

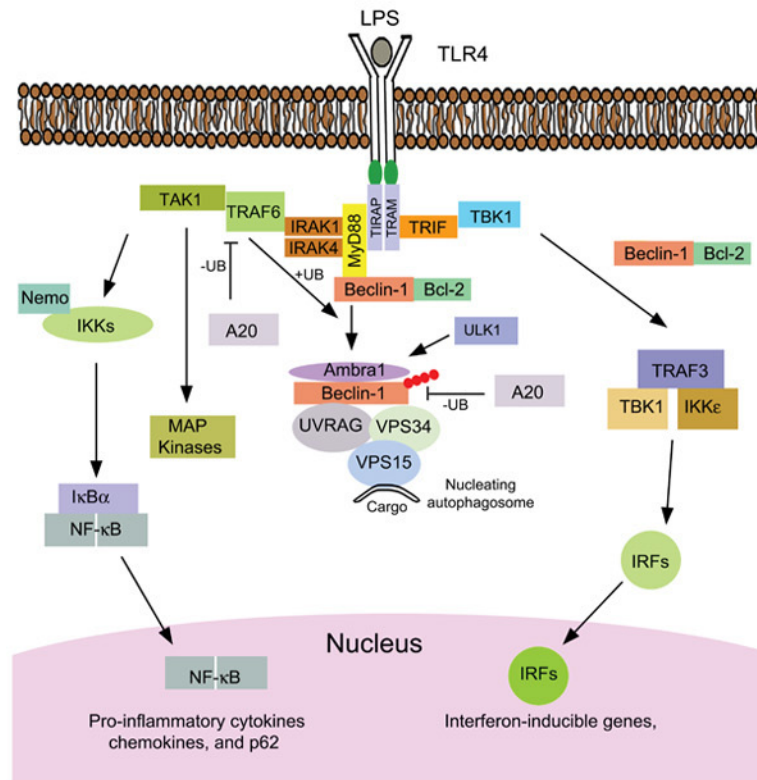
# AUTOPHAGY

CANCER, OTHER PATHOLOGIES,  
INFLAMMATION, IMMUNITY,  
INFECTION, AND AGING

VOLUME 6

EDITED BY

M. A. HAYAT



# AUTOPHAGY



This page intentionally left blank

# AUTOPHAGY

CANCER, OTHER PATHOLOGIES,  
INFLAMMATION, IMMUNITY,  
INFECTION, AND AGING

---

VOLUME 6

*Edited by*

M. A. HAYAT

*Distinguished Professor*

*Department of Biological Sciences*

*Kean University*

*Union, New Jersey*



ELSEVIER

AMSTERDAM • BOSTON • HEIDELBERG • LONDON  
NEW YORK • OXFORD • PARIS • SAN DIEGO  
SAN FRANCISCO • SINGAPORE • SYDNEY • TOKYO

Academic Press is an imprint of Elsevier



Academic Press is an imprint of Elsevier  
32 Jamestown Road, London NW1 7BY, UK  
525 B Street, Suite 1800, San Diego, CA 92101-4495, USA  
225 Wyman Street, Waltham, MA 02451, USA  
The Boulevard, Langford Lane, Kidlington, Oxford OX5 1GB, UK

Copyright © 2015 Elsevier Inc. All rights reserved.

No part of this publication may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, recording, or any information storage and retrieval system, without permission in writing from the publisher. Details on how to seek permission, further information about the Publisher's permissions policies and our arrangements with organizations such as the Copyright Clearance Center and the Copyright Licensing Agency, can be found at our website: [www.elsevier.com/permissions](http://www.elsevier.com/permissions).

This book and the individual contributions contained in it are protected under copyright by the Publisher (other than as may be noted herein).

### Notices

Knowledge and best practice in this field are constantly changing. As new research and experience broaden our understanding, changes in research methods, professional practices, or medical treatment may become necessary.

Practitioners and researchers must always rely on their own experience and knowledge in evaluating and using any information, methods, compounds, or experiments described herein. In using such information or methods they should be mindful of their own safety and the safety of others, including parties for whom they have a professional responsibility.

To the fullest extent of the law, neither the Publisher nor the authors, contributors, or editors, assume any liability for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions, or ideas contained in the material herein.

ISBN: 978-0-12-801032-7

### British Library Cataloguing-in-Publication Data

A catalogue record for this book is available from the British Library

### Library of Congress Cataloging-in-Publication Data

A catalog record for this book is available from the Library of Congress

For information on all Academic Press publications  
visit our website at [store.elsevier.com](http://store.elsevier.com)

Printed and bound in the United States of America



Working together  
to grow libraries in  
developing countries

[www.elsevier.com](http://www.elsevier.com) • [www.bookaid.org](http://www.bookaid.org)

# Dedication

---

To:

Julio A. Aguirre-Ghiso, Patrice Codogno, Eduardo Couve, Ana Maria Cuervo,  
Guido R. Y. De Meyer, Vojo Deretic, Fred J. Dice, William A. Dunn, Jr, Eeva-Lisa Eskelinen,  
Sharon Gorski, Tomotake Kanki, Daniel J. Klionsky, Guido Kroemer, Beth Levine,  
Noboru Mizushima, Yoshinori Ohsumi, Brinda Ravikumar, David Rubinsztein, Isei Tanida,  
Sharon A. Tooze, Herbert W. Virgin, Eileen White, Tamotsu Yoshimori, and others.

The men and women involved in the odyssey of deciphering the molecular  
mechanisms underlying the complexity of the autophagy process that  
governs our lives.

This page intentionally left blank

# Mitophagy and Biogenesis

---

*mTOR and nutrient sensors control  
Autophagy processes in all of our cells;  
Dozens of proteins must play each their role  
To enable engulfment of bad organelles.*

*Those who are young may mistakenly think one  
Is safe and immune to the dangers of aging  
But if you are lacking in proper PINK1  
Mitochondrial fires are already raging.*

*For insight and knowledge some turn to the fly;  
Drosophila's genes can help us discover  
The causes of aggregates seen in the eye,  
And even find drugs to help us recover.*

*Ubiquitin's role in degeneration  
Is to set out red flags on relevant cargo  
Marking the junk that needs degradation  
At a pace that is presto rather than largo.*

*Mitochondria fear Parkin known as PARK2  
Whose ubiquitin tags on two mitofusins  
Determine the fate of one or a slew,  
For a lonely short life of network exclusion.*

*Their fate is ensured by sequestosome 1  
Who recruits membranes rich with LC3-II  
Autophagosome to lysosome a perfect home run  
Cellular housekeeping momentarily through.*

*But the work isn't over and the job isn't done  
Unless Paris is tagged with ubiquitin too  
Then repression is lifted from PGC1  
So biogenesis starts and mitos renew!*

*Roberta A. Gottlieb*



This page intentionally left blank

Life in the Balance, Longevity the Goal  
Self-eating, recycling, cash-for-your clunkers:  
Trade up to the mitochondrial equivalent Prius.  
The road to rejuvenation is paved with destruction  
For clearing the rubble precedes reconstruction  
But remember that life's circular dance  
Depends on opposite forces in balance  
Excess destruction, too much biogenesis,  
Brings heart failure, cancer or neurodegeneries.

*Roberta A. Gottlieb*

This page intentionally left blank

# Autophagy and Cancer

---

When speaking of cancer, autophagy's good  
By culling mitochondria and clearing deadwood  
Autophagy limits the radical chain  
That breaks DNA and mutates a gene  
That makes a cell double, so careless and mean  
In order for cells to malignant transform  
They lose mitochondria except for a few  
Using glycolysis as the source of their fuel  
How they achieve mitochondrial decimation  
Is nothing more than autophagic elimination

Then one cell is many, an ominous mass  
Demanding more glucose, hungry and crass,  
Directing formation of artery and vein  
'Til capsular fibers give way under strain  
Then cancer cells spread so far and so wide  
They demand blood vessels the body provide  
But until those are patent the tumor cells strive  
To rely on autophagy to neatly survive  
The hurdles required for metastasis  
Until blood flow's established for cancerous bliss.

Blocking autophagy sends them over the brink  
And how chloroquine works, we think  
But tumors are slowed by statin's effects  
Which induce autophagy and tumor cell death  
Autophagy's good, autophagy's bad  
The confusion's enough to drive us all mad  
So study we must, and learn ever more  
'Til enlightenment finally opens the door  
Oncologists must heed the tumor's agenda  
And decide whether autophagy is a friend or foe.

*Roberta A. Gottlieb*

This page intentionally left blank

# Contents

---

Foreword xvii  
Preface xix  
Contributors xxiii  
Abbreviations and Glossary xxv  
Autophagy: Volume 1 – Contributions xxxv  
Autophagy: Volume 2 – Contributions xxxvii  
Autophagy: Volume 3 – Contributions xxxix  
Autophagy: Volume 4 – Contributions xli  
Autophagy: Volume 5 – Contributions xliii

1. Introduction to Autophagy: Cancer,  
Other Pathologies, Inflammation,  
Immunity, Infection, and Aging, Volume 6

M.A. HAYAT

Introduction 2  
Specific Functions of Autophagy (a Summary) 4  
Autophagy in Normal Mammalian Cells 4  
Endoplasmic Reticulum Stress and Autophagy 5  
Major Types of Autophagies 7  
Autophagosome Formation 8  
Autophagic Lysosome Reformation 9  
Autophagic Proteins 10  
Monitoring Autophagy 15  
Reactive Oxygen Species (ROS) 15  
Mammalian Target of Rapamycin (mTOR) 16  
Role of Autophagy in Tumorigenesis and Cancer 17  
Role of Autophagy in Immunity 19  
Autophagy and Senescence 20  
Role of Autophagy in Viral Defense and  
Replication 21  
Role of Autophagy in Intracellular Bacterial  
Infection 22  
Role of Autophagy in Heart Disease 23  
Role of Autophagy in Neurodegenerative  
Diseases 24  
Cross-Talk between Autophagy and Apoptosis 26  
Autophagy and Ubiquitination 29

Aggresome: Ubiquitin Proteasome and Autophagy  
Systems 30  
Autophagy and Necroptosis 31  
Mitochondrial Fusion and Fission 31  
Selective Autophagies 32  
References 44

## I

---

### AUTOPHAGY AND MOLECULAR MECHANISMS

2. Regulation of Autophagy by  
Amino Acids

SÉVERINE LORIN, ALFRED J. MEIJER AND  
PATRICE CODOGNO

Introduction 56  
Overview of the Insulin-Amino Acid-MTOR  
Signaling Pathway 56  
Amino Acids, mTOR Signaling and the Regulation  
of Autophagy 59  
Amino Acids, Beclin-1 and the Regulation  
of Autophagy 64  
Conclusion 66  
References 67

3. Regulation of Autophagy by Amino  
Acid Starvation Involving  $\text{Ca}^{2+}$

GHITA GHISLAT AND ERWIN KNECHT

Introduction 70  
Regulation of Autophagy by Amino Acids 72  
 $\text{Ca}^{2+}$ -Dependent Activation of Autophagy by  
Amino Acid Starvation 74  
 $\text{Ca}^{2+}$ /CaMKK- $\beta$ -Dependent Autophagy and Energy 75  
Conclusion 77  
Acknowledgments 78  
References 78

#### 4. Regulation of Autophagy by microRNAs

KUMSAL AYSE TEKIRDAG, DENIZ GULFEM OZTURK AND  
DEVIRIM GOZUACIK

Introduction	82
Molecular Mechanisms of Autophagy	82
Major Signaling Pathways Regulating Autophagy	85
Small Regulators: microRNAs, their Biogenesis and Biological Functions	88
microRNAs: Novel Regulators of Autophagy	90
microRNA Regulation of Autophagy-Related Signaling Pathways	96
Conclusion	97
Acknowledgments	99
References	99

#### 5. Mechanisms of Cross-Talk between Intracellular Protein Degradation Pathways

GRAEME HEWITT, BERNADETTE CARROLL AND  
VIKTOR I. KOROLCHUK

Introduction	104
The Ubiquitin-Proteasome System: Selective Degradation of Cytoplasmic Proteins	104
The Three Branches of Autophagy: Diverse Regulation of Lysosome-Dependent Degradation	107
Regulation of Intracellular Proteolysis by Cross-Talk Between Degradation Pathways	110
Functional Implications of Cross-Talk: Autophagy Can Compensate for UPS Impairment but Not Vice Versa	112
Insights into the Physiological Consequences of Perturbed Proteolysis: Focus on Aging	115
Conclusion	118
Acknowledgments	118
References	118

#### 6. Cross-Talk between Autophagy and Apoptosis in Adipose Tissue: Role of Ghrelin

AMAIA RODRÍGUEZ, LEIRE MÉNDEZ-GIMÉNEZ AND  
GEMA FRÜHBECK

Introduction	122
Apoptosis and Autophagy in Adipose Tissue	123
Role of Ghrelin in the Regulation of Apoptosis and Autophagy in Adipose Tissue	127
Discussion	129
Acknowledgments	130
References	130

## II

### AUTOPHAGY AND INTRACELLULAR PATHOGENS

#### 7. Intracellular Pathogen Invasion of the Host Cells: Role of $\alpha$ -Hemolysin- Induced Autophagy

MARÍA MILAGROS LÓPEZ DE ARMENTIA AND  
MARÍA I. COLOMBO

Introduction	136
<i>Staphylococcus aureus</i>	136
The <i>S. aureus</i> $\alpha$ -Hemolysin, a Key Secreted Virulence Factor	140
Discussion	142
References	143

#### 8. Modulation of Autophagy by Herpesvirus Proteins

MARION LUSSIGNOL AND AUDREY ESCLATINE

Introduction	146
Inhibition of Autophagy by Herpesvirus Proteins	147
Autophagy Activation by Herpesviruses	153
Conclusion	156
Acknowledgments	156
References	156

#### 9. Autophagy Induced by Varicella-Zoster Virus and the Maintenance of Cellular Homeostasis

CHARLES GROSE

Introduction	160
Varicella-Zoster Virus	160
The Disease Varicella	161
Characteristic Exanthems of Varicella and Herpes Zoster	161
Autophagy and its Visualization by Confocal Microscopy	162
Autophagosomes in the Exanthems of Varicella and Herpes Zoster	163

Evidence for ER Stress and Unfolded Protein Response 165  
 Acknowledgments 166  
 References 166

### 10. Autophagy and Hepatitis B Virus

YONGJUN TIAN, LIN-YA WANG AND  
 JING-HSIUNG JAMES OU

Introduction 170  
 The HBV Life Cycle 170  
 Mechanism of HBV-Induced Autophagy 172  
 Autophagy on HBV Replication 173  
 Autophagy and HBV-Induced  
 Hepatocarcinogenesis 174  
 Conclusion 175  
 References 175

## III

### AUTOPHAGY AND IMMUNITY

### 11. Toll-Like Receptors Serve as Activators for Autophagy in Macrophages Helping to Facilitate Innate Immunity

ALI VURAL, CHONG-SHAN SHI AND JOHN H. KEHRL

Introduction 180  
 Toll-Like Receptors 181  
 Autophagy 182  
 TLR-Induced Autophagy 184  
 Discussion 187  
 Acknowledgments 188  
 References 188

### 12. Autophagy in Antigen Processing for MHC Presentation to T Cells

CHRISTIAN MÜNZ

Introduction 192  
 Cytosolic Antigen Presentation on MHC  
 Class II Molecules 193  
 Autophagy Regulation of Phagocytosis 195  
 Antigen Packaging for Cross-Presentation via  
 Macroautophagy 196

Regulation of MHC Class I Antigen Processing by  
 Macroautophagy 196  
 Autophagy and Autoimmunity 197  
 Discussion 197  
 Acknowledgments 198  
 References 198

### 13. Autophagy Controls the Production and Secretion of IL-1 $\beta$ : Underlying Mechanisms

CELIA PERAL DE CASTRO, SARAH A. JONES AND  
 JAMES HARRIS

Introduction 202  
 Interleukin-1 $\beta$ : Biological Functions  
 and Regulation 202  
 Role of Autophagy in Interleukin-1 $\beta$  Secretion 203  
 Autophagy and Innate Th17 Immune  
 Responses 205  
 Autophagy and Inflammatory Diseases 206  
 Conclusion 207  
 References 208

### 14. Role of Autophagy in P2X7 Receptor- Mediated Maturation and Unconventional Secretion of IL-1 $\beta$ in Microglia

TAKATO TAKENOUCI, KAZUNARI SEKIYAMA,  
 MITSUTOSHI TSUKIMOTO, YOSHIFUMI IWAMARU,  
 MASAYO FUJITA, SHUEI SUGAMA, HIROSHI KITANI  
 AND MAKOTO HASHIMOTO

Introduction 212  
 Role of Lysosomes in the Maturation of IL-1 $\beta$  213  
 Autophagy Might Regulate the Maturation and  
 Secretion of IL-1 $\beta$  215  
 P2X7R-Mediated Maturation and Unconventional  
 Secretion of IL-1 $\beta$  217  
 Acknowledgments 221  
 References 221

### 15. Autophagy Restricts Interleukin-1 $\beta$ Signaling via Regulation of P62 Stability

JONGDAE LEE AND EYAL RAZ

Introduction 224  
 Discussion 226  
 Acknowledgments 228  
 References 228



## 16. Roles of Autophagy in the Thymic Epithelium

LEOPOLD ECKHART AND SUPAWADEE SUKSEREE

Introduction	232
Evidence for Autophagy in the Thymic Epithelium	234
Evaluation of Epithelial Autophagy in T Cell Selection	236
Conclusion	239
References	239

## IV

### AUTOPHAGY: GENERAL APPLICATIONS

## 17. The Role of Autophagy Receptors in Mitophagy

MIJA MARINKOVIĆ AND IVANA NOVAK

Introduction	244
Autophagy Receptors	247
Mitophagy	250
Discussion	254
Acknowledgments	254
References	254

## 18. The Role of Parkin and PINK1 in Mitochondrial Quality Control

MATTHEW Y. TANG AND THOMAS M. DURCAN

Introduction	258
Parkinson's Disease and Mitochondrial Dysfunction	259

Parkin and PINK1 Mutant Flies	260
Stabilization of PINK1 on Mitochondria	261
PINK1 Activity on the Mitochondria	264
Parkin: A PD-Associated E3-Ubiquitin Ligase	264
PINK1-Mediated Recruitment of Parkin onto Mitochondria	266
Parkin-Mediated Ubiquitination of Mitochondrial Proteins	266
Parkin/PINK1-Mediated Mitophagy	268
Mitophagy and Neurons	268
Discussion	269
References	270

## 19. Autophagy Degrades Endocytosed Gap Junctions

MATTHIAS M. FALK

Introduction	274
Results	275
Discussion	281
Conclusion	282
Acknowledgments	282
References	283

## Index 287

# Foreword

---

It is with great pleasure that I introduce Volume 6 of the impressive seven-volume series on autophagy edited by M.A. (Eric) Hayat. This volume addresses a number of mechanistic advances in our understanding of the regulation of autophagy, particularly the importance of nutrient availability. Regulatory mechanisms through micro-RNAs and cross-talk with other protein degradation pathways are presented. Several chapters cover the expanding role of autophagy in host immunity and the ways in which various intracellular pathogens repurpose the pathway for their own benefit. Finally, this volume addresses selective autophagy for degradation of mitochondria and endocytosed gap junctions.

