biology, University of Toronto (External Examiner)

Advisory Committee:
Dr. Robert Mullen (Advisor)
Dr. Cezar Khursigara
Dr. Annette Nassuth

Abstract: Plant oils are important for humans nutritionally, but also in terms of their potential for use as biofuels and various bioproducts. Plant oil is stored primarily as triacylglycerol in cytoplasmic lipid droplets (LDs), evolutionarily conserved organelles that function not only in neutral lipid storage, but also in several other cellular processes. LDs are especially abundant in plant seeds, where they provide energy required for germination and growth, but they are also found in virtually all other plant tissues. However, the molecular mechanisms underlying LD biogenesis, maintenance, and turnover in plant cells are generally unknown, primarily because relatively few LD proteins have been studied.

To address this, results were compiled from protein-protein interaction screens and LD proteomics studies from *Arabidopsis thaliana* tissues and surveyed for potentially new protein players in plant LD biology. In total, seven new families of plant LD proteins were identified based on their localization to LDs when expressed in plant cells. Other LD-related proteins were also identified based on partial localization to LDs and/or dysregulation of lipid homeostasis in mutant Arabidopsis plants.

Two newly identified LD proteins were selected for further characterization using cellular, genetic, and proteomic approaches, namely LIPID DROPLET PROTEIN OF SEEDS (LDPS) and EARLY RESPONSIVE TO DEHYDRATION 7 (ERD7). LDPS is a seed-specific protein that was shown to localize to LDs via a hydrophobic hairpin motif. *ldps* mutant seeds and seedlings had abnormally small LDs that failed to fuse together during early seedling growth, suggesting that LDPS regulates the size and number of LDs, perhaps via protein-mediated LD-LD fusion. ERD7 was detected in the LD proteome of drought-stressed Arabidopsis and was shown to localize to LDs via its C-terminal senescence domain. A

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yeast two-hybrid screen indicated that ERD7 interacts with various stress-related proteins, suggesting that ERD7 is an important link between LDs and the plant stress response. Taken together, these and other results for LDPS and ERD7 provide new insights to the molecular mechanisms underlying the roles of LDs in plant cells. Furthermore, the characterization of new LD proteins will inform future bioengineering efforts targeted at increasing oil yields in plants.

Curriculum Vitae: Nathan completed his B.Sc. (Hons.) in Biochemistry at Queen's University in 2014 where he conducted research in the lab of Dr. William Plaxton. He completed his M.Sc. in Molecular Genetics in 2018 at the University of Toronto under the supervision of Dr. James Rini. He then began his Ph.D. degree in September 2018 under the supervision of Dr. Robert Mullen.

Awards: Canadian Society for Plant Biologists (CSPB) award for outstanding poster presentation at the Plant Biology 2022 meeting (2022); Dr. Donald Robert Phillips Molecular and Cellular Biology Scholarship (2022); ICI Scholarship in Biotechnology (2022); Director's award for best poster presentation at the CSPB Eastern Regional Meeting (2021); President's award for best oral presentation at the CSPB General Meeting (2021); Molecular and Cellular Biology Plant Science Travel Grant (2021); Ontario Graduate Scholarship (2020, 2021); University of Guelph Graduate Tuition Scholarship (2018)

Publications: Krawczyk, HE, Sun, S, **Doner, NM**, Scholz, P, Schmitt, K, Valerius, O, Hillmer, S, Braus, G, Mullen, RT, Ischebeck, T. SLDP and LIPA mediate lipid droplet-plasma membrane tethering in Arabidopsis thaliana. (2022) The Plant Cell 34(6): 2424–2448.

Pyc, M, Gidda, SK, Seay, D, Esnay, N, Kretzschmar, FK, Cai, Y, **Doner, NM**, Greer, MS, Hull, JJ, Coulon, D, Bréhélin, C, Yurchenko, O, de Vries, J, Valerius, O, Braus, GH, Ischebeck, T, Chapman, KD, Dyer, JM, Mullen, RT. LDIP cooperates with SEIPIN and LDAP to facilitate lipid droplet biogenesis in plants (2021) The Plant Cell 33(9):3076-3103.

Doner, NM, Seay, D, Mehling, M, Sun, S, Gidda, SK, Schmitt, K, Braus, GH, Ischebeck, T, Chapman, KD, Dyer, JM, Mullen, RT. Arabidopsis thaliana EARLY RESPONSIVE TO DEHYDRATION 7 localizes to lipid droplets via its senescence domain. (2021) Frontiers in Plant Science 12:658961.

Kretzschmar, FK*, **Doner, NM***, Krawczyk, HE, Scholz, P, Schmitt, K, Valerius, O, Braus, GH, Mullen, RT, Ischebeck, T. Identification of new, low-abundance lipid droplet proteins Arabidopsis seeds and seedlings. (2020) Plant Physiology 182(3):1326-45. (*authors contributed equally).

Price, AM, **Doner, NM**, Gidda, SK, Jambunathan, S, James, CN, Schami, A, Yurchenko, O, Mullen, RT, Dyer, JM, Puri, V, Chapman, KD. Mouse Fat-Specific Protein 27 (FSP27) expressed in plant cells localizes to lipid droplets and promotes lipid droplet accumulation and fusion. (2020) Biochimie 169:41-53.