THE ROLE OF DIET IN THE REGULATION OF *DROSOPHILA* OVARIAN STEM CELLS AND THEIR PROGENY

By

Leesa Marie LaFever

Dissertation

Submitted to the Faculty of the

Graduate School of Vanderbilt University

in partial fulfillment of the requirements

for the degree of

DOCTOR OF PHILOSOPHY

in

Cell and Developmental Biology

December, 2010

Nashville, Tennessee

Approved:

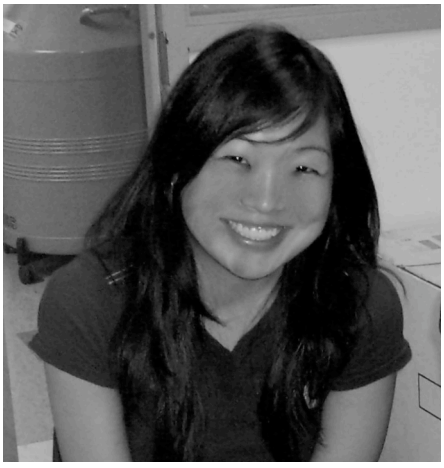
Daniela Drummond-Barbosa, Ph.D.

Chin Chiang, Ph.D

Laura Lee, M.D. Ph.D.

Lila Solnica-Krezel, Ph.D.

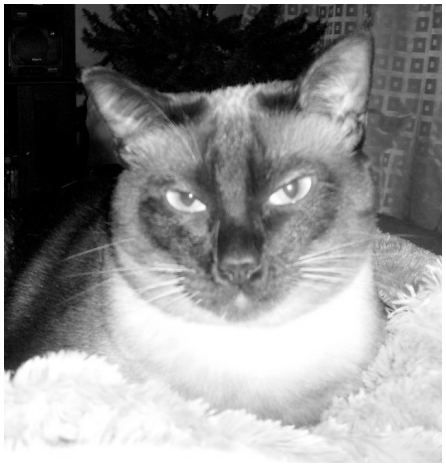
Jim Goldenring, M.D. Ph.D.



To Kimi Song, who kept our lab running smoothly

and

To Mai-Li, my 6:30am alarm clock



ACKNOWLEDGEMENTS

Many have been involved in my graduate school training, but none have played a role as great as my thesis advisor, Dr. Daniela Drummond-Barbosa. I thank Daniela for her patience and encouragement and for occasionally knowing me better than I know myself. She is to be credited greatly for my success as a graduate student.

My lab members have served not only as coworkers, but as friends. I am greatly thankful for the wonderful people who have shared time with me in the Drummond-Barbosa Lab: Dr. Jessica Rivera Von Stetina, Lily Zhang, Dr. Hwei-Jan Hsu, Rachel Gretchen, Dr. Elizabeth Ables, Alexander "Sasha" Feoktistov, Min-Young "Minnie" Kim, Kimberly LaFever, Kate Laws, and Kareshma Mohanty.

My thesis committee tested me, advised me, and wrote recommendation letters, which helped me progress in my scientific career. Thank you to Drs. Chin Chiang, Laura Lee, Lila Solnica-Krezel, and Jim Goldenring.

Thank you to Pierre Coulombe and the members of Johns Hopkins School of Public Health, Department of Molecular Biology and Biochemistry, for providing me with an intellectually stimulating and supportive home in my last year as a graduate student.

I thank Dr. Chris Wright and Kim Kane of the Vanderbilt Developmental Biology Program for two years of support on a Developmental Biology training grant. Thank you to the IGP for one year of support on a Reproductive Biology training grant and to Daniela for funding my last few years on an American Cancer Society grant.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

β-gal- β galactosidase (LacZ)

2D LC-MS/MS- 2-Dimensional Liquid Chromatography Tandem Mass Spectrometry

4E-BP- Eukaryotic translation initiation factor eIF4e binding protein (*Thor* in *Drosophila*)

A2BP1- Ataxin 2-binding protein 1

AdipoR- Adiponectin receptor

Adp- Adipose

AKT/PKB- Protein kinase B

ALS- Acid labile subunit (CG8561)

AMP- 5' adenosine monophosphate

AMPK- 5' adenosine monophosphate-activated protein kinase

AMPKK- AMPK kinase

APC- Anaphase promoting complex

APC- Anaphase promoting complex

arm-LacZ- Armadillo LacZ

Atg1,13, and 17- Autophagy-related genes

ATP- adenosine triphosphate

Bam- Bag of marbles

BGCN- Benign gonial cell neoplasm

BMI- Body Mass Index

Bmm- Brummer

BMP- Bone morphogenic protein

BrdU- 5-bromo-2-deoxyuridine

C. elegans- *Caenorhabditis elegans*

CaMKK- Calmodulin-dependent protein kinase kinase

Cdc- cell division cycle

Cdk- cyclin-dependent kinase

CG5315- *Drosophila* adiponectin receptor (dAdipoR)

