UNIVERSITY OF CALIFORNIA

LOS ANGELES

Genome-wide Analysis of Groucho Function in *Drosophila* Embryogenesis

A dissertation submitted in partial satisfaction

of the requirements for the degree Doctor of Philosophy

in Biochemistry and Molecular Biology

by

Michael Douglas Chambers

2015

© Copyright by

Michael Douglas Chambers

2015**ABSTRACT OF THE DISSERTATION**

Regulatory Function of the Groucho Corepressor in *Drosophila* Development

By

Michael Douglas Chambers

University of California, Los Angeles, CA 2009

Professor Albert J. Courey, Chair

Animal developmental patterning is a complex and intricate process, requiring the interpretation of multiple temporally and spatially regulated signals to define the transcriptional profile of each cell. In *Drosophila*, Groucho (Gro), a transcriptional corepressor, participates in these processes through long-range silencing of distant enhancers. Lacking any innate DNA-binding activity, Gro is targeted to these elements through interaction with multiple repressors.

Using a combination of ChIP-seq and RNA-seq techniques, I sought to characterize Groucho activity at multiple stages spanning the initial nine hours of embryonic development, and thereby gain insight into both the mechanisms and extent of Groucho-mediated repression. These data reveal that Groucho is recruited to thousands of sites at each stage of development. Most Gro peaks are < 1kb in width, consistent with recruitment of one or a small number of Gro complexes to a regulatory element, indicating that spreading of Gro along stretches of chromatin is not a common feature of repression, as previously thought. Gro binding is frequently observed as clusters of discrete peaks, which supports a model in which Gro self-association facilitates crosslinking of non-contiguous regions of chromatin. In some cases, Gro occupancy is observed at transcriptional start sites multiple kilobases from known Gro-binding silencing elements, suggesting that interactions with these sites via crosslinking and looping of chromatin is one method of Gro-mediated repression.

Both Gro gain- and loss-of-function embryos exhibit extensive perturbations in gene expression from the onset of zygotic transcription. Integration of these differentially expressed genes with ChIP-seq-derived Gro occupancy data facilitated the identification of Gro targets within each developmental stage, including known Gro-regulated genes and novel targets. Gro target genes are enriched for transcription factors involved in multiple developmental processes, as well as regulators of multiple signaling pathways. The activity of Gro in regulating both the inputs and outputs of signaling pathways suggests that Gro is utilized to regulate the cellular response to signaling on multiple levels by facilitating crosstalk between pathways.

Gro binds to many genes that it does not repress as exemplified by genes that are regulated by Dorsal, a bifunctional transcription factor that activates some targets and represses others. Gro is essential for repression, but dispensable for activation by Dorsal. However, data presented in this thesis suggest that Dorsal recruits Gro to both the repressed and the activated targets. This shows that Gro recruitment is not sufficient for repression.

Utilizing a technique to isolate and sequence the nascent transcripts of actively transcribed genes provides an accurate profile of the transcriptional activity of the *Drosophila* embryo at multiple stages of development. Nascent transcripts of Gro target genes are significantly enriched for promoter-proximal read density, indicating that Gro targets are enriched for stalled PolII. Coupled with the observation that Gro often binds overlapping or shortly downstream of start sites, we propose that Gro may repress genes by favoring the stalling of PolII, potentially through direct or mediated interaction with PolII or alteration of chromatin structure within transcribed regions.

Finally, I have contributed to a study characterizing the Gro interactome. This study showed that Gro interacts with U1 snRNP. I compared the effect of knocking down a U1 snRNP subunit with that of knocking down Gro on the S2 cell transcription profile. The results suggest that Gro-mediated repression of some targets may require U1 snRNP.

The dissertation of Michael Douglas Chambers is approved.

