ANNOUNCING

Doctoral Dissertation Defense

Nathan Eaton

“Dynamin 2-Dependent Endocytosis in Platelet and Megakaryocyte Signaling”

Candidate for Doctor of Philosophy in Cell Biology, Neurobiology, and Anatomy
Graduate School of Biomedical Sciences
Medical College of Wisconsin

Committee in Charge:
Hervé Falet, PhD (Advisor and Chair)
Nancy Dahms, PhD
Sridhar Rao, MD, PhD
Xuelin Lou, PhD
Sidney Whiteheart, PhD

Tuesday, May 25th, 2021, 11:00 AM (CST)

Live Public Viewing:
https://mcw.hosted.panopto.com/Panopto/Pages/Viewer.aspx?id=22f78715-ebce-4e1a-982e-ad02010897a2
GRADUATE STUDIES

- Introduction to Biomedical Research
- Molecular and Cellular Biology
- Biochemistry of the Cell
- Techniques in Molecular & Cell Biology
- Mechanisms of Cellular Signaling
- Classical and Molecular Genetics
- Ethics & Integrity in Science
- Advanced Cell Biology
- Current Concepts of Cancer Biology
- Research Ethics Discussion Series
- Cellular Signal Transduction
- Reading and Research
- Doctoral Dissertation
DISSERTATION
“Dynamin 2-Dependent Endocytosis in Platelet and Megakaryocyte Signaling”

Abstract:

Receptor-mediated endocytosis (RME) contributes to numerous physiological functions, including receptor signaling, cell adhesion, and migration. In megakaryocytes (MKs) and platelets, RME mediates the internalization and degradation of circulating thrombopoietin (TPO) and the uptake of plasma-derived fibrinogen into α-granules, thereby regulating hematopoiesis and hemostasis. RME requires the membrane fission activity of the large GTPase dynamin 2 (DNM2). Mice lacking DNM2 in MKs and platelets (Dnm2<sup>Plt<sup>−/−</sup> mice) exhibit hallmarks of myelofibrosis (MF), including elevated plasma thrombopoietin (TPO), MK and hematopoietic stem and progenitor cell (HSPC) expansion, bone marrow fibrosis, extramedullary hematopoiesis, and splenomegaly. We sought to determine whether the hyperproliferative phenotype of Dnm2<sup>Plt<sup>−/−</sup> mice was due to constitutive JAK2-STAT activation in MKs or elevated circulating TPO levels. Unstimulated Dnm2<sup>Plt<sup>−/−</sup> platelets showed constitutively phosphorylated STAT3, but impaired signaling following TPO stimulation. To assess the effects of elevated TPO on HSPCs, we crossed Dnm2<sup>Plt<sup>−/−</sup> mice in the Mpl<sup>−/−</sup> background to generate mice lacking DNM2 in MKs and platelets, and Mpl ubiquitously, including HSPCs. Mpl deletion abrogated the MK and HSPC compartments of Dnm2<sup>Plt<sup>−/−</sup> mice, confirming its role in the observed MK and HSPC hyperplasia. Mpl deletion also induced severe anemia due to impaired erythroid maturation, leading to premature lethality. The data shows that DNM2-dependent TPO uptake by MKs and platelets is required for normal hematopoiesis.

In addition to their hyperproliferative phenotype, Dnm2<sup>Plt<sup>−/−</sup> mice display severe macrothrombocytopenia, due to impaired platelet production by Dnm2<sup>Plt<sup>−/−</sup> MKs. We sought to determine the role of DNM2 in hemostasis and thrombosis using a combination of genetic and pharmacological approaches. Dnm2<sup>Plt<sup>−/−</sup> mice displayed a severe bleeding diathesis associated with defective platelet adhesion and aggregation to immobilized collagen under arterial shear flow in vitro. Inhibition of DNM2 in platelets diminished intracellular GPVI signaling and activation of the proximal kinase Lyn and showed GPVI-specific functional defects in GPIIba downregulation, spreading, α-granule release, and integrin αIIbβ3 activation. Loss of DNM2 also prevented the endocytosis and incorporation of fibrinogen into platelet α-granules. Taken together, the data illustrate a requirement for DNM2 in platelet GPVI signaling and hemostatic function.

