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 ***Master of Science*** *of*

MATTHEW CARSWELL

on Friday, January 8, 2021 at 9:30 a.m. (online)

Thesis Title: **A regulatory role for 14-3-3 proteins in the starch biosynthetic pathway of *Zea mays*.**

Examination Committee:

Dr. Matthew Kimber, Dept. of Molecular and Cellular Biology (Exam Chair)
Dr. Ian Tetlow, Dept. of Molecular and Cellular Biology
Dr. Steffen Graether, Dept. of Molecular and Cellular Biology
Dr. Nina Jones, Dept. of Molecular and Cellular Biology

Advisory Committee:
Dr. M. Emes (Advisor)
Dr. I. Tetlow
Dr. S. Graether

Abstract: Starch sustains most of humanity's caloric requirements and serves as a renewable raw material for several industrial applications. During carbon partitioning of photoassimilates, storage starch is deposited within the amyloplasts of heterotrophic tissues such as seeds, roots, and stems. Two glucose polymers, amylose and amylopectin, arrange into highly compact semi-crystalline starch granules, which are osmotically inactive and capable of long-term energy storage. Synthesizing amylopectin requires the coordinated action of several enzyme classes including starch synthases (SSs), starch branching enzymes (SBEs) and starch debranching enzymes (DBEs). In *Zea mays*, previous studies have shown phosphorylation directs the assembly of a large heteromeric enzyme complex (HEC) between two starch synthases and a starch branching enzyme. The trimeric complex that forms (SSI-SSIIa-SBEIIb) subsequently becomes entrapped within the amylopectine matrix of the growing nascent granule. The precise nature of these interactions remains elusive. However, a class of regulatory proteins called 14-3-3 are speculated to facilitate these protein-protein interactions (PPI). Ubiquitous within all eukaryotes, 14-3-3s bind to numerous targets at specified phosphorylated motifs. Such interactions can affect enzyme activities, alter subceullar localization or provide scaffolding for PPI. Here, a combination of bioinformatics and *in vitro* approaches were used to establish a regulatory role for 14-3-3 proteins in starch biosynthesis. Biochemical analysis and tandem mass spectrometry (MS/MS) identified a single 14-3-3 isoform, GF14-6, as becoming subcellularly localized within the maize amyloplast stroma but was absent from the starch granule matrix. *GF14-6* was cloned, and the recombinant protein expressed with an N-terminal His-tag. Affinity-bait chromatography identified SSI, SBEIIa and SBEIIb as interactors of recombinant GF14-6. Reciprocal experiments using an S-tagged recombinant SBEIIb also demonstrated interactions with endogenous 14-3-3s and that this was stimulated under phosphorylating conditions.

Curriculum Vitae: Matt completed his Bachelor of Science (Hons.) at the University of Guelph in the summer of 2017, and then began his MSc in the lab of Dr. Mike Emes in the fall of the same year.