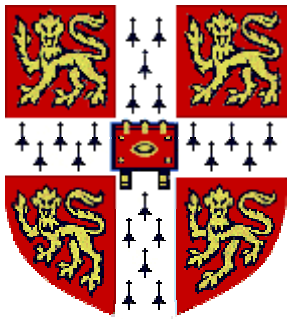
UNIVERSITY OF CAMBRIDGE

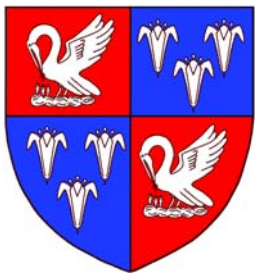


The role of the CapZ complex in vertebrate sarcomere integrity

Submitted in accordance with the requirements of the University of Cambridge for the degree of Doctor of Philosophy by

Annabelle Scott

The Wellcome Trust Sanger Institute



Corpus Christi College

Declaration

This thesis describes work undertaken in the laboratory of Dr Derek Stemple, at the Wellcome Trust Sanger Institute, in fulfillment of the requirements for the degree of Doctor of Philosophy, at Corpus Christi College, the University of Cambridge. This dissertation is entirely my own work and contains nothing that is the outcome of work done in collaboration with others, except as specified in the text. The material described here has not been submitted for a degree or diploma or any other qualification at any other University or Institution. I confirm that this thesis does not exceed 300 single sided pages of double spaced text, or 80,000 words.

Annabelle Scott

October 2007

Summary

Muscle cells provide the contractile force essential for internal organ function and body movement in all complex animals. Skeletal and cardiac muscle are composed of bundles of myofibrils containing highly organized arrays of individual contractile units called sarcomeres. Although many myofibrillar and sarcomeric proteins have been identified, we are only just beginning to elucidate the complex processes and interactions required for muscle formation and function. Mutations in components of the myofibril are responsible for a large proportion of human myopathies, therefore determining how the muscle develops and is maintained will be crucial for understanding the pathology of myopathies and developing treatments for these debilitating disorders.

The analyses of model organisms that carry mutations in sarcomeric and myofibrillar components can assist in our current understanding of muscle development and function. The aim of my project was to positionally clone and characterize the zebrafish (*Danio rerio)* muscle mutant *schnecke*, in order to investigate myofibrillogenesis *in vivo*. I identified a mutation in the *schnecke* locus at the exon 9 donor splice site of *capZα1*. CapZα1 is a subunit of the heterodimer CapZ, which caps the barbed end of polymerizing actin filaments at the sarcomeric Z-line. Characterization of the *schnecke* mutant and morpholino oligonucleotide knockdowns of the other CapZ subunits (CapZα2 and CapZβ) suggests that although loss of CapZ function alone does not affect sarcomere assembly, it is essential for the integrity of the myofibrillar and sarcomeric structure of zebrafish skeletal muscle. Additional double knockdown studies with the other components associated with the actin thin filament (Nebulin and Tropomodulin) indicate that CapZ is required in conjunction with these proteins for maintenance of the Z-line and thin filament assembly. The results presented in this thesis describe an additional role for CapZ in maintaining the stability of the sarcomeric and myofibrillar architecture and provides further insight into muscle integrity and function in vertebrates.

Acknowledgements

Firstly, I would like to thank my supervisor Derek Stemple, for allowing me to do a PhD in his lab, and providing me with guidance, encouragement and a fountain of ideas over the past three and a half years. I would also like to convey special thanks to Elisabeth Busch-Nentwich for assisting me in all aspects of the 'muscle' project, teaching me the art of positional cloning and collaborating on the double knockdown experiments. Thanks to past and present members of Team 31 and Team 30: Gareth, Steve, Huw, Yung Yao, Mariella, Christian, Carlos, Richard, Amanda, Fruzsina, John, Kevin, Emma, Kathy H, Kathy J, Evelyn, Ho-Yon, Ross, Colin, Marie, Isabel, Sam, Wei and Matt for help in proof reading this thesis, as well as plenty of experimental advice and lots of laughs over the years. A special mention to Morgan building friends Arun and Song for always cheering me up and sorting me out in computer crises!! Thanks to the staff in the RSF who have done a great job of keeping my lines healthy and happy: Dee, Rory, Davyd, Paul, Carl and Nick. Thanks to my committee members: Jim Smith, Jyoti Choudhary, John McCafferty and Gavin Wright, who have made me a better scientist (I hope!!!) and taught me how to be more organized and focused. I would also like to thank the Wellcome Trust and the Sanger for giving me the opportunity to work in a fantastic environment. Finally, the biggest thank you goes out to my husband Vardhman, who has supported me through all the ups and downs of this project and never lost faith in me.

Contents

List of Figures

Chapter 1

Chapter 3

Chapter 4

Chapter 5

Chapter 6

Appendix

List of Tables

Appendix