ANALYSIS OF THE MOLECULAR MECHANISM OF AUTOPHAGOSOME FORMATION IN SACCHAROMYCES CEREVISIAE

by

Wei-Lien Yen

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy (Molecular, Cellular, and Developmental Biology) in The University of Michigan 2009

Doctoral Committee:

Professor Daniel J. Klionsky, Chair Professor Lois S. Weisman Associate Professor Anuj Kumar Assistant Professor Yanzhuang Wang © Wei-Lien Yen 2009

DEDICATION

This thesis is dedicated to all the people who have helped and supported me during my pursuit of an academic degree through the years: to my parents and grandparents, for their unconditional love and support, to my teachers for teaching and training me, and to TC, for his encouragement and unquestioned love, for being my best friend, husband, and family. Without your support, I would not have made it this far. Finally, to the luck that let me have all these people in my life.

ACKNOWLEDGEMENTS

I would like to thank my mentor, Dr. Daniel J. Klionsky, for giving me invaluable guidance and training. I have learned a lot from his research expertise, his enthusiasm toward science and education, critical thinking, and extraordinary patience and openness. I would also like to thank my thesis committee members, Dr. Lois S. Weisman, Dr. Anuj Kumar and Dr. Yanzhuang Wang, for their insightful suggestions as well as persistent encouragement toward my research.

It has been wonderful to work together with all the former and current members of the Klionsky lab. I would like to thank Dr. Katy Tucker for her help and training during my rotation. My research projects were carried out with the collaborative efforts of many past and current members of the Klionsky lab. I would like to thank them all for their discussion, ideas, and generous assistance whenever I encountered problems in my experiments. I am especially grateful to have Dr. Ju Huang, Dr. Usha Nair, Dr. Congcong He, Dr. Tomotake Kanki, and Zhifen Yang as passionate colleagues and good friends. Special thanks are due to my undergrad supervisors, Dr. Sheng-Ping Huang at Academia Sinica and Dr. Jenn-Kan Lu for showing me what research is about and how fascinating it is. I am also grateful to Dr. John Kuwada and Dr. Amy Chang for giving me the opportunity to do rotations in their labs. Final thanks go to the Rackham Graduate School at the University of Michigan, and the MCDB department for supporting me financially and providing me a research opportunity in completing my PhD study.

Chapter 1 is reprinted from *Physiology*, 2009, Volume 23, Wei-Lien Yen and Daniel J. Klionsky. How to Live Long and Prosper: Autophagy, Mitochondria, and Aging, pg.248-262, Copyright (2008), with the permission of *Physiology*.

Chapter 2 is reprinted from *Molecular Biology of the Cell*, Volume 18, Wei-Lien Yen, Julie E. Legakis, Usha Nair and Daniel J. Klionsky. Atg27 Is Required for Autophagy-dependent Cycling of Atg9, pg. 581-593, Copyright (2007). Dr. Julie E. Legakis performed experiments in Fig. 2.6A and B, and Dr. Usha Nair contributed Fig. 2.3A. I contributed the rest of the data and wrote the paper.

Chapter 3 is reprinted from *Autophagy*, Volume 3, Julie E. Legakis, Wei-Lien Yen and Daniel J. Klionsky. A Cycling Protein Complex Required for Selective Autophagy, pg. 422-432, Copyright (2007), with the permission. I performed experiments in Fig. 3.1, Fig. 3.4D, Fig. 3.5, and Table 3.2 yeast two-hybrid analysis of Atg27 interactions. Dr. Julie E. Legakis contributed the rest of the figures.

Chapter 4 is a manuscript prepared for *Journal of Cell Biology*, 2009, Wei-Lien Yen, Takahiro Shintani, Cao Yang, Brian C. Richardson, Zhijian Li, Frederick M. Hughson, Misuzu Baba, and Daniel J. Klionsky. The Conserved Oligomeric Golgi complex functions as a tethering factor in autophagy. Dr. Takahiro Shintani contributed Fig. 4.1A, 4.1B, 4.1C, 4.1E. Dr. Yang Cao performed the experiments in Fig. 4.2. Dr. Frederick M. Hughson, Dr. Brian C. Richardson, and Zhijian Li provided yeast strains for

iv

autophagy phenotypic analysis. Dr. Misuzu Baba performed the experiments in Fig. 4.3C. I contributed the rest of the data.

TABLE OF CONTENTS

DEDICATIO	Nii	
ACKNOWLE	EDGEMENTSiii	
LIST OF TAE	BLESvii	
LIST OF FIG	URESviii	
CHAPTER		
1	Introduction1	
2	Atg27 Is Required for Autophagy-dependent Cycling of Atg9	
3	A Cycling Protein Complex Required for Selective Autophagy78	
4	The Conserved Oligomeric Golgi Complex Functions as a Tethering	
	Factor in Autophagy108	
5	Conclusions and Contributions150	
REFERENCE	ES156	

LIST OF TABLES

<u>Table</u>

1.1	Evidence to support the ROS theory of aging	26
1.2	Evidence contradicting the ROS theory of aging	27
2.1	Yeast strains used in this study	47
3.1	Yeast strains used in this study	85
3.2	Atg23, Atg27, and Atg9 interactions by yeast two-hybrid assay	100
4.1	Yeast strains used in this study	.114
4.2	PAS localization of Cog1-GFP and Cog6-GFP	135
4.3	PAS localization of Cog2-GFP and <i>cog2-1-GFP</i>	136
4.4	Yeast two-hybrid interactions between lobe A COG components and	
	Atg proteins	138

LIST OF FIGURES

<u>Figure</u>

1.1	Schematic diagram of selective and nonselective autophagy5
1.2	Schematic representation of signaling pathways regulating
	autophagy17
1.3	Pathways activated in response to different degrees of oxidative
	stress
2.1	Atg27 is a type I transmembrane protein46
2.2	The $atg27\Delta$ mutant is defective for autophagy-related pathways54
2.3	The $atg27\Delta$ mutant generates fewer autophagosomes
2.4	Atg27 cycles among the PAS, mitochondria, and Golgi complex61
2.5	Atg27 localizes to mitochondria and the Golgi complex62
2.6	Atg27 functions before Atg1 and is required for Atg9 cycling65
2.7	Binding to PtdIns(3)P is not required for Atg27 function
2.8	Mutation of the Atg27 putative PtdIns(3)P binding site has no effect on
	function
3.1	Atg9, Atg23, and Atg27 colocalize
3.2	Atg9 and Atg23 colocalize at or near the mitochondria
3.3	Atg23 is required for Atg9 localization at the PAS91
3.4	Atg23 localization requires Atg1, Atg9, and Atg2793

3.5	Atg1 and Atg23 are necessary for proper localization of Atg2795
3.6	Atg23 interacts with Atg9 and Atg27100
3.7	Summary of Atg9, Atg23, and Atg27 interactions and requirements for
	proper cycling104
4.1	The Cvt, autophagy and pexophagy pathways are defective in cog
	mutants
4.2	Anterograde movement of Atg9 is partially blocked in the $cog1\Delta$ and
	$cog6\Delta$ strains
4.S1	Antrograde movement of Atg27 is partially blocked in the $cog1\Delta$ and
	$cog6\Delta$ strains
4.3	The <i>cog</i> mutants affect sequestering vesicle formation130
4.4	Cog2 localizes to the PAS
4.S2	Cog1 and Cog6 localize to the PAS134
4.S3	COG partially localizes to the Golgi complex137
4.5	COG subunits associate with Atg proteins141