Role of Bmi-1 in Epigenetic Regulation During Early Neural Crest Development

Thesis by

Jane I. Khudyakov

In Partial Fulfillment of the Requirements

for the Degree of

Doctor of Philosophy

California Institute of Technology

Pasadena, California

2009

(Defended April 30, 2009)

ACKNOWLEDGEMENTS

First and foremost, I would like to express gratitude to my thesis advisor, Marianne Bronner-Fraser, without whose constant support, motivation, encouragement, and unfailing optimism it would have been impossible to navigate through a complex and challenging project. I am also grateful to my co-advisor, Tatjana Sauka-Spengler, for giving me the opportunity to study an exciting developmental question, providing me with a good balance of independence and support, facilitating my development as a mature scientist, and constantly encouraging me to believe in my own abilities. I would also like to thank members of my thesis committee: Scott Fraser, Angela Stathopoulos, Paul Sternberg, and Kai Zinn, for their critical advice and guidance through my graduate project.

I am grateful to my family, especially my father, Igor Khudyakov, for support during my time at Caltech and for the helpful advice and input that they have provided as fellow scientists. I would also like to thank my fiancé, Joseph Schramm, without whose persistent encouragement, support, kindness, and infinite patience I would never have been able to reach this point. I would like to thank the current and former members of the Bronner-Fraser group that have enriched my years in the lab with interesting discussions, helpful suggestions, motivation, friendship, and constant entertainment. In particular, I am exceedingly grateful to the support and friendship of Meghan Adams, Sonja McKeown, Sujata Bhattacharyya, Lisa Taneyhill, Saku Jayasena, Marcos Simoes-Costa, Ed Coles, and Natalya Nikitina. I am also especially grateful for the invaluable assistance of Mary Flowers and Matt Jones during my time in the Bronner-Fraser lab.

I would also like to thank my undergraduate advisor, Larysa Pevny, for inspiring me to pursue a graduate degree at Caltech, as well as Beverly Koller, in whose lab at UNC-Chapel Hill I first acquired an interest in scientific research. Last but not least, I would like to thank Cortney Tribu, Michael Brown, Jennifer Klamo and the wonderful Schramm family for their encouragement, friendship, cheerleading, and support.

ABSTRACT

The neural crest is a transient, multipotent cell population in the developing vertebrate embryo that migrates extensively and contributes to a staggering diversity of cell lineages. Neural crest progenitors are specified at the neural plate border during gastrulation; however, commitment to the neural crest lineage does not occur for some time. I find that the chick neural plate border is characterized by co-expression of several neural crest specifier genes, previously considered "late" signals, which often overlap with "early" neural plate border genes. This suggests that continuously expressed members of the neural crest gene regulatory network may be modulated or repressed for proper maintenance of the multipotent state. Consistent with this possibility, several members of the Polycomb Group of epigenetic repressors are expressed at these early stages. For example, the stem cell factor Bmi-1 is expressed in the neural plate border, dorsal neural folds, and migrating neural crest, but is extinguished in differentiated derivatives. Morpholino-mediated knock-down of Bmi-1 causes early upregulation of *Msx1*, *FoxD3*, and *Sox9* in the chick neurula without affecting cell proliferation. Conversely, Bmi-1 over-expression causes a downregulation of *Msx1*, suggesting that it negatively regulates neural crest network genes. I find that several alternatively spliced variants of Bmi-1 are expressed in the developing chick and that a truncated N-terminal variant, V4, acts as a dominant-negative regulator of the full-length protein by up-regulating *Msx1* expression. Taken together, these results suggest that neural crest progenitors are exposed to numerous signals during gastrulation, some of which are regulated by Polycomb Group factors such as Bmi-1. The activity of Bmi-1, in turn, is modulated by alternatively spliced variants, demonstrating an additional level of regulatory complexity acting during early neural crest development.

TABLE OF CONTENTS

Chapter 4:

Chapter 5:

LIST OF FIGURES

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 5