

To Aunt Brenda and Uncle Daryl

Acknowledgements

First and foremost, I would like to thank my advisor Dr. John Kuwada. His scientific rigor, insight, and patience were the influences allowing me to grow as a scientist. My time in the Kuwada Lab has given me opportunities to learn ideas and techniques that shape my thinking and attitudes toward experiments and science. In addition, I would like to thank the former post-doc Dr. Hiromi Hirata who initiated the exciting project I pursued during my degree. Hiromi is a constant source of invaluable scientific insight and advice. Another former post-doc, Dr. Louis Saint-Amant, contributed significantly to my project, exploring many early physiological possibilities for my project. From Louis and other students influenced by him, I learned the art and power of electrophysiology. I would like to acknowledge and thank other current and past Kuwada Lab member: Dr. Sean Low, Jeremy Linsley, Kenichi Iwasaki, Shawn Sprague, Alex Midga, Barabara Wagner, Dr. Weibin Zhou, and Dr. Wilson Cui. Dr. Sean Low is a good friend who is a constant source of advice and input. Jeremy Linsley helped significantly to shape the work and ideas behind this thesis. I would also like to thank my committee: Dr. Rich Hume, Dr. Mohammad Akaaboune, Dr. Haoxing Xu, and Dr. Miriam Meisler. Through the years all of you have been sources of excellent advice and constructive criticism that assisted in molding my research and grow as a scientist. In addition, I would like to acknowledge my previous undergraduates, Maggie Burke and

Elizabeth Andraska, who helped through the years. Meeting the wonderful people I have during my years in the Kuwada Lab has been a privilege with many lasting friendships.

Lastly, I want to thank my family. My father James Horstick, mother Sherri Bell, step-father Steve Bell, and sisters Amber Bromley and Alicia Bell. All of you have influenced me in one way or the other throughout the years. I would also like to thank the many friends who were part of this journey with me in some fashion: Dr. Mark Miller, Jason and Kim Amburgey, Dr. Andy Heglie, Ceyda Bilgir, Ed Kiddel, Jordan Ward, Lisa Adams, Dr. Frank Schaffer, Dr. Dan Smith, Dr. Tim Marzullo, Dr. Greg Gage, Dr. Dave Rousso, Dr. Jim Dowling, Jesse Skinner, Dr. Jake Jedynak, Woody Horeuf, Dr. Carl Hansen, Dr. Janet Robishaw, Jasper Humbert, B. Louies, Inna Nechipurenko, Dennis and Susan Amburgey, Ken and Cindy Bylsma, Ingrid Hansen, Dr. Clay Corbin, Dr. Kristen Brubaker, Dr. George Davis, Sarah Liebert, Jessica Beamer, Mary Carr, Greg Sobincinski, Diane Durfy, and any that may have missed this list.

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Abstract

From an ENU mutagenesis screen an embryonic lethal mutant, *mi34*, was isolated with progressive loss of motility during early development. *In vivo* electrophysiology demonstrated that input from the nervous system to the muscle was normal, implicating a defect in muscle. Indeed, the contraction of mutant skeletal muscle in response to depolarization was significantly reduced compared to siblings. *In vivo* calcium imaging demonstrated that evoked activity in mutant muscle produced significantly smaller calcium transients. These findings indicated that the functional defect in *mi34* was in excitation-contraction (EC) coupling, the conversion of electrical to chemical signals in muscle. Positional cloning identified the gene responsible for the *mi34* phenotype, *stac3*. The *stac3* gene encodes a novel adapter-like protein, with no known function. We found the Stac3 protein is specifically expressed in muscle and is co-localized with known EC coupling proteins within triads. Rescue by wildtype *stac3* confirmed that it is responsible for the *mi34* phenotype. Based on the myopathic features of *mi34* mutant skeletal muscle, such as SR swelling and sarcomere breakdown, we investigated whether mutations in human STAC3 could have a role in disease. Human *STAC3* maps within the chromosome locus 12q13-14, a region reported to contain the gene responsible for a rare congenital myopathy known as Native American

Myopathy (NAM). Analysis of seven NAM families, including 5 affected and 16 unaffected individuals identified a G>C base substitution causing a missense mutation within a conserved region of *STAC3*. The missense mutation segregated with the disease families, and was not observed in more than 200 control individuals, indicating that it is responsible for NAM. The homologous tryptophan to serine substitution in zebrafish *Stac3* failed to rescue the abnormal behavior of *mi34* mutant embryos, or fully restore protein localization to triads. Furthermore, *in vivo* calcium imaging of *Stac3*^{NAM} expressing muscle fibers showed reduced calcium transients compared to *Stac3*^{WT} rescued skeletal muscles. These data suggest that *Stac3*^{NAM} is a partial loss of function protein incapable of supporting normal EC coupling.