The importance of autophagy in host defense represents an exciting emerging field. Autophagy facilitates antigen presentation, participates in thymic development, and shares many regulatory nodes with innate immunity, including cross-talk with Toll-like receptors, reflecting its important role in

regulating the immune response. Autophagy is also a participant in the dynamic struggle between intracellular pathogens and the host. While cells often use autophagy to eliminate intracellular pathogens and to activate innate and adaptive immunity, bacterial and viral pathogens have evolved defensive mechanisms, enabling them to subvert autophagy for their own purposes. As mitochondria can be viewed as domesticated intracellular bacteria, it is not surprising that autophagy plays a significant role in their removal.

The state of current knowledge on these important topics is summarized in the chapters of Volume 6, with contributions from experts from around the world. Researchers in immunology and infectious disease will find this volume to be particularly valuable, as well as those interested in selective autophagy and its regulation.

*Roberta A. Gottlieb M.D.*  
Cedars-Sinai Heart Institute

This page intentionally left blank

# Preface

---

It is becoming clear that cancer is an exceedingly complex molecular network, consisting of tumor cells at different stages of differentiation and noncancerous cells from the tumor microenvironment, both of which play a role in sustaining cancer progression. The latter cells maintain a proinflammatory environment conducive to cancer progression through induction of angiogenesis and evasion of the innate immune system. Although induction of cancer cell death by apoptosis, autophagy and necroptosis has been the main system exploited as anticancer strategies, an understanding of the role of the alterations in cellular metabolism is necessary for the development of new, more effective anticancer therapies. For example, it is known that cancer cells switch towards aerobic glycolysis from mitochondrial oxidative phosphorylation.

Autophagy, on the other hand, also possesses mechanisms that can promote cancer cell survival and growth of established tumors. Regarding cell survival, tumor cells themselves activate autophagy in response to cellular stress and/or increased metabolic demands related to rapid cell proliferation. Autophagy-related stress tolerance can enable cell survival by maintaining energy production that can lead to tumor growth and therapeutic resistance. Tumors are often subjected to metabolic stress due to insufficient vascularization. Under these circumstances, autophagy is induced and localized to these hypoxic regions where it supports survival of tumors. Aggressive tumors have increased metabolic demands because of

their rapid proliferation and growth. Thus, such tumors show augmented dependency on autophagy for their survival.

Defective autophagy causes abnormal mitochondria accumulation and reduced mitochondrial function in starvation, which is associated with reduced energy output. Because mitochondrial function is required for survival during starvation, autophagy supports cell survival. The recycling of intracellular constituents as a result of their degradation serves as an alternative energy source for tumor survival, especially during periods of metabolic stress. In this context, in tumor cells with defective apoptosis, autophagy allows prolonged survival of tumor cells. However, paradoxically, as mentioned above, autophagy is also associated with antitumorigenesis. Autophagy induced by cancer therapy can also be utilized by cancer cells to obtain nutrients for their growth and proliferation. Therefore, such treatments are counterproductive to therapeutic efficacy.

This is the sixth volume of the seven-volume series, *Autophagy: Cancer, Other Pathologies, Inflammation, Immunity, Infection and Aging*. This series discusses in detail almost all aspects of the autophagy machinery in the context of cancer and certain other pathologies. Emphasis is placed on maintaining homeostasis during starvation or stress conditions by balancing the synthesis of cellular components and their degradation by autophagy.

Both autophagy and ubiquitin-proteasome systems degrade damaged and superfluous proteins. Degradation of intracellular

components through these catabolic pathways results in the liberation of basic building blocks required to maintain cellular energy and homeostasis. However, less than or more than optimal protein degradation can result in human pathologies. An attempt is made in this volume to include information on the extent to which various protein degradation pathways interact, collaborate or antagonize one another.

It is known that conditions resulting in cellular stress (e.g., hypoxia, starvation, pathogen entry) activate autophagy, but dysregulation of autophagy at this stage might result in pathological states including cancer. MicroRNAs are non-protein-coding small RNAs that control levels of transcripts and proteins through post-transcriptional mechanisms. Current knowledge of microRNA regulation of autophagy is presented in this volume.

Autophagy (macroautophagy) is strictly regulated and the second messenger  $\text{Ca}^{+2}$  regulates starvation-induced autophagy. Withdrawal of essential amino acids increases intracellular  $\text{Ca}^{+2}$ , leading to the activation of adenosine monophosphate-activated protein kinase and the inhibition of the mTORC1, which eventually results in the enhanced formation of autophagosomes. The importance of this signaling pathway and other pathways (AMPK, AKT) within the autophagy signaling network is emphasized in this volume.

Recent discoveries of autophagic receptors that recognize specific cellular cargo have opened a new chapter in the autophagy field. Receptors are indispensable for the initiation and finalization of specific cargo removal by autophagy. For example, BNIP3L/NIX mediates mitochondrial clearance, which is discussed in this volume. It is pointed out that, in the absence of such clearance, accumulation of ROS can severely damage the mitochondrial

population within the neuron and ultimately cause apoptosis of the affected neurons. Mitochondrial dysfunction is implicated in Parkinson's disease. Toll-like receptors (TLRs) play critical roles in host defense by recognizing specific molecular patterns from a wide variety of pathogens. In macrophages, TLR signaling induces autophagy, limiting the replication of intracellular pathogens. How TLRs activate autophagosome formation in macrophages and enhance immunity is discussed in this volume.

Autophagy plays an important role during viral and bacterial infection. Autophagy can act either as a part of the immune defense system or as a pro-viral or pro-bacterial mechanism. In other words, although autophagy suppresses the replication of some viruses, it enhances the replication of others. Several examples of the latter viruses are discussed in this volume. For example, *Herpes viridae* family members encode autophagy-regulating proteins, which contribute to the host antiviral defenses, either by enhancing innate immunity or by helping antigen presentation. Herpes viruses have also evolved proteins that are able to inhibit this cellular mechanism. Positive or negative impact of autophagy on viral infection is explained in this volume.

Another example of the role of a virus in inducing autophagy is varicella-zoster virus (VZV); this human herpes virus causes chickenpox. Infected cells show a large number of autophagosomes and an enlarged endoplasmic reticulum (ER) indicating its stress, which is a precursor to autophagy through the inositol requiring enzyme-1 pathway and PERK pathway. Hepatocellular  $\beta$  virus (HBV) also activates the autophagic pathway while avoiding lysosomal, protein degradation.

As in the case of VZV, ER stress also plays a positive role in HBV replication.

The possible effect of autophagy on HBV-induced hepatocarcinogenesis is also included in this volume. *Staphylococcus aureus* pathogen not only induces an autophagic response in the host cell (localizing in LC3 decorated components), but also benefits from that state.

Although inflammatory responses are essential for eradicating intracellular pathogens and tissue repair, they can be detrimental to the host when uncontrolled. Therefore, inflammation needs to be tightly controlled to prevent excessive inflammation and collateral damage. Cytokine IL-1 $\beta$  (produced by microglia in the CNS) is one of the pro-inflammatory mediators. The pivotal role of autophagy in regulating the production and secretion of the IL-1 family members is explained in this volume. Atg6L1, an essential component of autophagy, suppresses pro-inflammatory signaling. Better understanding of the role of the autophagy-lysosomal pathway in the maturation and secretion of IL-1 should provide a new strategy for targeting inflammation in various pathological conditions.

Excess adiposity contributes to the development of obesity-associated metabolic disturbances such as insulin resistance, type 2 diabetes, or metabolic syndrome. It is pointed out that imbalance between ghrelin (a gut-derived hormone) and tumor necrosis factor in states of insulin resistance may contribute to altered apoptosis and autophagy found in the adipose tissue of patients with type 2 diabetes.

By bringing together a large number of experts (oncologists, physicians, medical research scientists and pathologists) in the field of autophagy, it is my hope that substantial progress will be made against terrible diseases that inflict humans. It is difficult for a single author to discuss effectively

and comprehensively various aspects of an exceedingly complex process such as autophagy. Another advantage of involving more than one author is to present different points of view on various controversial aspects of the role of autophagy in health and disease. I hope these goals will be fulfilled in this and future volumes of this series.

This volume was written by 46 contributors representing 11 countries. I am grateful to them for their promptness in accepting my suggestions. Their practical experience highlights the very high quality of their writings, which should build and further the endeavors of the readers in this important medical field. I respect and appreciate the hard work and exceptional insight into the role of autophagy in disease provided by these contributors.

It is my hope that subsequent volumes of this series will join this volume in assisting in the more complete understanding of the complex process of autophagy and eventually in the development of therapeutic applications. There exists a tremendous urgent demand by the public and the scientific community to develop better treatments for major diseases. In the light of the human impact of these untreated diseases, government funding must give priority to researching cures over global military superiority.

I am grateful to Dr. Dawood Farahi and Phillip Connelly for recognizing the importance of medical research and publishing through an institution of higher education. I am thankful to my students for their contributions to the final preparation of this volume.

M. A. Hayat  
July 2014

This page intentionally left blank

# Contributors

---

- Bernadette Carroll** Ageing Research Laboratories, Institute for Ageing and Health, Newcastle University, Campus for Ageing and Vitality, Newcastle upon Tyne, United Kingdom
- Patrice Codogno** INSERM U1151-CNRS UMR 8253, Institut Necker Enfants-Malades, Paris, France
- María I. Colombo** School of Medicine, National University of Cuyo, Argentina
- Thomas M. Durcan** Montreal Neurological Institute and Hospital, Montreal, Quebec, Canada
- Leopold Eckhart** Department of Dermatology, Research Division of Biology and Pathobiology of the Skin, Medical University of Vienna, Vienna, Austria
- Audrey Esclatine** Institute for Integrative Biology of the Cell, Department of Virology, Gif sur Yvette, University Paris Sud, I2BC, France
- Matthias M. Falk** Department of Biological Sciences, Lehigh University, Bethlehem, Pennsylvania, USA
- Gema Frühbeck** Metabolic Research Laboratory Clínica Universidad de Navarra, University of Navarra Department of Endocrinology and Nutrition, University of Navarra, CIBERobn, Pamplona, Spain
- Masayo Fujita** Division of Sensory and Motor Systems, Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan
- Ghita Ghislat** Laboratorio de Biología Celular, Centro de Investigación Príncipe, Valencia, Spain
- Devrim Gozuacik** SABANCI University, Faculty of Engineering and Natural Sciences, Istanbul, Turkey
- Charles Grose** Virology Laboratory, University of Iowa Children's Hospital, Iowa City, Iowa, USA
- James Harris** Centre for Inflammatory Diseases, Faculty of Medicine, Nursing and Health Sciences, Monash University, Clayton, Victoria, Australia
- Makoto Hashimoto** Division of Sensory and Motor Systems, Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan
- M.A. Hayat** Kean University, Department of Biological Sciences, Union, New Jersey, USA
- Graeme Hewitt** Ageing Research Laboratories, Institute for Ageing and Health, Newcastle University, Campus for Ageing and Vitality, Newcastle upon Tyne, United Kingdom
- Yoshifumi Iwamaru** Prion Disease Research Center, National Institute of Animal Health, Ibaraki, Japan
- Sarah A. Jones** Centre for Inflammatory Diseases, Faculty of Medicine, Nursing and Health Sciences, Monash University, Clayton, Victoria, Australia
- John H. Kehrl** B-Cell Molecular Immunology Section, Laboratory of Immunoregulation, National Institutes of Health, Bethesda, Maryland, USA
- Hiroshi Kitani** Division of Animal Sciences, National Institute of Agrobiological Sciences, Ibaraki, Japan
- Erwin Knecht** Laboratorio de Biología Celular, Centro de Investigación Príncipe Felipe and CIBERER, C/Eduardo Primo Yufera 3, 46012 Valencia, Spain



- Viktor I. Korolchuk** Ageing Research Laboratories, Institute for Ageing and Health, Newcastle University, Campus for Ageing and Vitality, Newcastle upon Tyne, United Kingdom
- Jongdae Lee** Department of Medicine, University of California San Diego, San Diego, California, USA
- María Milagros López de Armentia** Instituto de Histología y Embriología Mendoza, Facultad de Ciencias Médicas U.N., Cuyo-CONICET, Argentina
- Séverine Lorin** EA4530, Faculté de Pharmacie, Châtenay-Malabry, France
- Marion Lussignol** Department of Infectious Diseases, Faculty of Life Sciences & Medicine, King's College London, London, UK
- Mija Marinković** School of Medicine, University of Split, Split, Croatia
- Alfred J. Meijer** Department of Medical Biochemistry, Academic Medical Center, Amsterdam, The Netherlands
- Leire Méndez-Giménez** Metabolic Research Laboratory, Clínica Universidad de Navarra, CIBERobn, Pamplona, Spain
- Christian Münz** Viral Immunobiology, Institute of Experimental Immunology, University of Zürich, Zürich, Switzerland
- Ivana Novak** School of Medicine, University of Split, Split, Croatia
- Jing-hsiung James Ou** Department of Molecular Microbiology and Immunology, University of Southern California, Keck School of Medicine, Los Angeles, California, USA
- Deniz Gulfem Ozturk** SABANCI University, Faculty of Engineering and Natural Sciences, Istanbul, Turkey
- Celia Peral de Castro** Immunology Research Centre, School of Biochemistry and Immunology, Trinity College Dublin, Ireland
- Eyal Raz** Department of Medicine, University of California San Diego, La Jolla, California, USA
- Amaia Rodríguez** Metabolic Research Laboratory, Clínica Universidad de Navarra, CIBERobn, Pamplona, Spain
- Kazunari Sekiyama** Division of Sensory and Motor Systems, Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan
- Chong-Shan Shi** Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA
- Shuei Sugama** Department of Physiology, Nippon Medical School, Tokyo, Japan
- Supawadee Sukserree** Research Division of Biology and Pathobiology of the Skin, Department of Dermatology, Medical University of Vienna, Vienna, Austria
- Takato Takenouchi** Division of Animal Sciences, National Institute of Agrobiological Sciences, Ibaraki, Japan
- Matthew Y. Tang** Montreal Neurological Institute and Hospital, Montreal, Quebec, Canada
- Kumsal Ayse Tekirdag** Sabanci University, Department of Biological Sciences and Bioengineering, Turkey
- Yongjun Tian** Department of Molecular Microbiology and Immunology, University of Southern California Keck School of Medicine, Los Angeles, California, USA
- Mitsutoshi Tsukimoto** Faculty of Pharmaceutical Sciences, Tokyo University of Science, Chiba, Japan
- Ali Vural** B-Cell Molecular Immunology Section, Laboratory of Immunoregulation, National Institutes of Health, Bethesda, Maryland, USA
- Lin-ya Wang** Department of Molecular Microbiology and Immunology, University of Southern California Keck School of Medicine, Los Angeles, California, USA

# Abbreviations and Glossary

---

<b>1AP</b>	inhibitor of apoptosis protein
<b>3-MA</b>	3-methyladenine, an autophagy inhibitor
<b>3-methyladenine</b>	an autophagic inhibitor
<b>5-Fu</b>	5 fluorouracil
<b>AAP</b>	protein that mediates selective autophagy
<b>ACF</b>	aberrant crypt foci
<b>aggrephagy</b>	degradation of ubiquitinated protein aggregates
<b>aggresome</b>	inclusion body where misfolded proteins are confined and degraded by autophagy
<b>AIF</b>	apoptosis-inducing factor
<b>AIM</b>	Atg8-family interacting motif
<b>Akt</b>	protein kinase B regulates autophagy
<b>Alfy</b>	autophagy-linked FYVE protein
<b>ALIS</b>	aggresome-like induced structures
<b>ALR</b>	autophagic lysosome reformation
<b>AMBRA-1</b>	activating molecule in Beclin 1-regulated autophagy
<b>AMP</b>	adenosine monophosphate
<b>amphisome</b>	intermediate compartment formed by fusing an autophagosome with an endosome
<b>AMPK</b>	adenosine monophosphate-activated protein kinase
<b>aPKC</b>	atypical protein kinase C
<b>APMA</b>	autophagic macrophage activation
<b>apoptosis</b>	programmed cell death type 1
<b>ARD1</b>	arrest-defective protein 1
<b>ASK</b>	apoptosis signal regulating kinase
<b>AT1</b>	Atg8-interacting protein
<b>ATF5</b>	activating transcription factor 5
<b>ATF6</b>	activating transcription factor 6
<b>Atg</b>	autophagy-related gene or protein
<b>Atg1</b>	serine/threonine protein 1 kinase
<b>Atg2</b>	protein that functions along with Atg18
<b>Atg3</b>	ubiquitin conjugating enzyme analogue
<b>Atg4</b>	cysteine protease
<b>Atg5</b>	protein containing ubiquitin folds
<b>Atg6</b>	component of the class III PtdIns 3-kinase complex
<b>Atg7</b>	ubiquitin activating enzyme homologue
<b>Atg8</b>	ubiquitin-like protein
<b>Atg9</b>	transmembrane protein

