Cascading Small GTPases in Insulin Action

by

Tingting Xiong

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
(Molecular and Integrative Physiology)
in The University of Michigan
2012

Doctoral Committee:

Professor Alan R. Saltiel, Chair
Assistant Professor Ken Inoki
Associate Professor Jiandie Lin
Professor Jessica Schwartz
Professor John Williams

ACKNOWLEDGEMENTS

I would like to give my deepest gratitude to my advisor, Dr. Alan Saltiel, for his support, guidance and encouragement in science and life. I feel extremely fortunate to have an advisor who gave me both the freedom to explore my ideas and the guidance to not sway from the right path. I would also like to thank my thesis committee members, Drs. Ken Inoki, Jiandie Lin, Jessica Schwartz, and John Williams for their valuable discussions and advice throughout my graduate career.

I would like to thank all of my past and current colleagues in the Saltiel lab, including Dave Bridges, David Buchner, Louise Chang, Xiao-Wei Chen, Alan Cheng, Shian-Huey Chiang, Stuart Decker, Jen Delproposto, Lynn Geletka, Irit Hochberg, Jonathan Hung, Mayumi Inoue, Dara Leto, Irfan Lodhi, Binbin Lu, Carey Lumeng, Melissa McGill, Nicole Maher, Jon Mowers, Xiao-Ling Peng, Christina Sherry, Shannon Reilly, Maeran Uhm, Jamie Yost, and Mei Zhang for their friendship, advice and help during my graduate training. I'm especially grateful to Xiao-Wei for the tremendous amount of help over the course of my graduate career.

Of course, I could not overcome many of the difficulties in the past years without the love and support from my husband, Weiyi. I am indebted to him for his patience and understanding while I pursed my graduate degree.

Finally, I would like to thank my parents for their love and support.

TABLE OF CONTENTS

LIST OF FIGURES

ABSTRACT

Insulin stimulates glucose uptake into adipocytes and muscle cells by stimulating the translocation of the glucose transporter 4 Glut4 from intracellular storage vesicles to the plasma membrane. This process is tightly regulated by insulin signaling cascades in concert with vesicle transport machineries. Small GTPases such as Rab10 and RalA play important roles in insulin-stimulated Glut4 translocation by functioning at the intersection of insulin signaling and vesicle trafficking. A novel effector pull-down assay to evaluate the intracellular activity of Rab10 was developed and used to characterize Rab10 as a functional target of the Akt substrate protein AS160. AS160 contains a GAP (GTPase-activating protein) domain that directly stimulates guanosine triphosphate hydrolysis of Rab10, leading to inactivation of the small GTPase. We show that Akt-catalyzed phosphorylation of AS160 inhibits the GAP activity, as activated Akt relieves the inhibitory effect of AS160 on Rab10 activity.

Activated Rab10 promotes Glut4 translocation to the plasma membrane by recruiting downstream effector proteins. One potential downstream target of Rab10 is the RalA GTPase, which has been reported to mobilize the exocyst complex, targeting complex that guides the Glut4 vesicle to the plasma membrane. Overexpression of constitutively active Rab10 increases RalA activity, while depletion of Rab10 with siRNA-mediated knockdown significantly reduces RalA activation by insulin in adipocytes. Rab10 potentially regulates RalA activity through an as-yet-unidentified guanine-nucleotide exchange factor for RalA, which facilitates guanosine triphosphate binding to RalA, leading to activation of the small GTPase and mobilization of the exocyst complex.

Taken together, these data suggest that a small GTPase cascade of Rab10 and RalA, together with their regulatory GAP and GEF proteins, connects the insulin/Akt signaling pathway with the transport machineries in Glut4 translocation.