CHIPS- Complete Hierarchical Integration of Protein Searches

CNS- central nervous system

CycB- Cyclin B

CycE- Cyclin E

Dap- Dacapo

DAPI- 4',6-diamidino-2-phenylindole

DGC- *Drosophila* Gene Collection

DGRC- *Drosophila* Genomics Resource Center

DIAP- *Drosophila* inhibitor of apoptosis protein

DILP- *Drosophila* insulin-like peptide

DNA- deoxyribonucleic acid

DPP- Decapentaplegic

Drk- *Drosophila* homolog of the SH2-SH3 domain adaptor protein Grb2

DSHB- Developmental Studies Hybridoma Bank

DTC- Distal tip cell

Dup- Double-parked

E2F- E2F transcription factor

E-Cad- E-Cadherin

EdU- 5-ethynyl-2-deoxyuridine

eIF4E- Eukaryotic initiation factor 4E

eIF4G- Eukaryotic initiation factor 4G

ExPASy- Expert Protein Analysis System

F-6-P- Fructose-6-phosphate

Fas III- Fasciclin III

FAT- Focal adhesion kinase targeting

FATC- C-terminal FAT kinase domain

FBF1,2- *C. elegans* relative of *Drosophila* Pumilio

Flp- Flipase

FOXO- Forkhead box, sub-group "O"

FRB- FKBP12-rapamycin binding domain

FRT- Flipase recognition target

FSC- Follicle stem cell

FSH- Follicle-stimulating hormone

G-6-P- Glucose-6-phosphate

GBB- Glass bottom boat

GDNF- Glial cell-line-derived neurotropic factor

Gel-C-MS/MS- In-gel tryptic digestion followed by liquid chromatography-tandem mass spectrometry

GFP- Green fluorescent protein

GLD1,3- *C. elegans* ortholog of *Drosophila* Bicaudal-C

GLUT4- Glucose transporter type 4

GSC- Germline stem cell

HEAT- Huntingtin elongation factor 3, protein phosphatase 2A, PI3-kinase TOR1

Hh- Hedgehog

HS- Heat-shock

HSC- Hematopoietic stem cell

Hts- Hu-li tai shao

hts-RC- Hu-li tai shao ring canal

IDGF- Imaginal disc growth factor

IGF- Insulin-like growth factor

Imp-L2- Ecdysone-inducible gene L2

InR- Insulin receptor

IRS- Insulin receptor substrate

iTRAQ- isobaric tags for relative and absolute quantitation

LamC- Lamin C

LKB1/STK11- Serine/threonine kinase 11

Lsd2,1- Lipid storage droplet 1 and 2

LSM- Laser Scanning Microscope

LSP- Larval serum protein

MAPK- Mitogen-activated protein kinase

Mdm2- Murine double minute oncogene

MRLC- Non-muscle regulatory light chain

MSC- Mesenchymal stem cell

MudPIT- Multidimensional Protein Identification Technology

Nos- Nanos

NSC- Neural stem cell

Orb- OO18-RNA binding

PAQR- Progestin and adipoQ receptor

PBS- Phosphate buffered saline

Pelo- Pelota

PH- Pleckstrin homology domain

PHH3- Phosphohistone H3

PHO36 (IZH2)- Plasma membrane protein involved in zinc homeostasis and osmotin-induced apoptosis

PI3K- Phosphoinositide 3-kinase

PIKK- Phosphoinositol kinase-related kinase

PMSF- Phenylmethanesulfonylfluoride

pSORT- program for subcellular localization prediction

PTEN- Phosphatase and tension homolog

Pum- Pumilio

Put- Punt

PX- Phox homology domain

Raptor- Regulatory-associated protein of TOR

Rb- Retinoblastoma

Rheb- Ras homolog enriched in brain

Rictor- Rapamycin-insensitive companion of TOR

RNA- Ribonucleic acid

RP49- Ribosomal protein 46 (standard control for RT-PCR)

RT-PCR- Reverse Transcription Polymerase Chain Reaction

S. cerevisae- Saccharomyces cerevisiae

S6K- Ribosomal protein S6-p70-protein kinase

Sax- Saxophone

SCF- Skp, Cullin, F-box containing complex

Sema-2a- Semaphorin-2a

Slif- Slimfast

SLIP- Self-Ligation of Inverse PCR Products

STAT3- Signal transducer and activator of transcription 3

Stg- String

TAG- Triacylglycerol or Triglyceride

TargetP- program for subcellular location of eukaryotic proteins

TCA- Tricarboxylic acid cycle

TCA- Trichloroacetic acid

TGFβ- Transforming growth factor β

Tkv- Thickveins

TMHMM- program for prediction of transmembrane helices in proteins

TNFα- Tumor necrosis factor-alpha

TOR- Target of rapamycin

TOS- TOR signaling motif

TSC1/2- Tuberous sclerosis protein 1 and 2 complex

TUNEL- Terminal deoxynucleotidyl transferase dUTP nick end labeling

UAS- Upstream activating sequence

Vas- Vasa

VDRC- Vienna *Drosophila* RNAi Center

Vps34- Vacuolar protein sorting 34

WDTC1- WD and tetratricopeptide repeats 1 (human homolog of adipose)

YP- Yolk protein