<b>Atg10</b>	ubiquitin conjugating enzyme analogue
<b>Atg11</b>	fungal scaffold protein
<b>Atg12</b>	ubiquitin-like protein
<b>Atg13</b>	component of the Atg1 complex
<b>Atg14</b>	component of the class III PtdIns 3-kinase complex
<b>Atg15</b>	vacuolar protein
<b>Atg16</b>	component of the Atg12-Atg5-Atg16 complex
<b>Atg17</b>	yeast protein
<b>Atg18</b>	protein that binds to PtdIns
<b>Atg19</b>	receptor for the Cvt pathway
<b>Atg20</b>	PtdIns P binding protein
<b>Atg21</b>	PtdIns P binding protein
<b>Atg22</b>	vacuolar amino acid permease
<b>Atg23</b>	yeast protein
<b>Atg24</b>	PtdIns binding protein
<b>Atg25</b>	coiled-coil protein
<b>Atg26</b>	sterol glucosyltransferase
<b>Atg27</b>	integral membrane protein
<b>Atg28</b>	coiled-coil protein
<b>Atg29</b>	protein in fungi
<b>Atg30</b>	protein required for recognizing peroxisomes
<b>Atg31</b>	protein in fungi
<b>Atg32</b>	mitochondrial outer membrane protein
<b>Atg33</b>	mitochondrial outer membrane protein
<b>Atg101</b>	Atg13-binding protein
<b>ATM</b>	ataxia-telangiectasia mutated protein
<b>autolysosome protein</b>	lysosomal associated membrane protein 2
<b>autolysosome</b>	formed by fusion of the autophagosome and lysosome, degrading the engulfed cell components
<b>autophagic body</b>	the inner membrane-bound structure of the autophagosome
<b>autophagic flux</b>	the rate of cargo delivery to lysosomes through autophagy
<b>autophagosome</b>	double-membrane vesicle that engulfs cytoplasmic contents for delivery to the lysosome
<b>autophagosome maturation</b>	events occurring post-autophagosome closure followed by delivery of the cargo to lysosomes
<b>autophagy</b>	programmed cell death type 2
<b>AV</b>	autophagic vacuole
<b>axonopathy</b>	degradation of axons in neurodegeneration
<b>BAD</b>	Bcl-2 associated death promoter protein
<b>Bafilomycin</b>	inhibitor of the vacuolar-type ATPase
<b>Bafilomycin A1(BAF-A1)</b>	an autophagy inhibitor
<b>BAG</b>	Bcl-2-associated athanogene
<b>BAG3</b>	Bcl-2-associated athanogene 3
<b>BAK</b>	Bcl-2 antagonist/killer
<b>Barkor</b>	Beclin 1-associated autophagy-related key regulator

<b>BATS</b>	Barkor/Atg14(L) autophagosome targeting sequence
<b>BAX</b>	Bcl-2-associated X protein
<b>Bcl-2</b>	B cell lymphoma-2
<b>Beclin 1</b>	mammalian homologue of yeast Atg6, activating macroautophagy
<b>Beclin 1</b>	Bcl-2-interacting protein 1
<b>BH3</b>	Bcl-2 homology domain-3
<b>BH3-only proteins</b>	induce macroautophagy
<b>BHMT</b>	betaine homocysteine methyltransferase protein found in the mammalian autophagosome (metabolic enzyme)
<b>BID</b>	BH3-interacting domain death agonist
<b>Bif-1 protein</b>	interacts with Beclin 1, required for macroautophagy
<b>Bim</b>	Bcl-2 interacting mediator
<b>BNIP</b>	pro-apoptotic protein
<b>BNIP3 protein</b>	required for the HIF-1-dependent induction of macroautophagy
<b>bortezomib</b>	selective proteasome inhibitor
<b>CaMKK<math>\beta</math> protein</b>	activates AMPK at increased cytosolic calcium concentration
<b>CaMK</b>	calcium/calmodulin-dependent protein kinase
<b>CASA</b>	chaperone-assisted selective autophagy
<b>caspase</b>	cysteine aspartic acid specific protease
<b>CCI-779</b>	rapamycin ester that induces macroautophagy
<b>CD46 glycoprotein</b>	mediates an immune response to invasive pathogens
<b>chloroquine</b>	an autophagy inhibitor which inhibits fusion between autophagosomes and lysosomes
<b>c-Jun</b>	mammalian transcription factor that inhibits starvation-induced macroautophagy
<b>Clg 1</b>	a yeast cyclin-like protein that induces macroautophagy
<b>CMA</b>	chaperone-mediated autophagy
<b>COG</b>	functions in the fusion of vesicles within the Golgi complex
<b>COP1</b>	coat protein complex1
<b>CP</b>	20S core particle
<b>CRD</b>	cysteine-rich domain
<b>CSC</b>	cancer stem cell
<b>CTGF</b>	connective tissue growth factor
<b>Cvt</b>	cytoplasm-to-vacuole targeting
<b>DAMP</b>	damage-associated molecular pattern molecule/danger-associated molecular pattern molecule
<b>DAP1</b>	death-associated protein 1
<b>DAPK</b>	death-associated protein kinase
<b>DAPK1</b>	death-associated protein kinase 1
<b>DDR</b>	DNA damage response
<b>DEPTOR</b>	DEP domain containing mTOR-interacting protein
<b>DFCP1</b>	a PtdIns (3) P-binding protein
<b>DISC</b>	death-inducing signaling complex

---

DMV	double-membrane vesicle
DOR	diabetes- and obesity-regulated gene
DRAM	damage-regulated autophagy modulator
DRAM-1	damage-regulated autophagy modulator 1 induces autophagy in a p53-dependent manner.
DRC	desmin-related cardiomyopathy
DRiP	defective ribosomal protein
DRP1	dynamain-related protein 1
DUB	deubiquitinases that accumulate proteins into aggresomes
E2F1	a mammalian transcription factor
efferocytosis	phagocytosis of apoptotic cells
EGFR	epidermal growth factor receptor
EIF2 $\alpha$	eukaryotic initiation factor 2 alpha kinase
endosomes	early compartments fuse with autophagosomes to generate amphisomes
ERAA	endoplasmic reticulum-activated autophagy
ERAD	endoplasmic reticulum-associated degradation pathway
ERK	extracellular signal regulated kinase
ERK1/2	extracellular signal regulated kinase 1/2
ERT	enzyme replacement therapy
ESCRT	endosomal sorting complex required for transport
everolimus	mTOR inhibitor
FADD	Fas-associated death domain
FKBP12	FK506-binding protein 12
FoxO3	Forkhead box O transcription factor 3
FYCO1	FYVE and coiled domain containing 1
GAA	acid $\alpha$ -glucosidase
GABARAP	gamma-aminobutyric acid receptor-associated protein
GAS	group A streptococcus
GATE-16	Golgi-associated ATPase enhancer of 16 kDa
GFP	green fluorescent protein
glycophagy	degradation of glycogen particles
GPCR	G protein-coupled receptor
GSK-3 $\beta$	glycogen synthase kinase 3 beta regulates macroautophagy
GST-BHMT	BHMT fusion protein used to assay macroautophagy in mammalian cells
HAV	heavy autophagic vacuole
HCV	hepatitis C virus
HDAC	histone deacetylase
HDAC6	histone deacetylase 6
HIF	hypoxia-inducible factor
HIF1	hypoxia-inducible factor 1
HMGB1	high mobility group box 1
HR-PCD	hypersensitive response programmed cell death
Hsc70	heat shock cognate protein

<b>HSP</b>	heat shock protein
<b>Hsp90</b>	heat shock protein 90
<b>HspB8</b>	heat shock cognate protein beta-8
<b>Htraz</b>	high temperature requirement factor Az is a pro-apoptotic protein
<b>I13P</b>	phosphatidylinositol
<b>IAP</b>	inhibitor of apoptosis protein
<b>IKK</b>	inhibitor of nuclear factor $\kappa$ B
<b>IL3</b>	interleukin-3
<b>IM</b>	isolation membrane
<b>inflammasome</b>	an intracellular protein complex that activates caspase-1
<b>IRF</b>	interferon regulatory factor
<b>IRGM</b>	immunity-associated GTPase family M
<b>IRS</b>	insulin receptor substrate
<b>JNK/SAPK</b>	c-Jun N-terminal kinase/stress-activated protein kinase
<b>KRAS</b>	an oncogene that induces autophagy in cancer cells
<b>LAMP</b>	lysosome-associated membrane protein
<b>LAMP1</b>	lysosome marker, lysosome-associated membrane protein 1
<b>LAMP2</b>	lysosomal-associated membrane protein 2
<b>LAMP-2A</b>	lysosomal-associated membrane protein 2A
<b>LAP</b>	LC3-associated phagocytosis
<b>LAV</b>	light autophagic vacole
<b>LC3 (MAP1LC3B)</b>	autophagosome marker microtubule-associated protein 1 light chain 3B
<b>LC3</b>	microtubule-associated protein light chain 3
<b>LET</b>	linear energy transfer
<b>lipophagy</b>	selective delivery of lipid droplets for lysosomal degradation
<b>LIR</b>	LC3 interacting region
<b>LKB</b>	liver kinase B
<b>LSD</b>	lysosomal storage disorder
<b>lysosomotropic agent</b>	compound that accumulates preferentially in lysosomes
<b>macroautophagy</b>	autophagy
<b>macrolipophagy</b>	regulation of lipid metabolism by autophagy
<b>MALS</b>	macroautophagy-lysosome system
<b>MAPK</b>	mitogen-activated protein kinase
<b>MARF</b>	mitofusion mitochondrial assembly regulatory factor
<b>MCU</b>	mitochondrial calcium uptake uniporter pore
<b>MDC</b>	monodansylcadaverine to measure autophagic flux <i>in vivo</i>
<b>MEF</b>	mouse embryonic fibroblast
<b>MFN2</b>	mitofusin 2, a mitochondrial outer membrane protein involved in fusion/fission to promote mitochondrial segregation and elimination
<b>MHC</b>	major histocompatibility complex
<b>MHC-II</b>	major histocompatibility complex class II
<b>MiCa</b>	mitochondrial inner membrane calcium channel

<b>micropexophagy or macropexophagy</b>	peroxisome degradation by autophagic machinery
<b>MIPA</b>	micropexophagy-specific membrane apparatus
<b>mitofusion</b>	mitochondrial fusion-promoting factor
<b>mitophagy</b>	degradation of dysfunctional mitochondria
<b>MOM</b>	mitochondrial outer membrane
<b>MPS</b>	mucopolysaccharide
<b>MPT</b>	mitochondrial permeability transition
<b>mPTP</b>	mitochondrial permeability transition pore
<b>MSD</b>	multiple sulfatase deficiency
<b>MTCO2</b>	mitochondrial marker
<b>MTOC</b>	microtubule organizing center
<b>mTOR</b>	mammalian target of rapamycin, which inhibits autophagy and functions as a sensor for cellular energy and amino acid levels
<b>mTORc1</b>	mammalian target of rapamycin complex 1
<b>MTP</b>	mitochondrial transmembrane potential
<b>MTS</b>	mitochondrial targeting sequence
<b>MVB</b>	multivesicular body
<b>NBR1</b>	neighbor of BRCA1 gene 1
<b>NDP52</b>	nuclear dot protein 52 kDa
<b>NEC-1</b>	necrostatin-1
<b>necroptosis</b>	a form of programmed cell death by activating autophagy-dependent necrosis
<b>Nix</b>	a member of the Bcl-2 family required for mitophagy
<b>NLR</b>	NOD-like receptor
<b>NOD</b>	nucleotide-binding oligomerization domain
<b>NOS</b>	nitric oxide synthase
<b>NOX</b>	NADPH oxidase
<b>Nrf2</b>	nuclear factor 2
<b>OCR</b>	oxygen consumption rate
<b>omegasome</b>	PI(3)P-enriched subdomain of the ER involved in autophagosome formation
<b>OMM</b>	outer mitochondrial membrane
<b>OPA1</b>	mitofusin 1 is required to promote mitochondrial fusion
<b>Ox-LDL</b>	oxidized low density lipoprotein is a major inducer of ROS, inflammation, and injury to endothelial cells
<b>p62</b>	an autophagy substrate
<b>p62/SQSTM1</b>	sequestosome 1
<b>PAMP</b>	pathogen-associated molecular pattern molecule
<b>PAS</b>	pre-autophagosomal structure
<b>PB1 domain</b>	Phox and Bem1 domain
<b>PCD</b>	programmed cell death
<b>PDI</b>	protein disulfide isomerase
<b>PE</b>	phosphatidyl ethanolamine



<b>PERK</b>	protein kinase-like endoplasmic reticulum kinase
<b>PFI</b>	proteasome functional insufficiency
<b>phagophore</b>	a cup-shaped, double membraned autophagic precursor structure
<b>PI(3)K-PKB-FOXO</b>	a growth factor that inhibits autophagy and increases apoptosis by regulating glutamine metabolism
<b>PI3K</b>	phosphatidylinositol 3-kinase
<b>PI3KC3</b>	phosphatidylinositol-3-kinase class III
<b>PINK1</b>	PTEN (phosphatase and tensin homologue deleted on chromosome 10)-induced putative kinase 1
<b>PKA</b>	protein kinase A
<b>PKB</b>	protein kinase B
<b>PKC</b>	protein kinase C
<b>polyQ</b>	polyglutamine
<b>PQC</b>	protein quality control
<b>prion disease</b>	transmissible spongiform encephalopathy
<b>PRR</b>	pathogen recognition receptor
<b>PS</b>	phosphatidyl serine
<b>PSMB5</b>	proteasome subunit beta type-5
<b>PtdIns</b>	phosphatidylinositol
<b>PTGS</b>	post-transcriptional gene silencing
<b>PUMA</b>	p53 upregulated modulator of apoptosis
<b>R1G</b>	retrograde signaling pathway
<b>Rag</b>	GTPase that activates TORC1 in response to amino acids
<b>RAGE</b>	receptor for advanced glycation end product
<b>rapamycin</b>	a well-known autophagy inducer by suppressing mTOR
<b>RAPTOR</b>	regulatory-associated of mTOR
<b>RE</b>	recycling endosome
<b>residual body</b>	lysosome containing undegraded material
<b>reticulophagy</b>	degradation of endoplasmic reticulum
<b>ribophagy</b>	degradation of ribosomes
<b>RIP</b>	receptor-interacting protein
<b>RISC</b>	RNA-induced silencing complex
<b>RLS</b>	reactive lipid species
<b>RNAi</b>	RNA interference
<b>RNS</b>	reactive nitrogen species
<b>ROS</b>	reactive oxygen species
<b>ROT</b>	rottlerin used as a protein kinase C-delta inhibitor
<b>RP</b>	19S regulatory particle
<b>Rubicon</b>	RUN domain and cysteine-rich domain-containing Beclin 1-interacting protein
<b>selective autophagy</b>	selective recruitment of substrates for autophagy
<b>sequestosome 1</b>	an autophagy substrate
<b>sequestosome 1 (p62/SQSTM1)</b>	a multifunctional adapter protein implicated in tumorigenesis



<b>sequestosome (SQSTM1)</b>	p62 protein, a ubiquitin-binding scaffold protein
<b>SESN2</b>	sestrin-2
<b>shRNA</b>	small/short hairpin RNA
<b>siRNA</b>	small interference RNA
<b>sirt 1</b>	sirtuin 1 class III histone deacetylase, prevents Alzheimer's disease
<b>SMIR</b>	small molecule inhibitor of rapamycin
<b>SNARE</b>	soluble N-ethylmaleimide-sensitive factor attachment receptor
<b>SNP</b>	single nucleotide polymorphism
<b>SQSTM1</b>	sequestosome 1
<b>Syt1</b>	synaptotagmin 1
<b>T1DM</b>	type 1 diabetes mellitus
<b>TAKA</b>	transport of Atg9 after knocking-out Atg1
<b>TASCC</b>	TOR-autophagy spatial coupling compartment
<b>TCN</b>	trans-Golgi network
<b>TCR</b>	T cell receptor
<b>TECPR1</b>	tectonin beta-propeller repeat containing 1
<b>tensinrolimus</b>	mTOR inhibitor
<b>TFEB</b>	transcript factor EB
<b>TGF<math>\beta</math></b>	transforming growth factor $\beta$ that activates autophagy
<b>TGN</b>	trans-Golgi network
<b>TIGR</b>	TP53 (tumor protein 53)-induced glycolysis and apoptosis regulator
<b>TK</b>	tyrosine kinase
<b>TKI</b>	tyrosine kinase inhibitor
<b>TLR</b>	Toll-like receptor
<b>TMD</b>	transmembrane domain
<b>TMEM166</b>	transmembrane protein 166 that induces autophagy
<b>TNF</b>	tumor necrosis factor
<b>TNF-<math>\alpha</math></b>	tumor necrosis factor alpha
<b>Torin1</b>	ATP-competitive mTOR inhibitor
<b>TRAIL</b>	tumor necrosis factor-regulated apoptosis-inducing ligand
<b>TSC</b>	tuberous sclerosis complex
<b>TSC2</b>	tuberous sclerosis complex 2
<b>TSP</b>	thrombospondin
<b>UBA domain</b>	ubiquitin-associated domain
<b>UBAN</b>	ubiquitin-binding domain
<b>ubiquitin</b>	a small protein that functions in intracellular protein breakdown and histone modification
<b>ubiquitination</b>	a well-established signal for inducing autophagy of protein aggregates
<b>Ubl</b>	ubiquitin-like
<b>ULK</b>	Unc-51-like kinase complex
<b>ULK1</b>	putative mammalian homologue of Atg1p
<b>UPR</b>	unfolded protein response

<b>UPS</b>	ubiquitin–proteasome system
<b>UVRAG</b>	UV-irradiation resistance-associated gene
<b>VAchT</b>	vesicular acetylcholine transporter
<b>VAMP</b>	vesicle-associated membrane protein
<b>VCP/p97</b>	valosin-containing protein involved in endosomal trafficking and autophagy
<b>VEGF</b>	vascular endothelial growth factor
<b>VEGFR</b>	vascular endothelial growth factor receptor
<b>VMP1</b>	vacuole membrane protein 1, promotes formation of autophagosomes
<b>VPS15</b>	vacuolar protein sorting 15 homologue
<b>VTA</b>	vascular targeting agent
<b>VTC</b>	vacuolar transporter chaperone
<b>wortmannin</b>	an autophagic inhibitor
<b>XBP1</b>	a component of the ER stress response that activates macroautophagy
<b>xenophagy</b>	degradation of invading bacteria, viruses and parasites
<b>YFP</b>	yellow fluorescent protein
<b>zymophagy</b>	lysosomal degradation of zymogen granules (digestive enzymes)

See also Klionsky, D. J., Codogno, P., Cuervo, A. M. *et al.* (2010). A comprehensive glossary of autophagy-related molecules and processes. *Autophagy* 6, 438–448.

