



**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*

**GREGORY MACNEILL**

**on Wednesday, January 6, 2021 at 9:30 a.m. (online)**

**Thesis Title:** Post-translational regulation of starch branching enzyme 2.2 from *Arabidopsis thaliana*

**Examination Committee:**

Dr. Annette Nassuth, Molecular and Cellular Biology (Exam Chair)  
Dr. Ian Tetlow, Dept. of Molecular and Cellular Biology  
Dr. Robert Mullen, Dept. of Molecular and Cellular Biology  
Dr. George van der Merwe, Dept. of Molecular and Cellular Biology  
Dr. Matthew Gentry, Dept. of Molecular and Cellular Biochemistry  
University of Kentucky (External Examiner)

**Advisory Committee:**

Dr. Mike Emes (Co-Advisor)  
Dr. Ian Tetlow (Co-Advisor)  
Dr. Robert Mullen  
Dr. Barry Micallef

**Abstract:** Starch is the most abundant carbohydrate storage molecule in plants. It is produced in chloroplasts in the light providing a source of fixed carbon when photosynthesis is not occurring. Its synthesis involves the coordinated activity of multiple enzyme classes, including starch branching enzymes (SBE) which introduce branch points onto linear glucan chains. Two functional SBE isoforms (SBE2.1 and SBE2.2) exist in *Arabidopsis*, with SBE2.2 accounting for most of the branching activity. Loss of both isoforms results in the abolition of starch production, with fixed carbon accumulating in high levels of maltose/maltodextrins. Previous studies have identified the role of protein phosphorylation in formation of heteromeric enzyme complexes between SBEs and starch synthases (SS). Here, a combination of bioinformatics, *in vitro* analysis, and *in vivo* complementation approaches were used to identify sites of post-translational regulation of AtSBE2.2 and analyse their importance. Two previously identified phosphorylation sites were investigated for their role in catalytic activity and protein-protein interactions. Phosphorylation of Ser<sup>290</sup> was shown to be involved in interactions with SS2, while phosphorylation of Ser<sup>301</sup> may affect catalytic activity by stabilizing a glucan binding site through interaction with a conserved domain (KCRRR). Redox regulation of recombinant SBE2.2 was investigated and three cysteines critical for enzyme activity (Cys<sup>375</sup>, Cys<sup>515</sup> and Cys<sup>744</sup>) were identified. Confounding factors in methodology commonly used to modify redox state and investigate SBE activity were identified, casting doubt on previous reports. Site directed mutagenesis of phosphorylation sites, the KCRRR domain and Cys<sup>744</sup> was performed and the products were expressed in either a wildtype or an SBE-null *Arabidopsis* genotype. Starch production and growth were partially restored by

complementation of the SBE-null genotype with the WT SBE2.2 protein, as well as by some of the mutated sequences. The results support the hypothesis that phosphorylation of Ser<sup>290</sup> and Ser<sup>301</sup> play distinct roles, governing protein interactions and glucan binding, respectively. Mutation of Cys<sup>744</sup> had no apparent effect on growth or starch production. Expression of SBE2.2 with a mutation to the KCRRR domain in the Landsberg *erecta* genotype resulted in unique floral and silique morphology, as well as increased seed production, with potential applications for crop production.

**Curriculum Vitae:** Greg obtained both his B.Sc.(H), Biochemistry/Biotechnology, and M.Sc., Biology, at the University of Waterloo, in 2008 and 2011, respectively. In the fall of 2014, he entered the Ph.D. program at the University of Guelph, under the supervision of Drs. Mike Emes and Ian Tetlow.

**Awards:** Canadian Society of Plant Biologists – Director’s Award – Student Poster Award, Eastern Regional Meeting (November 2018)

Canadian Society of Plant Biologists – President’s Award - Student Poster Award, Honourable Mention, Annual Meeting (July 2018)

Canadian Society of Plant Biologists – Director’s Award - Student Oral Presentation Award, Honourable Mention, Eastern Regional Meeting, (July 2017)

Ontario Graduate Scholarships (2015-2016 and 2016-2017)

Roche Molecular Biochemicals – Award for Best Graduate Student Seminar Presentation (April 2015)

**Publications:** Emes MJ, MacNeill GJ, Tetlow IJ. Heteromeric Protein Interactions in Starch Synthesis (Chapter 11). In *Enzymology of Complex Alpha-Glucans* (Nitschke, F. (Ed.)). CRC-Press, Taylor & Francis Group. Boca Raton. *In press*.

MacNeill, GJ, Mehrpouyan, S, Minow, MAA, Patterson, JA, Tetlow, IJ, Emes, MJ (2017) Starch as a source, starch as a sink: the bifunctional role of starch in carbon allocation, *Journal of Experimental Botany*, Volume 68, Issue 16, Pages 4433–4453.

Gerhardt, K.E., MacNeill, G.J., Gerwing, P.D., Greenberg, B.M. (2017) Phytoremediation of Salt-Impacted Soils and Use of Plant Growth Promoting Rhizobacteria (PGPR) to Enhance Phytoremediation. (Chapter 2). In *Phytoremediation: Management of Environmental Contaminants, Volume 5*, (Ansari, A., Gill, S., Gill, R., R. Lanza, G., and Newman, L. (Eds.)). Springer. (pp 19-51).

Greenberg, BM, X-D Huang, K Gerhardt, P Mosley, X-M Yu, S Liddycoat, X Lu, B McCallum, G MacNeill, N Knezevich, M Hannaberg, P Gerwing, T Obal and B Chubb (2012) Phytoremediation of petroleum and salt impacted soils: A scientifically-based innovative remediation process. *Proceedings of the Thirty-fifth AMOP Technical Seminar on Environmental Contamination Response*: 420-433.