We generated mice lacking JAK2 in MKs and platelets (Jak2<sup>Plt<sup>−/−</sup> mice) to investigate the role of JAK2 in platelet function and hemostasis. Jak2<sup>Plt<sup>−/−</sup> mice developed thrombocytosis and MK hyperplasia associated with bleeding and unstable thrombus formation in vivo. Jak2<sup>Plt<sup>−/−</sup> platelets showed defects in aggregation to collagen under arterial shear and in static spreading on collagen or in response to the GPVI-specific agonist collagen related peptide (CRP). Jak2<sup>Plt<sup>−/−</sup> platelets further displayed impairments in α-granule secretion, integrin αIIbβ3 activation, and aggregation in response to collagen and CRP or to the CLEC-2 agonist rhodocytin. Loss of JAK2 attenuated intracellular GPVI signaling and PLC-γ2 activation following CRP stimulation. The data identifies a novel role for JAK2 in platelet immunoreceptor tyrosine-based activation motif (ITAM) signaling in mouse hemostasis and suggests a contribution of JAK2 in disease-associated thrombosis.
CURRICULUM VITAE

Nathan Eaton, BSc
PhD Graduate Student, Medical College of Wisconsin
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EDUCATION

08/2016–Present
PhD Graduate Student, Interdisciplinary Biomedical Sciences Program (IDP), Department of Cell Biology, Neurology, and Anatomy, Medical College of Wisconsin, WI (Mentor: Hervé Falet, PhD. Expected graduation June 2021).

09/2013–12/2015
BSc in Biology, Cell & Molecular with Pre-Veterinary option, University of Wisconsin-Milwaukee, WI

09/2011–05/2013
Chemical Engineering (2011–2012), Animal Sciences (2012–2013), Oregon State University, OR

RESEARCH

03/2017–Present
Doctoral Dissertation Research
Versiti Blood Research Institute & Medical College of Wisconsin, Milwaukee, WI
Role of DNM2-dependent endocytosis and JAK2 in platelet GPVI signaling and Mpl-mediated bone marrow homeostasis
*Mentor: Hervé Falet, PhD, Investigator & Associate Professor*
My research involved determining the role of the endocytic GTPase dynamin 2 (DNM2) and endocytosis of the thrombopoietin (TPO) receptor Mpl in mouse platelet function and collagen receptor GPVI signaling, and in bone marrow homeostasis. Additionally, the contribution of the tyrosine kinase JAK2 in thrombosis and the GPVI pathway was also investigated. My responsibilities in these projects included the writing and revisions of their manuscripts and in data collection, I performed a variety of molecular and biochemical techniques to examine animal hematological parameters, in vivo platelet/hemostasis thrombosis assays, in vitro platelet function experiments, image/software analysis, and mentored 6 trainees.

01/2017–03/2017
Graduate Doctoral Rotation Student
Versiti Blood Research Institute & Medical College of Wisconsin, Milwaukee, WI
Role of sialyltransferases in bone marrow maintenance and hematopoiesis
*Mentor: Karin Hoffmeister, MD, Senior Investigator & Professor*
Investigated the contributions of sialic acid glycosylation in bone marrow megakaryocyte and platelet homeostasis using a Cre-LoxP mouse model lacking ST3GAL1, a sialyltransferase, in megakaryocytes and platelets. My duties in this research were to determine the role of ST3GAL1 in bone marrow inflammation and included mouse work, femur bone marrow collection, immunohistochemistry, fluorescent microscopy, and immunoblot analysis.

10/2016–12/2016
Graduate Doctoral Rotation Student
Medical College of Wisconsin, Milwaukee, WI
Viral vector engineering in ocular gene therapy
*Mentor: Daniel Lipinski, MSc, DPhil, Assistant Professor*
This research involved the cloning of single stranded DNA viral elements from different circoviruses (Canine, Human, and Porcine circovirus strains 1 and 2) into phagmids in order to determine their efficiencies as potential viral vectors in
gene therapy via cultured cell transfections. My responsibilities here combined molecular and viral biology techniques including vector construction and cloning, DNA isolation/PCR and cell culturing.

**08/2016–10/2016**
Graduate Doctoral Rotation Student  
Medical College of Wisconsin, Milwaukee, WI  
Exosomal microRNAs and ROS generation in Duchenne’s Muscular Dystrophy  
**Mentor: Jennifer Strande, MD, PhD, Associate Professor**  
Examined the contribution of exosomal microRNAs in Duchenne’s Muscular Dystrophy using patient-derived induced pluripotent stem cells. My work in this research included cell culturing, microRNA isolation, qRT-PCR, and the use of pharmacological inhibitors on exosomal microRNA secretion.