This page intentionally left blank

# Autophagy:

## Volume 1 – Contributions

---

- Mechanisms of Regulation of p62 in Autophagy and Implications for Health and Diseases
- Molecular Mechanisms Underlying the Role of Autophagy in Neurodegenerative Diseases
- Roles of Multiple Types of Autophagy in Neurodegenerative Diseases
- Autophagy and Crohn's Disease: Towards New Therapeutic Connections
- The Role of Autophagy in Atherosclerosis
- Treatment of Diabetic Cardiomyopathy through Upregulating Autophagy by Stimulating AMP-Activated Protein Kinase
- Hyperglycemia-Associated Stress Induces Autophagy: Involvement of the ROS-ERK/JNK-p53 Pathway
- Role of Autophagy in the Cellular Defense Against Inflammation
- Myophagy Plays a Protective Role in Fibroblasts from Patients with Coenzyme Q<sub>10</sub> Deficiency
- The Presence of Dioxin Kidney Cells Induces Cell Death with Autophagy
- Molecular Mechanisms Underlying the Activation of Autophagy Pathways by Reactive Oxygen Species and their Relevance in Cancer Progression and Therapy
- Induction of Autophagic Cell Death by Anticancer Agents
- Immunogenicity of Dying Cancer Cells – The Inflammasome Connection: Autophagic Death Arrives to the Scene
- Selenite-Mediated Cellular Stress, Apoptosis, and Autophagy in Colon Cancer Cells
- Enhancement of Cell Death in High-Grade Glioma Cells: Role of N-(4-Hydroxyphenyl) Retinamide-Induced Autophagy
- Cisplatin Exposure of Squamous Cell Carcinoma Cells Leads to Modulation of the Autophagic Pathway
- Autophagy, Stem Cells, and Tumor Dormancy
- Death-Associated Protein Kinase 1 Suppresses Tumor Growth and Metastasis via Autophagy and Apoptosis
- TRIM13, Novel Tumor Suppressor: Regulator of Autophagy and Cell Death
- Hypoxia-Induced Autophagy Promotes Tumor Cell Survival

This page intentionally left blank

# Autophagy:

## Volume 2 – Contributions

---

- Selective Autophagy: Role of Interaction between the Atg8 Family
- Mammalian Autophagy Can Occur Through an Atg5/Atg7-Independent Pathway
- Selective Autophagy: Role of Ubiquitin and Ubiquitin-Like Protein in Targeting Protein Aggregates, Organelles, and Pathogen
- Ubiquitin and p62 in Selective Autophagy in Mammalian Cells
- Role of the Golgi Complex and Autophagosome Biogenesis in Unconventional Protein Secretion
- Induction of Autophagy in HIV-1-Uninfected Cells: Role of Fusogenic Activity of GP41
- Non-Lipidated LC3 is Essential for Mouse Hepatitis Virus Infection
- Suppression of Innate Antiviral Immunity after Hepatitis C Virus Infection: Role of the Unfolded Protein Response and Autophagy
- Mycobacterial Survival in Alveolar Macrophages as a Result of Coronin-1A Inhibition of Autophagosome Formation
- Virulent Mycobacteria Upregulate Interleukin-6 (IL-6) Production to Combat Innate Immunity
- Autophagy in Parasitic Protists
- Cell Surface Pathogen Receptor CD46 Induces Autophagy
- Helicobacter pylori* Infection and Autophagy: A Paradigm for Host–Microbe Interactions
- Autophagy Is Required during Monocyte–Macrophage Differentiation
- Role of Autophagy Gene ATg5 in T Lymphocyte Survival and Proliferation
- Sepsis-Induced Autophagy Is a Protective Mechanism Against Cell Death
- Blockage of Lysosomal Degradation Is Detrimental to Cancer Cells Survival: Role of Autophagy Activation
- Autophagy as a Sensitization Target in Cancer Therapy
- Pathogenesis of Bile Duct Lesions in Primary Biliary Cirrhosis: Role of Autophagy Followed by Cellular Senescence
- Autophagy and NADPH Oxidase Activity Tends to Regulate Angiogenesis in Pulmonary Artery Endothelial Cells with Pulmonary Hypertension
- Role of Autophagy in Heart Disease
- Regulation of Autophagy in Obesity-Induced Cardiac Dysfunction
- Cytochrome P4502E1, Oxidative Stress, JNK, and Autophagy in Acute Alcohol-Induced Fatty Liver
- Autophagy-Independent Tumor Suppression: Role of UVRAG
- Chaperone-Mediated Autophagy and Degradation of Mutant Huntingtin Protein
- The Role of Atg8 Homologue in Lewy Disease

This page intentionally left blank

# Autophagy:

## Volume 3 – Contributions

---

- Autophagic Flux, Fusion Dynamics, and Cell Death
- Architecture of the Atg12–Atg5–Atg16 Complex and its Molecular Role in Autophagy
- The Molecular Mechanisms Underlying Autophagosome Formation in Yeast
- Role of Autophagy in Cell Survival in Liver Injury
- Polymorphisms in Autophagy-Related Genes in Crohn’s Disease: Impact on Intracellular Bacteria Persistence and Inflammatory Response
- Functional Relevance of Autophagins in Life and Disease
- Strategies to Block Autophagy in Tumor Cells
- Autophagic Dysfunction in Gaucher Disease and its Rescue by Cathepsin B and D Proteases
- Cargo Recognition Failure Underlies Macroautophagy Defects in Huntington’s Disease
- Hepatitis C Virus Infection, Autophagy, and Innate Immune Response
- Geranylgeranoic Acid Induces Incomplete Autophagy but Leads to the Accumulation of Autophagosomes in Human Hepatoma Cells
- Defense Against Proteotoxic Stress in the Heart: Role of p62, Autophagy, and Ubiquitin–Proteasome System
- Elimination of Intracellular Bacteria by Autophagy
- Protein Phosphatase 2A Has Positive and Negative Roles in Autophagy
- Erufosine Induces Autophagy and Apoptosis in Oral Squamous Cell Carcinoma: Role of the Akt–mTOR Signaling Pathway
- Emerging Role of Hypoxia-Induced Autophagy in Cancer Immunotherapy
- Involvement of Autophagy and Apoptosis in Studies of Anticancer Drugs
- Autophagy-Based Protein Biomarkers for In Vivo Detection of Cardiotoxicity in the Context of Cancer Therapy
- Inhibition of mTOR Pathway and Induction of Autophagy Block Lymphoma Cell Growth: Role of AMPK Activation
- Autophagy Regulates Osteoarthritis-Like Gene Expression Changes: Role of Apoptosis and Reactive Oxygen Species
- The Key Role of Autophagy and its Relationship with Apoptosis in Lepidopteran Larval Midgut Remodeling
- Interferon Regulatory Factor 1 Regulates both Autophagy and Apoptosis in Splenocytes during Sepsis
- The Interplay between Autophagy and Apoptosis



This page intentionally left blank

# Autophagy:

## Volume 4 – Contributions

---

- Molecular Process and Physiological Significance of Mitophagy
- Principles of Mitophagy and Beyond
- Quality Control in Mitochondria
- Mitophagy: An Overview
- Mitophagy Induction and Curcumin-Mediated Sonodynamic Chemotherapy
- Role of Nix in the Maturation of Erythroid Cells through Mitochondrial Autophagy
- Role of the Antioxidant Melatonin in Regulating Autophagy and Mitophagy
- Ubiquitin Ligase-Assisted Selective Autophagy of Mitochondria: Determining Its Biological Significance Using *Drosophila* Models
- Atg32 Confers Selective Mitochondrial Sequestration as a Cargo for Autophagy
- PARK2* Induces Autophagy Removal of Impaired Mitochondria via Ubiquitination
- Ubiquitination of Mitofusins in PINK1/Parkin-Mediated Mitophagy
- Mitochondrial Alterations and Mitophagy in Response to 6-Hydroxydopamine
- Role of Mitochondrial Fission and Mitophagy in Parkinson's Disease
- Mitophagy Controlled by the PINK1-Parkin Pathway Is Associated with Parkinson's Disease Pathogenesis
- Loss of Mitochondria during Skeletal Muscle Atrophy
- Role of Impaired Mitochondrial Autophagy in Cardiac Aging

This page intentionally left blank

# Autophagy:

## Volume 5 – Contributions

---

- Molecular Cross-Talk between the Autophagy and Apoptotic Networks in Cancer
- Inhibition of ErbB Receptors and Autophagy in Cancer Therapy
- Ginsenoside F2 Initiates an Autophagic Progression in Breast Cancer Stem Cells
- Role of Autophagy in Cancer Therapy
- Autophagy in Human Brain Cancer: Therapeutic Implications
- Blockage of Lysosomal Degradation Is Detrimental to Cancer Cell Survival: Role of Autophagy Activation
- Induction of Protective Autophagy in Cancer Cells by NAE Inhibitor MLN4924
- Effect of Autophagy on Chemotherapy-Induced Apoptosis and Growth Inhibition
- Autophagy Upregulation Reduces Doxorubicin-Induced Cardiotoxicity
- Autophagy in Critical Illness
- Autophagy in the Onset of Atrial Fibrillation
- Role of Autophagy in Atherogenesis
- Regulation of Autophagy in Insulin Resistance and Type 2 Diabetes
- Pancreatic Beta Cell Autophagy and Islet Transplantation
- Autophagy Guards Against Immunosuppression and Renal Ischemia-Reperfusion Injury in Renal Transplantation
- When the Good Turns Bad: Challenges in the Targeting of Autophagy in Neurodegenerative Diseases
- The  $\alpha$ -Tubulin Deacetylase HDAC6 in Aggresome Formation and Autophagy: Implications for Neurodegeneration

This page intentionally left blank

# Introduction to Autophagy: Cancer, Other Pathologies, Inflammation, Immunity, Infection, and Aging, Volume 6

M.A. Hayat

## OUTLINE

Introduction	2	<i>Abnormal Proteins</i>	11
Specific Functions of Autophagy (A Summary)	4	<i>Protein Degradation Systems</i>	12
		<i>Beclin 1</i>	13
Autophagy in Normal Mammalian Cells	4	<i>Non-Autophagic Functions of Autophagy- Related Proteins</i>	13
		<i>Microtubule-Associated Protein Light Chain 3</i>	14
Endoplasmic Reticulum Stress and Autophagy	5	Monitoring Autophagy	15
Major Types of Autophagies	7	Reactive Oxygen Species (ROS)	15
	7	Mammalian Target of Rapamycin (mTOR)	16
	7		
	7		
Autophagosome Formation	8	Role of Autophagy in Tumorigenesis and Cancer	17
Autophagic Lysosome Reformation	9	Role of Autophagy in Immunity	19
Autophagic Proteins	10		

Autophagy and Senescence	20	Mitochondrial Fusion and Fission	31
Role of Autophagy in Viral Defense and Replication	21	Selective Autophagies	32
Role of Autophagy in Intracellular Bacterial Infection	22	<i>Allophagy</i>	33
Role of Autophagy in Heart Disease	23	<i>Axonopathy (neuronal autophagy)</i>	34
Role of Autophagy in Neurodegenerative Diseases	24	<i>Crinophagy</i>	35
Cross-Talk Between Autophagy and Apoptosis	26	<i>Glycophagy</i>	35
Autophagy and Ubiquitination	29	<i>Lipophagy</i>	36
Aggresome: Ubiquitin Proteasome and Autophagy Systems	30	<i>Mitophagy</i>	38
Autophagy and Necroptosis	31	<i>Nucleophagy</i>	39
		<i>Pexophagy</i>	40
		<i>Reticulophagy</i>	41
		<i>Ribophagy</i>	42
		<i>Xenophagy</i>	43
		<i>Zymophagy</i>	43
		References	44

## Abstract

Autophagy plays a direct or indirect role in health and disease. A simplified definition of autophagy is that it is an exceedingly complex process which degrades modified, superfluous (surplus) or damaged cellular macromolecules and whole organelles using hydrolytic enzymes in the lysosomes. It consists of sequential steps of induction of autophagy, formation of autophagosome precursor, formation of autophagosomes, fusion between autophagosome and lysosome, degradation of cargo contents, efflux transportation of degraded products to the cytoplasm, and lysosome reformation.

This chapter discusses specific functions of autophagy, the process of autophagy, major types of autophagy, influences on autophagy, and the role of autophagy in disease, immunity, and defense.

## INTRODUCTION

Aging has so permeated our lives that it cannot be stopped, but it can be delayed. Under the circumstances, time is our only friend. Because the aging process is accompanied by disability and disease (for example, Alzheimer's and Parkinson's conditions) and cannot be prevented, it seems that slow aging is the only way to have a healthy longer life. In general, aging can be slowed down by not smoking or chewing tobacco, by preventing or minimizing perpetual stress (anger, competition), by abstinence from alcoholic beverages, by regular exercise, and by having a healthy diet. There is no doubt that regular physical activity is associated with a reduced risk of mortality and contributes to the primary and secondary prevention of many types of diseases. Discipline is required to attain this goal.

# Autophagy Degrades Endocytosed Gap Junctions

Matthias M. Falk

## OUTLINE

<b>Introduction</b>	274	<i>Potential Other Degradation Pathways for Endocytosed Gap Junctions</i>	279
<i>Gap Junction Structure and Function</i>	274	<i>Signals that Prime Gap Junctions for Endocytosis and Direct them to Autophagic Degradation</i>	280
<b>Results</b>	275	<b>Discussion</b>	281
<i>Gap Junction Endocytosis Generates     Cytoplasmic Double-Membrane     Vesicles</i>	275	<b>Conclusion</b>	282
<i>Endocytosed Gap Junctions are     Degraded by Autophagy</i>	277	<b>Acknowledgments</b>	282
<i>Structural Elements Warrant the     Autophagic Degradation of     Endocytosed Gap Junctions</i>	278	<b>References</b>	283

## Abstract

Four principal categories of cell-cell junctions connect cells in vertebrates and form the basis for shaping distinct tissues and organs. Gap junctions (GJs), one of the four junction types, provide direct cell-to-cell communication by mediating passive diffusion of small hydrophilic signaling molecules between neighboring cells. Gap junction mediated intercellular communication (GJIC) has been shown to play a crucial role for all aspects of multicellular life, including embryonic development, tissue function, and cellular homeostasis; and mutations in the GJ forming proteins, connexins (Cx), have been linked to severe human diseases that include inherited and sporadic nonsyndromic hearing loss, neuropathies, eye lens cataracts, cardiac diseases, craniofacial malformations, and a number of acute skin disorders. Clearly, biosynthesis and degradation significantly contribute to GJ function and need to be controlled precisely. We have previously shown that GJs are removed from the plasma membrane via the internalization of entire GJ plaques (or portions thereof) in a cellular process that resembles clathrin-mediated endocytosis. GJ endocytosis results in

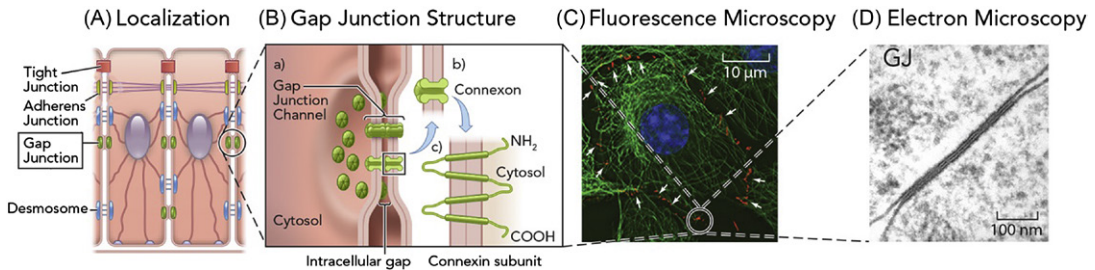


the formation of double-membrane vesicles (termed annular gap junctions [AGJs] or connexosomes) in the cytoplasm of one of the coupled cells. A set of recent independent studies consistent with earlier ultrastructural analyses demonstrate the degradation of endocytosed AGJs by autophagy. Some other reports, however, describe AGJ degradation by endo-/lysosomal pathways in cells that were treated with TPA. Here, I summarize evidence that supports the concept that autophagy serves as the principal cellular degradation pathway for internalized GJs under physiological and pathological conditions.

## INTRODUCTION

### Gap Junction Structure and Function

Cells in vertebrates including humans are linked together by four principal types of cell-cell junctions to form tissues and organs. Each type of cell-cell junction is considered to fulfill a special function (Figure 19.1A). Tight junctions (TJs) form a net-like belt of branched ridges of transmembrane proteins (claudins, occludins, tricellulin) around cells that tightly link cells together to separate apical from baso-lateral membrane domains, or (in case of epithelia and vascular endothelia) to separate outside from inside, or the lumen of blood vessels from the surrounding body, respectively. Desmosomes and adherens junctions (AJs) form patchy cell-cell contacts that connect cytoskeletal elements (intermediate and actin filaments, respectively) of neighboring cells to provide tissue strength, aid in tissue morphogenesis during development, and to maintain proper tissue organization. Gap junctions (GJs) consist of clusters of double-membrane spanning hydrophilic channels that provide direct cell-to-cell communication by allowing the passage of signaling molecules, ions, and electrical currents. Epithelia and endothelia, sheets of polarized single-cell layers that coat the outside and inside surface of organs such as the intestine, liver, kidneys, or the vasculature, are particularly rich in cell-cell junctions and exhibit a well-organized hierarchical architecture of these structures (Figure 19.1A).