James Bowie

Catherine Clarke

Albert J. Courey, Chair

University of California, Los Angeles

2015

**TABLE OF CONTENTS**

Abstract of the Dissertation4

Acknowledgements4

Vita4

Chapter 1: Introduction: Groucho – A multifunctional regulator of *Drosophila* development1

Figures2

References3

Chapter 2: Groucho activity in the developing embryo4

Abstract5

Introduction5

Materials and Methods5

Results5

Discussion5

Figures5

References6

Chapter 3: Investigating the dynamics of the embryonic transcriptome4

Abstract5

Introduction5

Materials and Methods5

Results5

Discussion5

Figures5

References6

Chapter 3: Investigating the dynamics of the embryonic transcriptome4

Abstract5

Introduction5

Materials and Methods5

Results5

Discussion5

Figures5

**LIST OF FIGURES**

Figure 1-1 1

Figure 2-1 2

Figure 2-2 3

Figure 2-3 4

Figure 2-4 5

Figure 2-5 6

Figure 2-6 7

Figure 2-7 8

Figure 2-8 9

Figure 2-9 10

Figure 2-10 11

Figure 2-11 12

Figure 2-12 13

Figure 2-13 14

Figure 2-14 15

Figure 2-15 16

Figure 2-16 17

Figure 2-17 18

Figure 2-18 19

Figure 2-19 20

Figure 2-20 21

Figure 2-21 22

Figure 2-22 23

Figure 2-23 24

Figure 2-24 25

Figure 2-25 26

Figure 2-26 27

Figure 2-27 28

Figure 3-1 29

Figure 3-2 30

Figure 3-3 31

Figure 3-4 32

Figure 3-5 33

Figure 3-6 34

Figure 3-7 35

Figure 3-8 36

**LIST OF TABLES**

Table 1-1 1

Table 3-1 2

**ACKNOWLEDGEMENTS**

I would like to thank Dr. Albert Courey for the opportunity to work in his lab and for all the guidance, support, and advice he has offered throughout my graduate career. His laboratory is a wonderful environment for learning and developing skillsets that will be invaluable in the career of any scientist.

I would like to thank Dr. Courey, Wiam Turki-Judeh, Mitchell Kim, and Kenny Chen for their enormous contributions to this project. Additionally, I would like to thank Jesse Cheung and Kenny Chen for working beside me as undergraduate researchers. I would like to thank Pak Kwong and Tak Yu Yau for the opportunity to collaborate on the work presented in chapter 4.

I would like to thank the former Courey lab members Alberto Ponce, Matthew Smith, Clint Winkler, and Dennis Kuo for the guidance, conversation, and friendship they provided during my graduate career.

I would like to thank Sean Gallaher and Kelvin Zhang for their extensive skills and advice. My forays into high-throughput sequencing would have been a much rougher journey without their valuable assistance.

I would like to thank my committee members, James Bowie, Michael Carey, Cathery Clarke, and Volker Hartenstein for their advice and encouragement throughout my time here.

I would like to acknowledge the American Society for Biochemistry and Molecular Biology for allowing me to reprint “The central region of the Drosophila co-repressor Groucho as a regulatory hub” [published online ahead of print October 19, 2015] *J Biol Chem*.

**Vita**

2006 B.S., Chemistry

Georgia Institute of Technology

Atlanta, GA

2007 M.S., Chemistry

Georgia Institute of Technology

Atlanta, GA

2007-2015 Graduate Student Researcher

Dr. Albert Courey Laboratory

University of California, Los Angeles

2010-2015 Teaching Assistant

Protein Purification and Characterization

Cold Spring Harbor Laboratory

Cold Spring Harbor, NY

2012-2015 Teaching Assistant

Biochemical Methods I

University of California, Los Angeles

2007-8, 2012 Teaching Assistant

Biochemistry I

University of California, Los Angeles

**Publications**

Kuo, D., Nie, M., Hoff, P., Chambers, M., Philips, M., Hirsch, A.M., and Courey, A.J. “A SUMO-Groucho Q domain fusion protein: characterization and *in vivo* Ulp-1-mediatec cleavage,” *Protein Expr Purif,* 2011; 76(1):65-71

Kwong, P.N., Chambers, M., Vashisht, A.A., Turki-Judeh, W., Yau, T.Y., Wolschlegel, J.A., and Courey, A.J. “The central region of the Drosophila co-repressor Groucho as a regulatory hub,” [published online ahead of print October 19, 2015] *J Biol Chem*