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*

MICHAL PYC

on Monday, June 18, 2018 at 9:30 a.m. in SSC 1511

Thesis Title: **The dynamics of leaf lipid droplets and the identification of a novel lipid droplet protein in *Arabidopsis thaliana.***

Examination Committee:

Dr. A. Bendall, Dept. of Molecular and Cellular Biology (Exam Chair)
Dr. I. Tetlow, Dept. of Molecular and Cellular Biology
Dr. S. Rothstein, Dept. of Molecular and Cellular Biology
Dr. A. Nassuth, Dept. of Molecular and Cellular Biology
Dr. O. Rowland, Dept. of Biology, Carleton University

Advisory Committee:
Dr. R. Mullen (Adv.)
Dr. I. Tetlow
Dr. S. Rothstein

Abstract: Lipid droplets (LDs) are unique, ER-derived organelles found in all eukaryotic organisms. Structurally, LDs contain a core of energy-rich neutral lipids, such as triacylglycerols (TAGs), that is enclosed by a single phospholipid monolayer and decorated with a diverse array of 'coat' proteins. In plants, LDs are found in high abundance in pollen and oilseeds, and are ultimately mobilized in the latter as a carbon and energy source during post-germinative growth. While some of the proteins associated with seed LDs have been well-characterized, relatively little is known about LDs in other plant tissue/cell types, particularly in vegetative organs, such as leaves, and the proteins that govern their formation and activity. In this study, *Arabidopsis thaliana* was used as a model organism to investigate LD activity and to identify and characterize novel LD coat proteins. Using a variety of imaging techniques, the abundance of LDs in leaf cells was found to vary throughout the diurnal cycle and increased in response to both heat and cold stress treatments. Extended light and dark treatments were also shown to affect leaf LD abundance. The number of LDs in leaves appears to be regulated, at least in part, by lipid droplet associated proteins (LDAPs), a three-member family of constitutively-expressed LD coat proteins. Loss-of-function *Arabidopsis ldap* mutants were found to produce fewer LDs in leaf cells and exhibited a marked decrease in LD proliferation during temperature stress treatments. Putative interactors of LDAP3 were also identified using a Y2H assay and investigated to determine their possible involvement in LD-related processes. One of these interactors, termed LDAP-interacting protein (LDIP) was determined to be a ubiquitously-expressed, *bona fide* LD coat protein that regulates LD size and neutral lipid homeostasis in both leaves and seeds. Subsequent localization experiments indicated that LDAP3 recruits LDIP to the LD surface during biogenesis and that LDIP may modulate the phospholipid composition of the LD monolayer. This research has allowed for the generation of a model for plant LD formation in non-seed tissues.

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Curriculum Vitae: Mike obtained his B.Sc. (Biology, Honours) with a minor in Psychology, and his M.Sc. (Biology) at Queen's University, in 2012 and 2014, respectively. In September 2014, Mike began his Ph.D. program at the University of Guelph, with Dr. Robert Mullen as his advisor.

Awards: Dr. Donald Robert Phillips Scholarship (2018), Dr. and Mrs. Kenneth F. Gregory OGS Award (2018); Ontario Graduate Scholarship (2017); I.C.I. Scholarship in Biotechnology (2017 and 2018); Canadian Society of Plant Biologists Director's Award for Best Oral Presentation (2016); Roche Molecular Biochemicals Award of Excellence (2015), for best 'brown bag seminar'.

Publications:

Huang, L., Pyc, M., Alseekh, S., Aarabi, F., McCarty, D., Crecy-Legard, V., Gregory, J., Henry, C., Fernie, A., Mullen, R.T., and Hanson, A.D. (2018). A plastidial pantoate transporter with a potential role in pantothenate synthesis. *Biochemical Journal,* BCJ-2017-0883R1. *In Press.*

Pyc, M., Cai, Y., Gidda, S., Yurchenko, O., Park, S., Kretschmar, F, Ischebeck, T., Valerius, O., Braus, G., Chapman, K.D, Dyer, J.M., and Mullen, R.T. (2017). An *Arabidopsis* LDAP-interacting protein (LDIP) influences lipid droplet size and neutral lipid homeostasis in both leaves and seeds. *The Plant Journal*, 92(6): 1182-1201.

Pyc, M., Cai, Y., Greer, M.S., Yurchenko, O., Chapman, K.D., Dyer, J.M., and Mullen, R.T. (2017). Turning Over a New Leaf in Lipid Droplet Biology. *Trends in Plant Science*, 22(7): 596-609.

Ying, S., Hill, A.T., Pyc, M., Anderson, E.A., Snedden, W.A., Mullen, R.T., She, Y-M., and Plaxton, W.C. (2017). Regulatory Phosphorylation of Bacterial-type PEP Carboxylase by the Ca2+-Dependent Protein Kinase RcCDPK1 in Developing Castor Oilseeds. *Plant Physiology*, 174(2): 1012-1027.

Chapman, K.D., Mullen, R.T., Pyc, M., and Dyer, J.M. (2017) Methods for increasing oil content in plant tissues by suppressing hydrophobic lipid droplet protein (2017). U.S. Patent Application 15/624,495.

Fedosejevs, E. T., Gerdis, S. A., Ying, S., Pyc, M., Anderson, E. M., Snedden, W. A., Mullen, R.T., She, Y.M., and Plaxton, W. C. (2016). The calcium-dependent protein kinase RcCDPK2 phosphorylates sucrose synthase at Ser11 in developing castor oil seeds. *Biochemical Journal*, 473(20), 3667-3682.

Gidda, S.K., Park, S., Pyc, M., Yurchenko, O., Cai, Y., Wu, P., Andrews, D.W., Chapman, K.D., Dyer, J.M., and Mullen, R.T. (2016). Lipid droplet-associated proteins (LDAPs) are required for the dynamic regulation of neutral lipid compartmentation in plant cells. *Plant Physiology*, 170: 2052-2071.

Cai, Y., Goodman, J. M., Pyc, M., Mullen, R. T., Dyer, J. M., and Chapman, K. D. (2015). Arabidopsis SEIPIN Proteins Modulate Triacylglycerol Accumulation and Influence Lipid Droplet Proliferation. *The Plant Cell*, 27(9): 2616-2636.