**FIGURE 19.1** Cellular location and structure of gap junctions (GJs). (A) GJs are assemblies of double-membrane spanning hydrophilic channels termed “plaques” that bridge the apposing plasma membranes of neighboring cells to provide direct cell-to-cell (or intercellular) communication as shown here for epithelial cells. (B) GJ channels form by the head-on docking of two hemi-channels or “connexons” each assembled and trafficked to the plasma membrane by one of the two contacting cells. Connexons are assembled from six four-pass trans-membrane proteins termed “connexins” (Cxs). (C) GJs can be detected by immunofluorescence light microscopy when stained with fluorescence-tagged antibodies, such as the ones shown here in T51B liver cells assembled from endogenously expressed Cx43 protein. (D) GJs also appear as structures with unique morphology in ultrathin sections when examined by electron microscopy (EM).

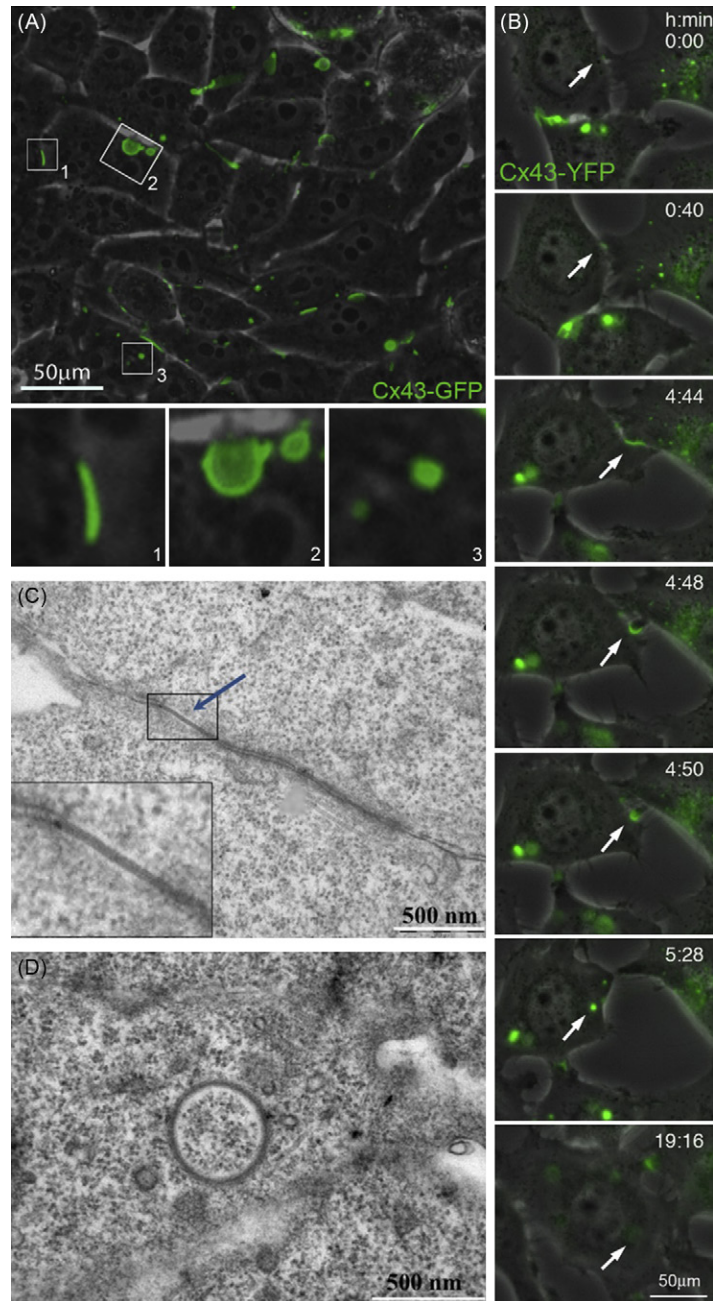
Direct cell-to-cell communication is a pivotal cellular function of multicellular organisms. It is established by GJ channels, which bridge apposing plasma membranes of neighboring cells. Typically, tens to thousands of GJ channels cluster into densely packed two-dimensional arrays, termed GJ plaques, that can reach several micrometers in diameter (Figure 19.1B). GJ channels are assembled from a ubiquitously expressed class of four-pass trans-membrane proteins, termed connexins (Cxs), with connexin 43 (Cx43) being the most abundantly expressed Cx type. Six Cx polypeptides oligomerize into a ring to form a hexameric trans-membrane structure with a central hydrophilic pore, called a hemi-channel or connexon. Once trafficked to the plasma membrane, two connexons, one provided by each of two neighboring cells, dock head-on in the extracellular space to form the complete double-membrane spanning GJ channel that is completely sealed off to the extracellular space (Thévenin *et al.*, 2013) (Figures 19.1, 19.2). Recruitment of additional GJ channels along the outer edge enlarges the GJ plaques, while simultaneous removal of older channels from plaque centers balances GJ channel turnover (Falk *et al.*, 2009; Gaietta *et al.*, 2002; Lauf *et al.*, 2002).

## RESULTS

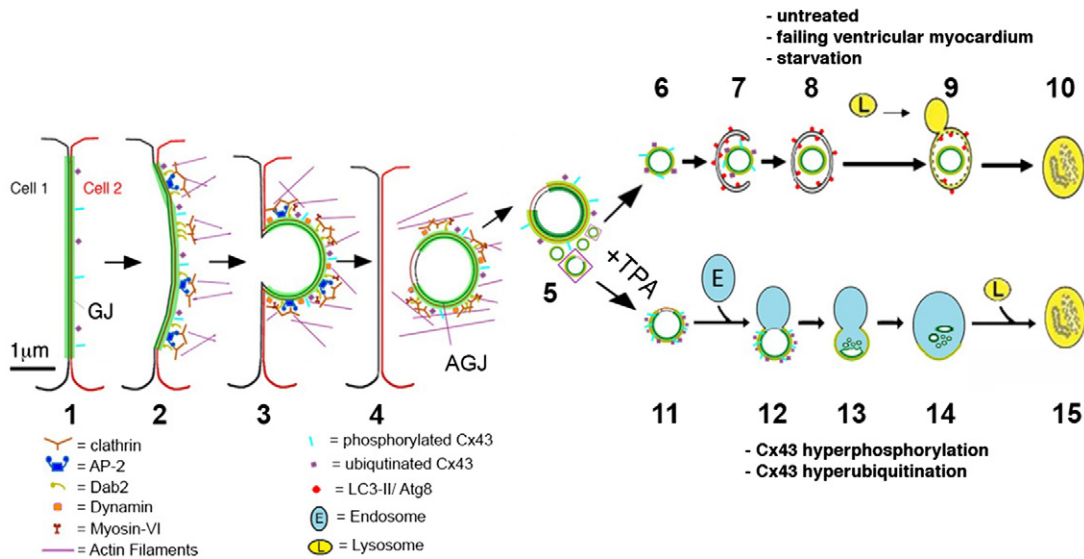
### Gap Junction Endocytosis Generates Cytoplasmic Double-Membrane Vesicles

Goodenough and Gilula (1974), and Ghoshroy *et al.* (1995) found that connexons, once docked, appear inseparable under physiological conditions (Ghoshroy *et al.*, 1995; Goodenough and Gilula, 1974), suggesting that cells may endocytose and degrade GJ plaques in whole. Indeed, we found that cells endocytose their GJs as complete double-membrane structures via a combined endo-/exocytic process (endocytic for the receiving cell, exocytic for the donating cell) (Baker *et al.*, 2008; Falk *et al.*, 2009; Gilleron *et al.*, 2008; Gumpert *et al.*, 2008; Piehl *et al.*, 2007) (Figure 19.3, steps 1–5). Internalization was found to occur preferentially into one of two coupled cells, indicating a highly regulated process (Falk *et al.*, 2009; Piehl *et al.*, 2007). Further analyses indicated that GJ internalization utilizes well-known components of the clathrin-mediated endocytosis (CME) machinery, including the classical endocytic coat protein clathrin, the clathrin-adaptors AP-2 and Dab2, the GTPase dynamin2, the retrograde actin motor myosin VI (myo6), as well as the process of actin polymerization (Gumpert *et al.*, 2008; Piehl *et al.*, 2007) (Figure 19.3, steps 1–4). A recent analysis from our lab revealed that two AP-2 binding sites are present in the C-terminus of Cx43 that cooperate to mediate GJ endocytosis (Fong *et al.*, 2013), suggesting a mechanistic model for clathrin's ability to internalize these large plasma membrane structures.

GJ internalization generates characteristic cytoplasmic double-membrane GJ vesicles, termed annular GJs (AGJs) or connexosomes (Figures 19.2, 19.3). Note that the outer membrane of the generated AGJ vesicles corresponds to the plasma membrane of the host cell, while the inner membrane and the vesicle lumen correspond to plasma membrane and cytoplasm of the neighboring donor cell (Figures 19.2, 19.3, steps 1–5). Extensive further analyses revealed that cells turn over their GJs constitutively (Falk *et al.*, 2009; Piehl *et al.*, 2007), and efficiently after treatment with inflammatory mediators such as thrombin and endothelin (Baker *et al.*, 2008); mitogens such as EGF and VEGF (Fong and Falk, and Nimlamool and Falk, unpublished); in response to treatment with the nongenomic



**FIGURE 19.2** Gap junctions and endocytosed gap junctions. (A) HeLa cells transfected with Cx43-GFP efficiently express and assemble GJs in the adjacent plasma membranes of transfected cells (visible as green fluorescent lines and puncta such as the one shown in insert 1). Over time, GJs bulge inward (insert 2), detach from the plasma membrane and form endocytosed cytoplasmic annular gap junction (AGJ) vesicles or connexosomes (insert 3). (B) Selected still images of a time-lapse recording of stably transfected Cx43-YFP expressing HeLa cells showing the formation of a GJ, its endocytic internalization into the cytoplasm of one of the previously coupled cells, and final degradation of the generated AGJ vesicle, indicated by the loss of its fluorescence (marked with arrows). Combined phase contrast and fluorescence images are shown in (A) and (B). Transmission electron micrographs of a gap junction (C) and an annular gap junction (D) in mouse embryonic stem cells.



**FIGURE 19.3** Mechanisms of gap junction endocytosis and degradation. Schematic representation of proposed steps that lead to GJ internalization (steps 1–3), cytoplasmic AGJ vesicle formation and fragmentation (steps 4, 5), and AGJ vesicle degradation by phago-/lysosomal (steps 6–10) and endo-/lysosomal pathways (steps 11–15) based on the previous work by others and us. Note the proposed nonjunctional membrane domains missing the green GJ label (shown in steps 4, 5, 11, 12), and the increased phosphorylation and ubiquitination on AGJ vesicles that fuse with endosomes (steps 11, 12 versus 6, 7).

carcinogen lindane (Gilleron *et al.*, 2008); and under pathological conditions such as in the failing canine ventricular myocardium (Hesketh *et al.*, 2010). Constitutive and acute endocytosis of GJ plaques correlates with the described short half-life of connexins of only 1–5 hours (Beardslee *et al.*, 1998; Berthoud *et al.*, 2004; Falk *et al.*, 2009; Fallon and Goodenough, 1981; Gaietta *et al.*, 2002).

## Endocytosed Gap Junctions are Degraded by Autophagy

Four recent studies by Hesketh *et al.* (2010), Lichtenstein *et al.* (2011), Fong *et al.* (2012), and Bejarano *et al.* (2012) report the degradation of endocytosed AGJ vesicles via autophagy (Figure 19.3, steps 6–10). Hesketh *et al.* (2010) report loss of GJs from the plasma membrane, and GJ endocytosis and AGJ degradation by autophagy in pacing-induced failing canine ventricular myocardium. Lichtenstein *et al.* (2011) report that autophagy contributes to the degradation of endogenously (NRK cells, mouse embryonic fibroblasts) and exogenously (HeLa cells) expressed Cx43 protein, and of wild-type and cataract-associated mutant Cx50 proteins in both un-induced cells and in cells in which autophagy was induced by starvation (Lichtenstein *et al.*, 2011). Fong *et al.* (2012) report the autophagic degradation of AGJ vesicles in normal, untreated HeLa cells that express exogenous fluorescently tagged Cx43; and in primary porcine pulmonary artery endothelial cells (PAECs) endogenously



expressing Cx43. Bejarano *et al.* (2012) report the Nedd4-mediated ubiquitin-dependent autophagic degradation of internalized GJs *in situ* (mouse liver) as well as in starved and fed cultured cells expressing Cx43 endogenously and exogenously (mouse embryonic fibroblasts, NIH3T3, COS7, and NRK cells).

In all four studies cytoplasmic AGJ vesicles were detected inside phagophores by ultrastructural analyses. Autophagosomes exhibit a highly characteristic, clearly recognizable double-membrane structure on ultra-thin sections (Figure 19.2D), making conventional electron microscopy a very reliable technique for the characterization of autophagosomes (Mizushima, 2004). Also, in all studies AGJs were observed to co-localize with the autophagy marker protein, LC3-II/Atg8, known to be one of the most useful generic marker proteins for the characterization of autophagosomes (Kabeya *et al.*, 2000). Microtubule-associated protein light chain 3 (LC3, the mammalian homologue of the yeast autophagic protein Atg8) is an abundant soluble cytoplasmic protein. It is proteolytically processed by the removal of a few N-terminal amino acid residues shortly after translation that generates LC3-I. LC3-I is recruited to developing phagophores, is covalently conjugated to phosphatidyl-ethanolamine (PE) of the phagophore membrane (termed LC3-II), and remains on autophagosomes for most of their lifetime (Kabeya *et al.*, 2000; Mizushima, 2004).

While the Lichtenstein *et al.* and Bejarano *et al.* studies were aimed more broadly at a potential role of autophagy contributing to Cx and GJ degradation in general, the Fong *et al.* and the Hesketh *et al.* studies were aimed specifically at investigating the fate of internalized AGJ vesicles that others and we had characterized previously (Baker *et al.*, 2008; Gumpert *et al.*, 2008; Jordan *et al.*, 2001; Piehl *et al.*, 2007). To further support their findings, Lichtenstein *et al.* and Bejarano *et al.* knocked down the autophagy-related proteins Atg5 and Atg7 in cells expressing either endogenous or exogenous Cx43, and used the drugs chloroquine and 3MA to inhibit autophagy. Fong *et al.* knocked down expression of the autophagy related proteins Beclin-1 (Atg6), LC3 (Atg8), LAMP-2 and p62/sequestosome 1 (SQSTM1), and used the drugs 3MA, Wortmannin, and Bafilomycin A1 in Cx43-GFP expressing HeLa cells.

As mentioned previously in the Lichtenstein *et al.*, Fong *et al.*, and Bejarano *et al.* studies the ubiquitin-binding protein p62/SQSTM1 was identified as a protein that targets internalized GJs to autophagic degradation. Knocking down p62/SQSTM1 protein levels as performed by Fong *et al.* resulted in a significantly increased accumulation of cytoplasmic AGJs (av. 55%, n = 4) and a significantly reduced co-localization (av. 69.5%, n = 3) of AGJs with autophagosomes. In summary, all four complementary studies (Bejarano *et al.*, 2012; Fong *et al.*, 2012; Hesketh *et al.*, 2010; Lichtenstein *et al.*, 2011) compellingly show that under physiological and pathological conditions GJ plaques are endocytosed from the plasma membrane, and that the generated AGJ vesicles are degraded by autophagy.

## Structural Elements Warrant the Autophagic Degradation of Endocytosed Gap Junctions

Since cytoplasmic vesicles normally can fuse with endosomes, at first glance, autophagic degradation of AGJ vesicles might not appear intuitive. However, considering the GJ internalization process that generates double-membrane vesicles in which both membranes are tightly linked to each other (not single membrane vesicles that typically are formed by the

endocytosis of cargo molecules on the plasma membrane), the structural organization of AGJ vesicles (multiprotein complexes with paracrystalline surface packing), and their cytoplasmic location, autophagic degradation emerges as the most apparent cellular degradation pathway. Finally, the unique structural composition of AGJ vesicles with lumen and inner membrane derived from the neighboring cell (being foreign to the AGJ-receiving host cell) may further direct AGJs to autophagic degradation. Taken together, the structural and functional characteristics of AGJ vesicles, along with the fact that autophagy serves as the generic degradation pathway for cytoplasmically localized structures (organelles and protein aggregates), renders autophagic degradation the most obvious cellular AGJ degradation pathway.

### Potential Other Degradation Pathways for Endocytosed Gap Junctions

Interestingly, a recent paper by [Leithe \*et al.\* \(2009\)](#) reports that in TPA-treated cells (a structural analogue of the secondary messenger molecule diacylglycerol [DAG]), internalized GJs may be degraded by the endo-/lysosomal and not the autophagosomal pathway ([Figure 19.3](#), steps 11–15). Recently, the Leithe lab identified the protein Smurf2 (the HECT E3 ubiquitin ligase smad ubiquitination regulatory factor-2) as a critical factor that regulates GJ internalization and endo-/lysosomal targeting in TPA-treated cells ([Fykerud \*et al.\*, 2012](#)). DAG is a known potent activator of protein kinase C (PKC), and PKC is known to phosphorylate and promote ubiquitination of Cx43 ([Leithe \*et al.\*, 2009](#); [Leithe and Rivedal, 2004b](#); [Postma \*et al.\*, 1998](#)). Based on these and our own results, it is tempting to speculate that cells might be able to regulate by which pathway (endo-/lysosomal versus phago-/lysosomal) specific cargo is sequestered and processed (e.g., endo-/lysosomal and phago-/lysosomal pathways might process internalized GJs in different ways). Furthermore, the level of cargo-phosphorylation and/or ubiquitination might determine which of these pathways is ultimately chosen (basic phosphorylation/ubiquitination signaling autophagic AGJ vesicle degradation; elevated phosphorylation/ubiquitination signaling endo-/lysosomal AGJ vesicle degradation) (see [Figure 19.3](#), steps 6–10 versus 11–15).