**08/2016–Present**  
PhD Graduate Student, Medical College of Wisconsin, Milwaukee, WI

**04/2015**  
Volunteer Toxicology Research Assistant  
University of Wisconsin-Milwaukee, WI  
Titanium Dioxide nanoparticle toxicity in C. elegans  
**Mentor: Hongbo Ma, PhD, Assistant Professor**  
Studied effects of TiO₂ nanoparticle poisoning on the nematode C. elegans samples by quantitative measurement of reproduction and mortality using predominantly microscopy.

**09/2014–09/2015**  
Independent Cell Biology Research Assistant  
University of Wisconsin-Milwaukee, WI  
Carbonic Anhydrase-Transporter interactions in Arabidopsis thaliana  
**Mentor: Dazhong Zhao, PhD, Associate Professor**  
Investigated degrees of genetic interaction between certain bicarbonate transporter-like genes and beta-family carbonic anhydrases in developing Arabidopsis ovules via transporter-like null mutants, mutant β-CA enzymes, and gene analysis. My responsibilities included molecular biology wet benchwork and techniques including DNA isolation/PCR and immunofluorescent microscopy, as well as student mentoring.

**OTHER WORK**

**07/2015**  
Molecular Biology Mentoring, University of Wisconsin-Milwaukee, Milwaukee, WI  
High School Student Mentor on Cellular & Molecular Biology Laboratory Techniques  
Mentored high school summer students in various molecular biology laboratory techniques performed in Arabidopsis thaliana biological research including gardening, seed sterilization and plating, and DNA isolation/PCR.

**Other Positions**

**09/2016–Present**  
Senior member, Enhancing Scholarly Culture Committee, Medical College of Wisconsin, Milwaukee, WI

**AWARDS & HONORS**

**2020**  
HTRS 2021 Abstract Award, Hemostasis & Thrombosis Research Society (HTRS) 2021 Scientific Symposium

**2020**  
Graduate Student Travel Award  
Graduate Student Office, Medical College of Wisconsin, Milwaukee, WI

**2020**  
ASH Abstract Achievement Award, American Society of Hematology (ASH) Virtual Annual Meeting

**2019**  
ASH Abstract Achievement Award, American Society of Hematology (ASH) Annual Meeting, Orlando, FL

**2019**  
Third Best Poster Award Winner, 2019 Great Lakes Translational Glycomics Symposium, Versiti Blood Research Institute, Milwaukee, WI
2019  Graduate Student Travel Award  
Graduate Student office, Medical College of Wisconsin, Milwaukee, WI  

2018  Winner, 2019 Platelets Journal Cover Competition  

2018  Top-rated Abstract, 2018 International Society on Thrombosis and Hemostasis (ISTH) Scientific and Standardization Committee (SSC) Meeting, Dublin, Ireland  

2015  Support for Undergraduate Research Fellows Summer (SURF) Award  
Biology Department, University of Wisconsin-Milwaukee, Milwaukee, WI  

2014  Acknowledgement of Exceptional Performance and Contribution in English for stunning performance in English & writing  
English Department, University of Wisconsin-Milwaukee, Milwaukee, WI  

PEER-REVIEW PRESENTATIONS  

Local/Regional  


National  


PUBLICATIONS  


STUDENTS MENTORED

1. Allison Gerk, BSc, Graduate Student, Medical College of Wisconsin, 01/2021–Present, Capacity (Researching/teaching mentor)
2. Ratnashree Biswas MD, Postdoc, Versiti Blood Research Institute, 09/2019–02/2020, Capacity (Researching/teaching mentor)
3. Lucia Rondini, University School of Milwaukee summer student, Versiti Blood Research Institute, 06/2019–08/2019, Capacity (Researching/teaching mentor)
4. Demetra Korkos BSc, Graduate Student, Medical College of Wisconsin, 10/2019–12/2019, Capacity (Research/teaching mentor)
5. Eric Fellin BSc, Research technician & medical student, Versiti Blood Research Institute, 06/2018–08/2018; 08/2019–07/2020, Capacity (Research/teaching mentor)
6. Lazaro Vergara, Marquette High School summer student, Medical College of Wisconsin ROADS program, 06/2018–08/2018, Capacity (Research/teaching mentor)