CHARACTERIZATION OF MOUSE MODELS TO STUDY PROTEIN TRAFFICKING IN THE EARLY SECRETORY PATHWAY

by

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DEDICATION

To my parents, with my deepest love,
admiration, respect and gratitude.

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ABSTRACT

CHARACTERIZATION OF MOUSE MODELS TO STUDY PROTEIN TRAFFICKING IN THE EARLY SECRETORY PATHWAY

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Chair: David Ginsburg

Newly synthesized proteins destined for secretion or trafficking to the lysosomes, secretory granules, or cell surface, exit the endoplasmic reticulum (ER) in coat protein complex-II (COPII) vesicles. The SEC24 subunit of the inner coat complex is believed to play an important role in cargo binding. COPII vesicle formation is coordinated with cargo selection via the interaction of SEC24 with the cytoplasmic portion of transmembrane cargo proteins. Transmembrane cargo adaptor proteins are also thought to have a critical role in linking soluble cargo proteins to SEC24. However, very few selective cargo receptors have been identified. In mammals, a family of paralogous genes gives rise to four different isoforms of SEC24 (SEC24A-D). Though their relative functions are unknown, these SEC24 isoforms likely expand the variety of COPII vesicles that can be generated and the range of cargo proteins that can be incorporated. In mammals, the LMAN1-MCFD2 complex is the only known example of a specific cargo receptor. LMAN1-MCFD2 is required for the ER-to-Golgi transport of

coagulation factors V and VIII, and mutations in *LMAN1* or *MCFD2* underlie the human bleeding disorder, combined deficiency of factor V and factor VIII (F5F8D).

The work described in this dissertation focuses on the process of selective cargo transport and the role of SEC24 and LMAN1-MCFD2. Through the generation and characterization of a mouse model deficient in SEC24D, we demonstrate that SEC24D is required for very early embryonic development, at least prior to the blastocyst stage. Mice heterozygous for a null allele of *Sec24d* exhibit normal growth, development and survival, and no obvious phenotypic abnormalities. We sought to identify additional cargo proteins whose transport relies on the LMAN1-MCFD2 complex by performing a quantitative mass spectrometry-based proteomic analysis on a mouse model of F5F8D. Our preliminary results suggest that differences between the protein "secretome" of *Lman1* null and wild-type mice should allow us to identify additional LMAN1-dependent cargo proteins. Taken together, these studies lay the groundwork for future studies of selective protein trafficking within the secretory pathway, with important implications for F5F8D and other related human disorders of protein transport.