Endo-/lysosomal degradation of AGJs as observed in TPA-treated cells by [Leithe \*et al.\* \(2009\)](#) of course raises an important question: How is it structurally possible for a double-membrane vesicle that consists of tightly bonded membrane layers and densely packed GJ channels to fuse with a single-membrane endosome? The Rivedal and Leithe laboratories suggest that subsequent to GJ internalization and AGJ formation, the inner AGJ membrane splits and peels away from the outer AGJ membrane, generating a single-membraned cytoplasmic AGJ vesicle that then can fuse with a single-membraned endosome ([Kjenseth \*et al.\*, 2010, 2012](#); [Leithe \*et al.\*, 2009, 2012](#)). However, since docked GJ channels cannot split into undocked connexons under physiological conditions ([Ghoshroy \*et al.\*, 1995](#); [Goodenough and Gilula, 1974](#)) – which appears to be the apparent reason for double-membrane GJ endocytosis – it is not clear how membrane separation could be initiated in the AGJ vesicles shortly after their generation. Clearly low pH, a characteristic of late endosomes and lysosomes, and a potential initiator of GJ splitting, can be excluded because AGJ vesicle membrane-separation needs to occur before AGJ/endosome fusion.

Interestingly, by electron microscopic (EM) examination, we found that AGJ vesicles examined by electron microscopy (EM) appear to include a small region where the two membranes are void of GJ channels and are not docked or linked to each other ([Falk \*et al.\*, 2012](#);

Piehl *et al.*, 2007) (shown schematically in Figure 19.3, steps 4, 5, 11 and 12). Similar small AGJ membrane separations were also observed in classical ultrastructural analyses of GJs and AGJ vesicles (see, e.g., Mazet *et al.*, 1985). Possibly, these nonjunctional membrane domains consist of plasma membrane that is derived from both neighboring cells, and we postulated that these areas might originate from plasma membrane regions that were located immediately adjacent to the GJ plaques and were internalized as well. To gain further support for this hypothesis, we incubated inducible stably Cx43-YFP expressing HeLa cells for 2–4 hours with a fluorescently tagged lectin, Alexa594-wheat germ agglutinin (WGA), and examined AGJ vesicles by high-resolution fluorescence microscopy. WGA binds specifically to sialic acid and N-acetylglucosaminyl carbohydrate moieties commonly found on extracellular-exposed carbohydrate side-chains of plasma membrane proteins. Due to its relatively large size (~38 kDa), WGA is not able to traverse the plasma membrane in living cells. However, WGA will bind to and label the extracellular surface of plasma membranes, and subsequently will be endocytosed and then will also label intracellular membrane compartments. Interestingly we found that a significant portion of AGJ vesicles (~50%, n = 80; the ones that likely were generated during the WGA-incubation period), exhibited red-fluorescent WGA-puncta (Falk *et al.*, 2012). These results support our hypothesis that the undocked membrane domains we detected by EM indeed represent plasma membrane areas that were located in the immediate vicinity of GJ plaques and were concomitantly internalized in the AGJ endocytosis process. It is very likely that these nonjunctional membrane domains provide the single membrane areas that allow double-membrane AGJ vesicles to fuse with single-membrane endosomes.

### Signals that Prime Gap Junctions for Endocytosis and Direct them to Autophagic Degradation

Post-translational modification of proteins is a widespread mechanism to fine-tune the structure, function, and localization of proteins. One of the most versatile and intriguing protein modifications is the covalent attachment of ubiquitin (Ub) or Ub-like modifications to target proteins. Ub is a small, 76-amino acid protein, and either single or multiple Ub moieties can be conjugated to lysine amino acid residues of target proteins. An incredible diversity of mono- and poly-Ub chains (in which Ub moieties can be linked to each other via the Ub residues Met1-, Lys6-, Lys11-, Lys27-, Lys29-, Lys33-, Lys48-, and Lys63-) conjugated to target proteins have been characterized that can range in function from protein activation to protein degradation (Fushman and Wilkinson, 2011). Multiple mono-Ubs, and Lys48- and Lys63-linked poly-Ubs, have been recognized as important signals for protein degradation. For example, conjugation of Ub moieties to proteins has been recognized as a signal for both proteasomal targeting (addition of Lys48-linked poly-Ub chains) and more recently as a sorting signal for internalized vesicles of the late endocytic pathway. This is achieved through the addition of multiple mono-Ub moieties or of Lys63-linked poly-Ub chains, which ultimately lead to degradation by lysosomes (Hicke, 2001; Hicke and Dunn, 2003; Schnell and Hebert, 2003). In addition, Lys-63-linked polyubiquitination can act as an internalization signal for clathrin-mediated endocytosis (CME) (Belouzard and Rouille, 2006; Geetha *et al.*, 2005). Lys63-polyubiquitinated target proteins are recognized by specific CME machinery protein components that associate with a subset of Ub-binding proteins, specifically Epsin1 and Eps15 (Barriere *et al.*, 2006; Hawryluk *et al.*, 2006; Madshus, 2006). Further

work has shown that the Ub-binding protein p62/SQSTM1 recognizes and interacts via its UBA-domain with polyubiquitinated proteins (Ciani *et al.*, 2003; Seibenhener *et al.*, 2004) and delivers polyubiquitinated (Lys63-linked) oligomeric protein complexes to the autophagic degradation pathway (Bjorkoy *et al.*, 2005; Pankiv *et al.*, 2007). Ubiquitination of Cx43-based GJs has been described previously (Catarino *et al.*, 2011; Girao *et al.*, 2009; Leithe *et al.*, 2009; Leithe and Rivedal, 2004b). The findings that Cx43-based GJs can become ubiquitinated (e.g., Lys63-polyubiquitinated; Kells and Falk, unpublished), the known affinity of p62/SQSTM1 for ubiquitinated protein complexes, its co-localization with plasma membrane GJs in HeLa, COS7, and PAE cells (Bejarano *et al.*, 2012; Fong *et al.*, 2012; Lichtenstein *et al.*, 2011), and its apparent involvement in targeting AGJ vesicles to autophagic degradation (Fong *et al.*, 2012) suggest that ubiquitination of Cx43 (and at least Cx50), besides serving as a likely signal for GJ internalization, may also serve as the signal for targeting AGJ vesicles to autophagic degradation. Future research will be required to determine the potentially numerous types (multiple mono-Ubs, Lys48- and Lys63-linked poly-Ubs, etc.) and functions of connexin ubiquitination (see Kjenseth *et al.*, 2010; Leithe *et al.*, 2012; Su and Lau, 2012 for recent reviews that discuss Cx-ubiquitination). Very recently, Kjenseth *et al.* (2012) described an additional, Ub-like post-translational modification of Cx43, SUMOylation (SUMO, small ubiquitin-like modifier) that appears to be involved in regulating GJ stability and turnover. The small Ub-like protein SUMO was found to be conjugated to lysines 144 and 237 of the Cx43-C-terminal domain, further widening the role of Ub and Ub-like signals in the maintenance and degradation of GJs.

## DISCUSSION

Cells have developed three principal degradation pathways: the proteasomal, the endo-/lysosomal, and the phago-/lysosomal system (termed macroautophagy or simply autophagy), and all three have been implicated previously at various steps in the regulation of GJ stability and Cx degradation (Hesketh *et al.*, 2010; Laing *et al.*, 1997; Leach and Oliphant, 1984; Leithe and Rivedal, 2004a; Musil *et al.*, 2000; Pfeifer, 1980; Qin *et al.*, 2003). While the two latter ones utilize the lysosome for final degradation and are designed for the degradation of protein aggregates, multiprotein complexes and cytoplasmic organelles, the proteasomal system is designed for the degradation of single polypeptide chains that require unfolding to be inserted into the tubular core of the cytoplasmically located proteasome. Since AGJ vesicles are highly complex multi-subunit protein assemblies, their degradation by the proteasome is highly unlikely, and no evidence appears to exist that would suggest a proteasome-mediated degradation of GJs or AGJ vesicles. Similarly, lysosomal inhibitors such as leupeptin, chloroquine, NH<sub>4</sub>Cl, and E-64, which previously have been used to gain evidence for endo-/lysosomal degradation of GJs (Berthoud *et al.*, 2004; Laing *et al.*, 1997; Musil *et al.*, 2000; Qin *et al.*, 2003), will also inhibit autophagic GJ degradation, and thus obtained results may not have been interpreted correctly. Experimental approaches that specifically target the autophagosomal degradation pathway that were used by others and us compellingly demonstrate that endocytosed GJs are degraded by autophagy.

Historically, autophagy has been known as a lysosomal degradation pathway that becomes essential to cell survival following nutrient depletion. However, substantial research over



the past decade has indicated that autophagy, besides its well-known function in organelle degradation during starvation, represents a much more common and highly conserved autonomous lysosome-based cellular degradation pathway that is specifically designed to remove and degrade protein aggregates, multiprotein complexes, organelles, and invading pathogens from the cytoplasm (Bjorkoy *et al.*, 2005; Hung *et al.*, 2009; Pohl and Jentsch, 2009; Ravikumar *et al.*, 2008). Recent studies have further shown that protein aggregates, such as the ones formed by huntingtin and  $\beta$ -amyloid protein, and cellular structures such as the mid-body ring, a mitotic cytokinesis leftover multiprotein complex, are all degraded by autophagy (Bjorkoy *et al.*, 2005; Hung *et al.*, 2009; Pohl and Jentsch, 2009; Ravikumar *et al.*, 2008). Clearly, these cellular structures are degraded by autophagy independent of starvation. In addition, autophagosomal degradation of membranous/vesicular organelles, as for example malfunctioning mitochondria, is common. Since the catabolic activity of lysosomes is used in this process, degradation-prone structures first need to be separated from the cytoplasm. This is necessary due to the destructive activity of lysosomal enzymes, which cannot be released directly into the cytoplasm. Thus, cytoplasmic structures targeted for degradation are first engulfed in double-membrane vesicles (autophagosomes) that allow lysosomal fusion, degradation, and subsequent recycling of the phagosome cargo and the phagosome membrane.

## CONCLUSION

In this article, I have summarized recent experimental results and discussed structural and functional considerations that all support the concept that autophagy serves as the default degradation pathway for endocytosed GJs. Indeed, in several classical ultrastructural analyses of various cells and tissues *in situ* including heart, dermis, and liver (Leach and Oliphant, 1984; Mazet *et al.*, 1985; Pfeifer, 1980; Severs *et al.*, 1989), autophagic degradation of GJs had been suggested. However, surprisingly back then not much attention was attributed to this evidently fundamental GJ degradation pathway. Autophagic degradation of GJs plays a significant role in the regulation of GJ function, as inhibition of cellular autophagy increases GJIC, prevents internalization of GJs, slows down the degradation of Cxs, and causes cytoplasmic accumulation of internalized GJ vesicles *in situ*, and in cells that either express endogenously or exogenously connexin proteins (Bejarano *et al.*, 2012; Fong *et al.*, 2012; Lichtenstein *et al.*, 2011). Hence, it is likely that certain disease-causing mutations in Cx proteins will impair physiological levels of GJ endocytosis and autophagosomal turnover, and that this will cause a detrimental misregulation of GJ function. Future research also will need to address the signals that specifically modify the Cx proteins to initiate GJ endocytosis and degradation. Post-translational modifications, such as phosphorylation, ubiquitination, and acetylation, the binding/release of regulatory proteins (e.g., ZO-1), and specific conformational changes of the Cx43-C-terminus that regulate access of modifying enzymes are all enticing possibilities.

## Acknowledgments

Work in my laboratory is supported by NIHs NIGMS (grant GM55725) and by Lehigh University. I wish to thank members of the Falk laboratory for critical comments on the manuscript.

## References

- Baker, S.M., Kim, N., Gumpert, A.M., et al., 2008. Acute internalization of gap junctions in vascular endothelial cells in response to inflammatory mediator-induced G-protein coupled receptor activation. *FEBS Lett.* 582, 4039–4046.
- Barriere, H., Nemes, C., Lechardeur, D., et al., 2006. Molecular basis of oligoubiquitin-dependent internalization of membrane proteins in mammalian cells. *Traffic* 7, 282–297.
- Beardslee, M.A., Laing, J.G., Beyer, E.C., et al., 1998. Rapid turnover of connexin43 in the adult rat heart. *Circ. Res.* 83, 629–635.
- Bejarano, E., Girao, H., Yuste, A., et al., 2012. Autophagy modulates dynamics of connexins at the plasma membrane in a ubiquitin-dependent manner. *Mol. Biol. Cell* 23, 2156–2169.
- Belouzard, S., Rouille, Y., 2006. Ubiquitylation of leptin receptor OB-Ra regulates its clathrin-mediated endocytosis. *EMBO J.* 25, 932–942.
- Berthoud, V.M., Minogue, P.J., Laing, J.G., et al., 2004. Pathways for degradation of connexins and gap junctions. *Cardiovasc. Res.* 62, 256–267.
- Bjorkoy, G., Lamark, T., Brech, A., et al., 2005. p62/SQSTM1 forms protein aggregates degraded by autophagy and has a protective effect on huntingtin-induced cell death. *J. Cell Biol.* 171, 603–614.
- Catarino, S., Ramalho, J.S., Marques, C., et al., 2011. Ubiquitin-mediated internalization of connexin43 is independent of the canonical endocytic tyrosine-sorting signal. *Biochem. J.* 437, 255–267.
- Ciani, B., Layfield, R., Cavey, J.R., et al., 2003. Structure of the ubiquitin-associated domain of p62 (SQSTM1) and implications for mutations that cause Paget’s disease of bone. *J. Biol. Chem.* 278, 37409–37412.
- Falk, M.M., Baker, S.M., Gumpert, A.M., et al., 2009. Gap junction turnover is achieved by the internalization of small endocytic double-membrane vesicles. *Mol. Biol. Cell* 20, 3342–3352.
- Falk, M.M., Fong, J.T., Kells, R.M., et al., 2012. Degradation of endocytosed gap junctions by autophagosomal and endo-/lysosomal pathways: a perspective. *J. Membrane Biol.* 245, 465–476.
- Fallon, R.F., Goodenough, D.A., 1981. Five-hour half-life of mouse liver gap-junction protein. *J. Cell Biol.* 90, 521–526.
- Fong, J.T., Kells, R.M., Falk, M.M., 2013. Two tyrosine-based sorting signals in the Cx43 C-terminus cooperate to mediate gap junction endocytosis. *Mol. Biol. Cell* 24, 2834–2848.
- Fong, J.T., Kells, R.M., Gumpert, A.M., et al., 2012. Internalized gap junctions are degraded by autophagy. *Autophagy* 8, 794–811.
- Fushman, D., Wilkinson, K.D., 2011. Structure and recognition of polyubiquitin chains of different lengths and linkage. *F1000 Biol. Rep.* 3, 26.
- Fykerud, T.A., Kjenseth, A., Schink, K.O., et al., 2012. Smad ubiquitination regulatory factor-2 controls gap junction intercellular communication by modulating endocytosis and degradation of connexin43. *J. Cell Sci.* 125, 3966–3976.
- Gaietta, G., Deerinck, T.J., Adams, S.R., et al., 2002. Multicolor and electron microscopic imaging of connexin trafficking. *Science* 296, 503–507.
- Geetha, T., Jiang, J., Wooten, M.W., 2005. Lysine 63 polyubiquitination of the nerve growth factor receptor TrkA directs internalization and signaling. *Mol. Cell* 20, 301–312.
- Ghoshroy, S., Goodenough, D.A., Sosinsky, G.E., 1995. Preparation, characterization, and structure of half gap junctional layers split with urea and EGTA. *J. Membrane Biol.* 146, 15–28.
- Gilleron, J., Fiorini, C., Carette, D., et al., 2008. Molecular reorganization of Cx43, ZO-1 and Src complexes during the endocytosis of gap junction plaques in response to a non-genomic carcinogen. *J. Cell Sci.* 121, 4069–4078.
- Girao, H., Catarino, S., Pereira, P., 2009. Eps15 interacts with ubiquitinated Cx43 and mediates its internalization. *Exp. Cell Res.* 315, 3587–3597.
- Goodenough, D.A., Gilula, N.B., 1974. The splitting of hepatocyte gap junctions and zonulae occludentes with hypertonic disaccharides. *J. Cell Biol.* 61, 575–590.
- Gumpert, A.M., Varco, J.S., Baker, S.M., et al., 2008. Double-membrane gap junction internalization requires the clathrin-mediated endocytic machinery. *FEBS Lett.* 582, 2887–2892.
- Hawryluk, M.J., Keyel, P.A., Mishra, S.K., et al., 2006. Epsin 1 is a polyubiquitin-selective clathrin-associated sorting protein. *Traffic* 7, 262–281.
- Hesketh, G.G., Shah, M.H., Halperin, V.L., et al., 2010. Ultrastructure and regulation of lateralized connexin43 in the failing heart. *Circ. Res.* 106, 1153–1163.

- Hicke, L., 2001. Protein regulation by monoubiquitin. *Nat. Rev. Mol. Cell Biol.* 2, 195–201.
- Hicke, L., Dunn, R., 2003. Regulation of membrane protein transport by ubiquitin and ubiquitin-binding proteins. *Annu. Rev. Cell Dev. Biol.* 19, 141–172.
- Hung, S.Y., Huang, W.P., Liou, H.C., et al., 2009. Autophagy protects neuron from Abeta-induced cytotoxicity. *Autophagy* 5, 502–510.
- Jordan, K., Chodock, R., Hand, A.R., et al., 2001. The origin of annular junctions: a mechanism of gap junction internalization. *J. Cell Sci.* 114, 763–773.
- Kabeya, Y., Mizushima, N., Ueno, T., et al., 2000. LC3, a mammalian homologue of yeast Apg8p, is localized in autophagosome membranes after processing. *EMBO J.* 19, 5720–5728.
- Kjenseth, A., Fykerud, T., Rivedal, E., et al., 2010. Regulation of gap junction intercellular communication by the ubiquitin system. *Cell Signal.* 22, 1267–1273.
- Kjenseth, A., Fykerud, T.A., Sirnes, S., et al., 2012. The gap junction channel protein connexin43 is covalently modified and regulated by SUMOylation. *J. Biol. Chem.* 287, 15851–15861.
- Laing, J.G., Tadros, P.N., Westphale, E.M., et al., 1997. Degradation of connexin43 gap junctions involves both the proteasome and the lysosome. *Exp. Cell Res.* 236, 482–492.
- Lauf, U., Giepmans, B.N., Lopez, P., et al., 2002. Dynamic trafficking and delivery of connexons to the plasma membrane and accretion to gap junctions in living cells. *Proc. Natl Acad. Sci. USA* 99, 10446–10451.
- Leach, D.H., Oliphant, L.W., 1984. Degradation of annular gap junctions of the equine hoof wall. *Acta Anat. (Basel)* 120, 214–219.
- Leithe, E., Kjenseth, A., Sirnes, S., et al., 2009. Ubiquitylation of the gap junction protein connexin-43 signals its trafficking from early endosomes to lysosomes in a process mediated by Hrs and Tsg101. *J. Cell Sci.* 122, 3883–3893.
- Leithe, E., Rivedal, E., 2004a. Epidermal growth factor regulates ubiquitination, internalization and proteasome-dependent degradation of connexin43. *J. Cell Sci.* 117, 1211–1220.
- Leithe, E., Rivedal, E., 2004b. Ubiquitination and down-regulation of gap junction protein connexin-43 in response to 12-O-tetradecanoylphorbol 13-acetate treatment. *J. Biol. Chem.* 279, 50089–50096.
- Leithe, E., Sirnes, S., Fykerud, T., et al., 2012. Endocytosis and post-endocytic sorting of connexins. *Biochim. Biophys. Acta* 1818, 1870–1879.
- Lichtenstein, A., Minogue, P.J., Beyer, E.C., et al., 2011. Autophagy: a pathway that contributes to connexin degradation. *J. Cell Sci.* 124, 910–920.
- Madhus, I.H., 2006. Ubiquitin binding in endocytosis – how tight should it be and where does it happen? *Traffic* 7, 258–261.
- Mazet, F., Wittenberg, B.A., Spray, D.C., 1985. Fate of intercellular junctions in isolated adult rat cardiac cells. *Circ. Res.* 56, 195–204.
- Mizushima, N., 2004. Methods for monitoring autophagy. *Int. J. Biochem. Cell Biol.* 36, 2491–2502.
- Musil, L.S., Le, A.C., VanSlyke, J.K., et al., 2000. Regulation of connexin degradation as a mechanism to increase gap junction assembly and function. *J. Biol. Chem.* 275, 25207–25215.
- Pankiv, S., Clausen, T.H., Lamark, T., et al., 2007. p62/SQSTM1 binds directly to Atg8/LC3 to facilitate degradation of ubiquitinated protein aggregates by autophagy. *J. Biol. Chem.* 282, 24131–24145.
- Pfeifer, U., 1980. Autophagic sequestration of internalized gap junctions in rat liver. *Eur. J. Cell Biol.* 21, 244–246.
- Piehl, M., Lehmann, C., Gumpert, A., et al., 2007. Internalization of large double-membrane intercellular vesicles by a clathrin-dependent endocytic process. *Mol. Biol. Cell* 18, 337–347.
- Pohl, C., Jentsch, S., 2009. Midbody ring disposal by autophagy is a post-abscission event of cytokinesis. *Nat. Cell Biol.* 11, 65–70.
- Postma, F.R., Hengeveld, T., Alblas, J., et al., 1998. Acute loss of cell-cell communication caused by G protein-coupled receptors: a critical role for c-Src. *J. Cell Biol.* 140, 1199–1209.
- Qin, H., Shao, Q., Igdoura, S.A., et al., 2003. Lysosomal and proteasomal degradation play distinct roles in the life cycle of Cx43 in gap junctional intercellular communication-deficient and -competent breast tumor cells. *J. Biol. Chem.* 278, 30005–30014.
- Ravikumar, B., Imarisio, S., Sarkar, S., et al., 2008. Rab5 modulates aggregation and toxicity of mutant huntingtin through macroautophagy in cell and fly models of Huntington disease. *J. Cell Sci.* 121, 1649–1660.
- Schnell, D.J., Hebert, D.N., 2003. Protein translocons: multifunctional mediators of protein translocation across membranes. *Cell* 112, 491–505.

- Seibenhener, M.L., Babu, J.R., Geetha, T., et al., 2004. Sequestosome 1/p62 is a polyubiquitin chain binding protein involved in ubiquitin proteasome degradation. *Mol. Cell Biol.* 24, 8055–8068.
- Severs, N.J., Shovel, K.S., Slade, A.M., et al., 1989. Fate of gap junctions in isolated adult mammalian cardiomyocytes. *Circ. Res.* 65, 22–42.
- Su, V., Lau, A.F., 2012. Ubiquitination, intracellular trafficking, and degradation of connexins. *Arch Biochem. Biophys.* 524, 16–22.
- Thévenin, A.F., Kowal, T.J., Fong, J.T., et al., 2013. Proteins and mechanisms regulating gap junction assembly, internalization and degradation. *Physiology* 28, 93–116.

This page intentionally left blank

# Index

---

*Note:* Page numbers followed by “*f*” and “*t*” refer to figures and tables, respectively.

## A

- Aberrant autophagy, 37
- Activating molecule in Beclin 1-regulated autophagy (AMBRA-1), 26–27, 84
- Acyl coenzyme A-binding protein (Acb1), 216–217
- Acyl coenzyme A-binding protein (ACBP), 196
- Adaptor proteins, 181–182
- Adenosine monophosphate-activated protein kinase (AMPK), 23, 73–75
  - and autophagy activation, 86
  - inhibition, and regulation of autophagy, 62–63
- A desintegrin and metalloprotease 10 (ADAM10), 141
- Adherens junction (AJ), 274
- Adipose tissue
  - apoptosis in, 123–124
  - autophagy in, 126
  - ghrelin as survival factor in, 128–129, 128*f*
  - mass, determination of, 122
- Aggresome
  - ubiquitin-proteasome system (UPS), 30
- Aging, 3
  - and changes in autophagy, 116
  - protein homeostasis and, 115
  - and reduction in UPS activity, 115–116
- AKT/PKB pathway, for autophagy activation, 86
- Alcohol-induced liver disease
  - lipophagy in, 37–38
- Allophagy, 33–34
- $\alpha$ -Hemolysin (Hla), 140–141
  - A desintegrin and metalloprotease 10, 141
  - inducing an autophagic response, 141–142
- $\alpha$ -Toxin, 136
- Alphaherpesvirinae*, 147
- Alpha-synuclein, 24–25
- Alzheimer’s disease (AD), 2, 24–25
  - unfolded/misfolded proteins and, 11–12
- Amino acids, 56
  - input to MTORC1/Beclin-1, 58*f*–59*f*
  - and regulation of autophagy, 59–64, 72–74
  - starvation, induction of autophagy by, 73
- Amyloid precursor protein (APP), 24
- Amyotrophic lateral sclerosis (ALS), 25
- Anderson disease, 35
- Annular gap junction (AGJ), 275–277
  - autophagic degradation of, 278–279
- Anticancer agents, 91*t*–93*t*
- Apoptosis, 122
  - adipocyte, 124
  - in adipose tissue
    - signaling pathways, 123–124
  - autophagy and, 26–29
- Argonaute protein (AGO), 88–90
- Atg12-Atg5 conjugate, 5
- Atg9 cycling system, 10–11
- Atg5-deficient mouse embryonic fibroblasts (MEF), 225
- Atg16L1* (autophagy-related 16-like 1), 224
  - role in TLR signaling, 225
- Autoimmune diseases, 206
- Autoimmunity, autophagy and, 197
- Autoinflammatory diseases, 206
- Autolysosomes, 82–83
- Autophagic lysosome reformation (ALR), 9–10
  - mTOR role in, 10
  - process steps, 10
- Autophagic proteins, 10–15
  - abnormal, 11–12
  - Beclin 1, 13
  - groups, 11
  - microtubule-associated protein light chain 3, 14–15
  - non-autophagic functions, 13–14
  - protein degradation pathways, 12–13
- Autophagosome, 34–35, 70, 82–83, 108, 160, 170, 216–217
  - detection by confocal microscopy, 163
  - elongation, 84–85
    - regulation of, 95–96
  - formation of, 8–9
    - initiation and, 83–84
    - in mammalian cells, 8–9
  - in herpes zoster dermatomal exanthem, 163–165
  - and IL-1 $\beta$ , 204–205
  - maturation/fusion with lysosomes, 85
    - Beclin-1 and, 152–153
    - miRNA and, 96

- Autophagosome (*Continued*)  
 miRNA regulation of, 94–96  
 in varicella exanthem, 163–165
- Autophagy, 122, 233–234  
 aberrant, 37  
 activation of  
   AKT/PKB pathway for, 86  
   AMPK pathway for, 86  
   BECN1 for, 87  
   FoxO3 pathway for, 86  
   by herpesviruses, 153–156  
   inositol pathway for, 86–87  
   mTOR pathway for, 85–86  
   p53 pathway for, 87–88  
   via PKR-eIF2 $\alpha$  signaling pathway, 148f  
   by viral nucleic acids, 155  
 in adipose tissue, 126  
 age-related changes in, 116  
 aging process, 3  
 and apoptosis, 26–29  
 and autoimmunity, 197  
 Ca<sup>2+</sup>-dependent activation, amino acid starvation  
   and, 74–75  
 and cellular senescence, 20–21  
 defects, 17  
 definition, 3  
 essential proteins. *See* Autophagic proteins  
 functions of, 4  
 general, 245–246  
 HBV-induced, 172–173  
 and heart disease, 23–24  
 hypoxia and, 87  
 induce, 18  
 induction by amino acid starvation, 73  
 inhibition  
   by amino acids, 72–73  
   by herpesvirus proteins, 147–153  
 interaction with *Staphylococcus aureus*, 137–140, 139f  
 and intracellular bacterial infection, 22  
 in mammalian cells, 4–5  
 mitochondrial fusion/fission, 31–32  
 molecular mechanisms of, 82–85  
 monitoring, 15  
 and necroptosis, 31  
 and neurodegenerative diseases. *See*  
   Neurodegenerative diseases  
 reactive oxygen species, 15–16  
 receptors, 247–250, 248f  
 regulation  
   miRNAs and, 90–96, 91f–93f  
   P2X7R-mediated, 219–220  
 regulation, amino acids and, 59–64, 72–74  
   AMPK inhibition and, 62–63  
   Beclin-1 and, 64–66  
   glutamate dehydrogenase and, 61–62  
   plasma membrane amino acid transporters, 63–64  
   Rag GTPases/v-ATPase/t-RNA synthetases and,  
     60–61  
   regulatory elements of, 125–126, 125f  
   role in immunity, 19–20  
   role in quality control, 3–4  
   role in tumorigenesis/cancer, 17–19  
 selective, 32–44, 246–247, 249f  
   allophagy, 33–34  
   axonopathy, 34–35  
   crinophagy, 35  
   glycophagy, 35–40  
   lipophagy, 36–38  
   mitophagy, 38–39  
   nucleophagy, 39–40  
   pexophagy, 40–44  
   reticulophagy, 41–42  
   ribophagy, 42–43  
   xenophagy, 43  
   zymophagy, 43–44  
 starvation-induced, 56  
 stress-responsive, 3–4, 82  
 TLR-induced, 184–186  
 types of, 7–8  
   chaperone-mediated autophagy, 7–8, 11, 70  
   macroautophagy, 7  
   microautophagy, 7, 70  
 and ubiquitination, 29–30  
 and ubiquitin-proteasome system, 112–115  
 and ubiquitin-proteasome system (UPS), 30  
 in viral defense/replication, 21–22  
 visualization by confocal microscopy, 162–163
- Autophagy-lysosome pathway, 12–13
- Autophagy-related gene (ATG), 10–11, 83–84, 122,  
 233–234  
 deletion, in thymic epithelial cells, 238–239  
 IBD risk variants, 224  
 loss of, 25
- Autophagy-related protein 8 (ATG8), 32–33
- Axonopathy, 34–35
- B**
- Basal/quality control autophagy, 4–5
- Basic leucine zipper (bZIP), 166
- B cells, 192
- Bcl-2, overexpression of, 6
- BCL2 homology 3 domain (BH3D), 12
- Beclin 1, 13, 27–28  
 amino acids input to, 58f–59f  
 complexes  
   and autophagy, 65

initiation, inhibition of, 150–152  
 regulation of activity, during starvation, 65–66  
 and maturation of autophagosome, 152–153  
 and regulation of autophagy, 64–66  
 BECN1 gene, 17–18, 64–65, 95  
 for autophagy activation, 87  
*Betaherpesvirinae*, 147  
 BNIP3L/NIX mitophagy receptor, 253  
*Burkholderia pseudomallei*, 22

## C

Ca<sup>2+</sup>, 72  
 and activation of autophagy by amino acid starvation, 74–75  
 Ca<sup>2+</sup>/Calmodulin-dependent kinase kinase- $\beta$  (CaMKK- $\beta$ ), 74–75  
 and activation of autophagy, 75–77, 76f  
 Cancer  
 miRNAs in, 88  
 role of autophagy in, 17–19  
 Cargo sequestration, 7  
 Caspases, 122  
 activation of, 123  
 Catabolism, 104  
 CD4<sup>+</sup> T cells, 193–195  
 Cell proteins, post-translation modifications of, 22  
 Cellular homeostasis, 170, 245  
 Cellular senescence  
 autophagy and, 20–21  
 types of, 20  
 Chaperone-assisted selective autophagy (CASA), 108  
 Chaperone Hsc70 proteins catalyze, 5  
 Chaperone-mediated autophagy (CMA), 7–8, 11, 70, 109–111  
 reduction in activity, aging and, 116  
 Chemotherapeutics, 18  
 c-jun N-terminal kinase 1 (JNK1), 65–66  
 Clathrin, 10  
 Closed circular DNA (cccDNA), 171  
 Confocal microscopy  
 autophagy visualization by, 162–163  
 Core particle (CP), 106  
 C-reactive protein (CRP), 193–195  
 Crinophagy, 35  
 Crohn's disease (CD), 197, 206, 224  
 C-terminus, 161  
 Cullin-3, 226  
 Cybrids, 259  
 Cyclooxygenase type 2 (COX-2), 202  
 Cytochrome c, 26  
 Cytokines  
 interleukin-1. *See* Interleukin-1 (IL-1)

## D

Death-associated protein kinase (DAPK), 28, 65  
 Death-inducing signaling complex (DISC), 26, 123–124  
 de Duve, Christian, 82–83  
 Dendritic cells (DCs)  
 HSV-1 infection of, 153  
 Dengue virus, 147  
 Deubiquitinating enzyme (DUB), 29–30  
 Disulfide isomerase, 5  
 DJ-1 oncogene, mutations in, 24–26  
 DNA viruses, 147  
 Dopaminergic neurons (DA), 258–259  
 Double FYVE domain-containing protein 1 (DFCP1), 5–6  
*Drosophila melanogaster*, 259–260  
 Dynamin-related protein 1 (DRP1), 244–245

## E

Electron transport chain (ETC), 258  
 Endoplasmic reticulum (ER), 3, 5–6, 9  
 function of, 5–6  
 reticulophagy and, 41–42  
 role in cell biosynthesis, 5  
 stress, 6  
 HBV and, 173  
 in VZV-infected cells, 160  
 Endosome-mediated autophagy, 236  
 Epigallocatechin gallate (EGCG), 37  
 Epigenetic dysregulation, 24  
 Epithelial cells  
 medullary, role of autophagy within, 237  
 thymic, deletion of autophagy-related genes in, 238–239  
 Epstein-Barr virus (EBV), 146–147  
 nuclear antigen 1, 146–147, 193–195  
*Escherichia coli*, 22, 136  
 Eukaryotic cells, 5–6  
 Exotoxin, 136

## F

Fas-associated death domain (FADD), 123–124  
 Fas ligand (FAS-L), 123–124  
 Fission protein 1 (FIS1), 244–245  
 FK506-binding protein 12-rapamycin-associated protein 1 (FRAP1). *See* Mammalian target of rapamycin (mTOR) kinase  
 FLIP (FLICE-like inhibitor protein), 152  
 FoxO3 pathway, for autophagy activation, 86

## G

GADD34 (growth arrest and DNA damage-inducible protein), 161  
 Gamma-aminobutyric acid A receptor-associated protein (GABARAP), 9



*Gammaherpesvirinae*, 147, 150–151

Gap junctions (GJs)

annular, 275–277

endocytosis

and cytoplasmic double-membrane vesicles

generation, 275–277, 276f, 277f

degradation by autophagy, 277–278

other degradation pathways for, 279–280

function, 274–275

structure, 274–275, 274f

gE (ORF68), 166

Genethonin 1, 36

Genome-wide association studies (GWAS), 224

GFP-LC3 protein, 15

gH (HSV-1 glycoprotein), 154

Ghrelin, 122, 127

and autophagy, 129

as survival factor in adipose tissue, 128–129, 128f

and tumor necrosis factor  $\alpha$ , 128–129

Ghrelin O-acyltransferase (GOAT) enzyme, 127

*GHRL* gene, 127

Glutamate decarboxylase 65 (GAD65), 197

Glutamate dehydrogenase

importance of, 61–62

and regulation of autophagy, 61–62, 72–73

Glycophagy, 35–40

Golgi complex, 8–9

Golgi reassembly stacking protein (GRASP) 55, 216

Gonadal protein (GnRHR), 12

G protein  $\beta$ -subunit-like protein (G $\beta$ L), 71–72, 85–86

G-protein-coupled taste receptor, 64

Green fluorescent protein (GFP)-microtubule-associated protein light chain 3 (GFP-LC3), 234–235

## H

HBV-induced hepatocarcinogenesis, 174–175

HBV X protein (HBx), 172–173

Heart disease, autophagy role in, 23–24

Heart failure (HF), 23

inflammation and, 24

*Helicobacter pylori*, 28

Hepatitis B virus (HBV)

and autophagy, 172–173, 172f

genomic organization, 170–171, 171f

hepatocytes infection, 171

life cycle, 170–171

NTCP as receptor for, 171

replication, autophagy on, 173–174

Hepatitis C virus (HCV), 22

Hepatocellular carcinoma (HCC) model

*MIR7* role in, 90–94

Herpes simplex virus type 1 (HSV-1), 21. *See also*

Herpesviruses

autophagy regulation by, 154

of dendritic cells, 153

xenophagy of, 146

*Herpesviridae*, 147

Herpesviruses, 147

autophagy activation by, 153–156

proteins, inhibition of autophagy by, 147–153

varicella-zoster virus. *See* Varicella-zoster virus (VZV)

Herpesvirus saimiri (HVS), 152

Herpes zoster dermatomal exanthem, 162

autophagosomes in, 163–165

Histone deacetylase 6 (HDAC6), 111–112

Histone deacetylase (HDAC) inhibitor, 24–26, 28

Hormonal regulators

autophagy, in mammalian cells, 70

Human cytomegalovirus (HCMV), 151–152, 155

Human immunodeficiency virus (HIV), 21

negative elongation factor protein, 21

Humoral barriers, 180

Huntington's disease (HD), 24–25

Hybrid cells, 259

Hyperinsulinism/hyperammonia (HHS)

syndrome, 61

Hypoxia, and autophagy, 87

Hypoxia inducible factor-1a (HIF-1a), 87

## I

ICP34.5 viral proteins, 151, 161

IL-1 $\beta$ , 202, 212

autophagosomes and, 204–205

biological functions and regulation, 202–203

maturation of

by autophagy, 215–217

P2X7R-mediated, 215–217, 218f

role of lysosomes in, 213–215, 214f

mature 17-kDa form (mIL-1 $\beta$ ), 212

production, 202–203

by peripheral blood mononuclear cells (PBMCs), 204

secretion, 215–217

P2X7R-mediated, 220–221

role of autophagy in, 203–205, 203f

as therapeutic target in neurodegenerative disease, 220–221

Immune system, innate. *See* Innate immune system

Immunity, autophagy role in, 19–20

Induce autophagy, 18

Inducible nitric oxide synthase (iNOS), 202

Inflammasomes, 202–203

activation, autophagy and, 204

components, degradation by autophagosome, 204–205

NALP3, 213–214

- Inflammation, 202  
and heart failure, 24
- Inflammatory bowel diseases (IBD), 224
- Inflammatory diseases  
autophagy and, 206–207  
classification, 206. *See also* Autoimmune diseases;  
Autoinflammatory diseases
- inhibitor of apoptosis proteins (IAPs), 124
- Innate immune system, 180  
components, 180  
role of autophagy in, 215
- Inositolphosphate multikinase (IPMK), 62–63
- Inositol signaling pathway, for autophagy activation,  
86–87
- Insulin-amino acid-MTOR signaling pathway  
overview of, 56–57, 58f–59f
- Insulin growth factor 1 (IGF-1), 128–129
- Interleukin-1 (IL-1), 202  
IL-1 $\beta$ . *See* IL-1 $\beta$
- Intracellular bacterial infection, autophagy role in, 22
- Intracellular protein degradation pathways  
chaperone-mediated autophagy, 109  
macroautophagy, 107–108  
microautophagy, 109–110  
ubiquitin-proteasome system, 104–107
- Intrinsically disordered regions proteins (IDRPs), 12
- IRE1 (inositol-requiring enzyme-1), 166
- IT15* gene, 25
- J**
- JNK-interacting-protein-1 (JIP-1), 65–66
- K**
- Kaposi's sarcoma-associated herpesvirus (KSHV), 147,  
152  
anti-autophagic protein of, 153  
KFERG-like motif, 8, 70
- L**
- Lafora disease, 35
- Latent membrane protein (LMP1), 154
- LC3-associated phagocytosis (LAP), 22, 195
- LC3-Interacting Regions (LIR) motifs, 111–112
- Legionella pneumophila*, 22
- Leucyl-tRNA synthetase (LRS), 72–73
- Light chain 3 (LC3)  
conjugation complex, inhibition of, 152
- Lipodystrophies, 124
- Lipophagy, 36–38, 147  
in alcohol-induced liver disease, 37–38
- Lipopolysaccharide (LPS), 181, 212
- Lipoprotein receptor-related protein-1 (LRP1), 28
- Listeria monocytogenes*, 22
- Lysosomal membrane protein (LAMP), 8, 10
- Lysosomes, 74  
conventional, 213  
maturation/fusion with  
autophagosome, 85  
IL-1 $\beta$ , 213–215, 214f  
and processing of pro-IL1 $\beta$ , 213–214  
secretory, 213
- M**
- Macroautophagy, 7, 56, 70, 107–108, 170, 182–184, 233.  
*See also* Autophagy  
antigen packaging for cross-presentation, 196  
MHC class I antigen regulation by, 196–197  
pharmacological inhibition of, 193–195  
reduction in activity, aging and, 116  
upregulation, UPS disruption and, 112–114, 113f
- Macrophages, 180–181  
and IL-1 $\beta$ , 202
- Major histocompatibility complex (MHC), 146–147  
classes of, 192  
class I antigen, regulation by macroautophagy, 196–197  
class II molecules, cytosolic antigen presentation,  
193–195
- Malignant neoplasms, 17
- Mammalian target of rapamycin complex 1 (mTORC1),  
70–72  
activation of, 73  
inactivation of, 73  
subunits of, 85–86
- Mammalian target of rapamycin (mTOR) kinase, 5,  
16–17  
and autophagy activation, 85–86  
nutrient-sensor, 126  
pathways upstream to, 90–94  
role in autophagic lysosome reformation, 10
- MAP4K3 protein, 63
- Matrix protein 1 (MP1), 193–195
- Mature 17-kDa form (mIL-1 $\beta$ ), 212  
P2X7R-mediated unconventional secretion, 218–219
- Medullary epithelial cells  
role of autophagy within, 237
- Membrane-derived vesicles (MVs), 140–141
- Messenger RNA (mRNA), 88–90
- 2-Methoxyestradiol-bis-sulfamate (2-MeDE2bis MATE),  
28
- MHC class II-containing compartments (MIICs), 192  
autophagy in delivering antigens to, 236
- Microautophagy, 7, 70, 109–110. *See also* Autophagy
- Micrococci, 136
- Microglia  
P2X7R functional expression, in, 217, 218f
- MicroRNA-224 (miR-224), 174–175

- MicroRNAs (miRNAs), 23–24, 88  
 biogenesis, 88–90, 89f  
 in cancer, 88  
 names of, 88  
 and regulation of autophagosome, 94–96  
 and regulation of autophagy, 90–97, 91f–93f
- Microtubule-associated histone deacetylase 6 (HDAC6), 30
- Microtubule-associated protein light chain 3 (LC3), 9, 14–15  
 limitations, 15
- MIR7 (miRNAs), 90–94
- MIR101 (miRNAs), 90–94
- MIR30A (miRNAs), 94–96
- MIR181A (miRNAs), 95–96
- Mitochondria, 26, 82–83, 244–245, 258  
 damaged, removal of, 251–252  
 dysfunction, Parkinson's disease and, 259–260  
 fusion/fission, 31–32  
 PINK1 stabilization on, 261–263  
 proteins, Parkin-mediated ubiquitination of, 266–268, 267f
- Mitochondrial DNA (mtDNA)  
 nDNA and, 245
- Mitochondrial processing peptidase (MPP), 262–263
- Mitofusins 1/2 (MFN 1/2), 244–245, 251
- Mitophagy, 38–39, 82–83, 250–254  
 and neurons, 268–269  
 Parkin/PINK1-mediated, 268  
 receptors, 252–254  
 BNIP3, 253–254  
 BNIP3L/NIX, 252–254  
 and removal of damaged mitochondria, 251–252
- Molecular mechanisms, of autophagy, 82–85
- Monocytes  
 and IL-1 $\beta$ , 202
- MPTP (1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine), 259
- MTORC1 (mammalian target of rapamycin complex 1), 56–57  
 amino acids input to, 58f–59f  
 regulation of  
 glutamate dehydrogenase and, 61–62  
 Rag GTPases/v-ATPase/t-RNA synthetases and, 60–61
- Murine gamma herpesvirus 68 ( $\chi$ HV68), 150–151
- Mycobacterium bovis*, 219
- Mycobacterium tuberculosis*, 22, 30, 146, 183–184
- Myeloid differentiation factor 88 (MyD88), 181–182
- Myocardial stress, 23
- N**
- NADPH, 62
- NALP3 inflammasome, 213–214
- NBR1 protein, 32–33, 111–112, 247–250
- NDP52, autophagy receptors, 250
- Necroptosis  
 autophagy and, 31
- Necrosis, 122
- Necrostatin-1 (Nec-1), 31
- NEDA (Nuclear Envelope-Derived Autophagy), 154
- Neurodegenerative diseases. *See also* Alzheimer's disease (AD); Huntington's disease (HD); Parkinson's disease (PD)  
 IL-1 $\beta$  as therapeutic target in, 220–221  
 role of autophagy in, 24–26
- Neuronal autophagy. *See* Axonopathy
- Neurons, mitophagy and, 268–269
- NOD-like receptors (NLRs), 202–203
- Nuclear antigen 1 of Epstein Barr virus (EBNA1), 193–195
- Nuclear DNA (nDNA)  
 mtDNA and, 245
- Nuclear export signal (NES), 64–65
- Nucleic acids  
 viral, autophagy activation by, 155
- Nucleophagy, 39–40
- Nutritional regulators  
 autophagy, in mammalian cells, 70
- O**
- Obesity, 124
- Oncogene-induced senescence, 20
- 1N3 molecule, 12
- Optic atrophy 1 (OPA1), 244–245
- Optineurin (OPTN), 250
- Outer mitochondrial membrane, 9
- Oxidative stress, 16
- P**
- Parkin (*PARK2*) gene, 259–261, 264–266  
 domain boundaries of, 262f  
 PINK1-mediated recruitment of, 266
- Parkinson's disease (PD), 2, 24–26, 111, 258–259  
 and mitochondrial dysfunction, 259–260
- Pathogen-associated molecular patterns (PAMPs), 180–182
- PAT proteins, 37
- Pattern recognition receptors (PRRs), 180–181
- Peptidylarginine deiminase (PAD), 195
- Peripheral blood mononuclear cells (PBMCs)  
 IL-1 $\beta$  production by, 204
- Peroxisomes, 82–83  
 degradation of. *See* Pexophagy
- Pexophagy, 40–44
- p53 gene, tumor suppressor, 3, 114  
 and autophagy activation, 87–88
- Phagocytosis, 180–181  
 autophagy regulation of, 195

Phagophore, 70  
 Phosphatidylethanolamine (PE), 9, 84–85  
 Phosphatidylinositol 3-kinase complex (PI3KC), 9–11, 159  
 Phosphatidylinositol 3-phosphate (PI3P), 84, 170  
 Phospholipase D, 62–63  
*Pichia pastoris*, 40–41  
 Piecemeal micronucleophagy of nucleus (PMN), 39–40  
 PINK1, 38  
 Plasma membrane amino acid transporters  
 and regulation of autophagy, 63–64  
 Pocket of  $\beta 5$  subunit (PSMB5), 118  
 Polymorphisms, 206–207  
 Polyubiquitylation, 106, 107f, 111  
 p62 protein, 32, 111–112, 114, 165–166, 224, 247–250  
 regulation of, 225–226  
 ubiquitination by *Atg16L1*, 226  
 p97 protein, 112  
 PRAS40 (proline rich Akt substrate of 40kDa), 85–86  
 Pregenomic RNA (pgRNA), 171  
 Premature-miRNAs (pre-miRNA), 88–90, 89f  
 Premature senescence, 20  
 Primary-miRNAs (pri-miRNAs), 88–90, 89f  
 Procaspase-8, 123–124  
 Pro-cytokine (pro-IL1 $\beta$ )  
 processing of, lysosomes in, 213–214  
 Programmed cell death, 122  
 forms of, 122. *See also* Apoptosis; Autophagy;  
 Necrosis  
 Proteasome, 82  
 inhibition, 112–114, 113f  
 molecular architecture of, 106  
 Proteasome functional insufficiency (PFI), 117  
 Protein homeostasis  
 and aging, 115  
 Proteins. *See also* Specific proteins  
 adapter, 181–182  
 aggregation of, 247–248  
 mitochondria, Parkin-mediated ubiquitination of,  
 266–268, 267f  
 targeted for degradation by UPS, 105–106  
 ubiquitination, 224–225  
 PtdIns 3-kinase (class III PI3K), 63, 84  
 PTEN-induced putative kinase 1 (PINK1), 251, 259–261  
 activity on mitochondria, 264  
 domain boundaries of, 262f  
 model for, 263f  
 stabilization on mitochondria, 261–263  
 Pterostilbene, 28  
 P2X7 receptor (P2X7R), 212–213  
 functional expression, in microglia, 217, 218f  
 in maturation and unconventional secretion of IL-1 $\beta$ ,  
 217–221, 218f

## R

Rag GTPases, 72–73  
 and regulation of autophagy, 60–61  
 Rapamycin-insensitive companion of mTOR (RICTOR),  
 71–72  
 RAPTOR (regulatory associated protein of mTOR),  
 71–72, 85–86  
 Reactive oxygen species (ROS), 15–16, 62, 87, 245  
 Receptor-interacting protein-1 (RIP1), 31  
 Receptors  
 autophagy, 247–250, 248f  
 NBR1, 247–250  
 NDP52, 250  
 optineurin, 250  
 p62, 247–250  
 mitophagy, 252–254  
 BNIP3, 253–254  
 BNIP3L/NIX, 252–254  
 Regulatory particle (RP), 106  
 Replicative senescence, 20  
 Reticulophagy, 41–42  
 RHEB (activator of mTORC1), 72–73  
 Rhesus monkey rhadinovirus (RRV), 154–155  
 Ribophagy, 42–43  
 RNA induced silencing complex (RISC), 88–90

## S

*Saccharomyces cerevisiae*, 16, 39  
*Salmonella enteritica typhimurium* (*S. typhimurium*), 250  
*Salmonella typhimurium*, 22  
 SAPK-interacting protein 1 (SIN1), 71–72  
 scAtg32 protein, 252  
 Secretory lysosomes, 213  
 Senescence. *See* Cellular senescence  
 Sepsis, 207  
 Sequestosome 1 gene (SQSTM1), 165–166, 236  
*Shigella flexneri*, 22  
 Sindbis virus, 21  
 Single nucleotide polymorphisms (SNPs), 160, 197  
 Skin, as physical barrier, 180  
 Small interfering RNAs (siRNAs), 88  
 Sodium taurocholate cotransporting polypeptide  
 (NTCP)  
 as receptor for HBV, 171  
*Staphylococcus aureus*, 22, 136–140, 139f  
 $\alpha$ -hemolysin, 140–141  
 A desintegrin and metalloprotease 10, 141  
 inducing an autophagic response, 141–142  
 interaction with autophagic pathway, 137–140, 139f  
 membrane-derived vesicles, 140–141  
 pathogen with dual lifestyle, 136–137  
 Starch-binding domain-containing protein 1  
 (Stbd 1), 36

- Starvation  
  amino acid, induction of autophagy by, 73  
  Beclin-1 complex during, activity of, 65–66  
  in mammalian cells, 5  
  Starvation-induced autophagy, 56  
*Streptococcus pyogenes*, 22, 183–184  
  Stress-responsive autophagy, 3–4, 82  
  Substantia nigra pars compacta (SNpc), 258–259  
  Systemic lupus erythematosus (SLE), 197, 206–207
- T**  
  TANK binding kinase 1 (TBK1), 252  
  Target of rapamycin complex 1 (TORC1), 83–84  
  Tarui disease, 35  
  Taurine-induced apoptosis, 27  
  T cell receptor (TCR), 193–195  
  T cells, 192  
  Th17 cells, 205–206  
  Thymus  
    autophagy-deficient transplants, 236–237  
    epithelium of, 232–233  
    evidence for autophagy in, 234–236  
    function of, 233  
    stroma of, 232  
  Tight junctions (TJ), 274  
  TNF-related apoptosis-induced ligand (TRAIL), 123–124  
  Toll-like receptor (TLR), 19–20, 146, 181–182, 195, 215  
    and autophagy, 184–186  
    signaling, *Atg16L1* role in, 225  
  Transcriptional dysregulation, 24  
  Transcription factor EB (TFEB), 57  
  Transporters associated with antigen processing (TAPs), 146–147, 196–197  
  TRIM50 gene, 30  
  t-RNA synthetases  
    and regulation of autophagy, 60–61  
  TIR1/TIR3 receptor, 64  
  Tuberous Sclerosis Complex 1/2 (TSC1/2 complex), 70  
  Tumorigenesis/cancer. *See* Cancer  
  Tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ), 123–124  
    ghrelin and, 128–129  
  Tumor suppressor  
    *p53* gene, 3  
  Type 2 diabetes, 127  
  Type 2 phospholipase A, 202
- U**  
  Ubiquitin, 29–30, 111–112  
  Ubiquitination, 22, 245–246  
    autophagy and, 29–30  
    of mitochondrial proteins, Parkin-mediated, 266–268, 267f  
    P62, by *Atg16L1*, 226  
    proteins, 224–225  
  Ubiquitin–proteasome system (UPS), 12–13, 30, 82, 104–107  
    age-associated changes in, 115–116  
    and autophagy, 112–115  
    disruption, macroautophagy upregulation in response to, 112–114, 113f  
    inhibition of, 114–115  
    and protein targeting for degradation, 105–106  
  Ulcerative colitis (UC), 224  
  UNC-51-like kinase 1 (ULK1), 71–72  
  Unfolded protein response (UPR), 6, 41–42, 113–114, 165–166, 173
- V**  
  Vacuole membrane protein 1 (VMP1), 162  
  Varicella disease, 160–161  
  Varicella exanthem, 161–162  
    autophagosomes in, 163–165  
  Varicella-zoster virus (VZV), 147, 160–161  
    herpes zoster dermatomal exanthem, 162  
    infection, and ER stress, 165–166  
    varicella exanthem, 161–162  
  v-ATPase  
    and regulation of autophagy, 60–61  
  vFLIP virus, 154–155  
  Viral KSHV Bcl-2 homologue (vBcl-2), 150–151  
  Viral nucleic acids  
    autophagy activation by, 155  
  Viruses  
    autophagy and, 21–22  
  Voltage-dependent anion channel 1 (VDAC1), 251–252  
  VPS34-containing vesicles, 5–6
- W**  
  Wortmannin, 138
- X**  
  X-box binding protein-1 (XBP1) mRNA, 166  
  Xenophagy, 43, 146, 170, 250
- Y**  
  *Yersinia pseudotuberculosis*, 22
- Z**  
  Zymophagy, 43–44