ESSAYS IN BIOCHEMISTRY

Other recent titles in the *Essays in Biochemistry* series:

The Role of Non-Coding RNAs in Biology: volume 54
edited by M.A. Lindsay and S. Griffiths-Jones
2013
ISBN 978 1 85578 190 0

Cell Polarity and Cancer: volume 53
edited by A.D. Chalmers and P. Whitley
2012
ISBN 978 1 85578 189 4

Lysine-Based Post-Translational Modification of Proteins: volume 52
edited by I. Scott
2012
ISBN 978 1 85578 185 6

Molecular Parasitology: volume 51
edited by R. Docampo
2011
ISBN 978 1 85578 184 9

ABC Transporters: volume 50
edited by F.J. Sharom
2011
ISBN 978 1 85578 181 8

Chronobiology: volume 49
edited by H.D. Piggins and C. Guilding
2011
ISBN 978 1 85578 180 1

Epigenetics, Disease and Behaviour: volume 48
edited by H.J. Lipps, J. Postberg and D.A. Jackson
2010
ISBN 978 1 85578 179 5

ESSAYS IN BIOCHEMISTRY

volume 55 2013

Autophagy: Molecules and Mechanisms

Edited by Jon D. Lane

portlandpresslimited
publishing innovation

Essays in Biochemistry is published by Portland Press Limited on behalf of the Biochemical Society

Portland Press Limited
Third Floor, Charles Darwin House
12 Roger Street
London WC1N 2JU
U.K.
Tel: +44 (0)20 7685 2410
Fax: +44 (0)20 7685 2469
email: editorial@portlandpress.com
www.portlandpress.com

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library
ISBN 978-1-85578-191-7
ISSN (print) 0071 1365
ISSN (online) 1744 1358

Typeset by Techset Composition Ltd, Salisbury, U.K.
Printed in Great Britain by Cambrian Printers Ltd, Aberystwyth

CONTENTS

PREFACE

Autophagy has emerged in recent years as one of the most exciting fields in cell biology. From its meagre beginnings as a curiosity reported by the pioneers of electron microscopy in the 1950s, through its first description in 1963 by the late Nobel Laureate, Christian DeDuve, to the latter day application of yeast genetics, cell biology and vertebrate model organism research, interest in this key cellular process has blossomed. Until quite recently, the mechanistic control of autophagy remained obscure, but with the molecular jigsaw pieces now being assembled at a terrific pace, there is a need to take stock and consider how far we have come and how much there is still to learn. This is particularly important for autophagy because very little background information is available in textbooks for consultation by students and early researchers interested in learning about this field.

To chart the increasing numbers of articles on autophagy in major journals over recent years tells only part of the story of the expanding interest in this burgeoning field. Only when one delves into the diversity of the scientific disciplines for which autophagy contributes, can one fully appreciate the broad influence of this essential biological process. Autophagy has roles in development, tissue and cell differentiation and maturation, cell division, homoeostasis and metabolic control, to name but a few. Appropriately enough, dysregulation of autophagy is now known to cause or contribute to a range of human diseases, further underlining the importance of studying the regulation of this pathway, and further explaining the explosion in interest in this topic.

Three major types of autophagy have been characterized to date, CMA (chaperone-mediated autophagy), microautophagy and macroautophagy. This collection of essays is dedicated to macroautophagy, the most studied and thus best understood form of autophagy, and the reader is directed to other sources for coverage of the other autophagy pathways. In this preface, and in the following chapters, the common practice of describing macroautophagy as simply 'autophagy' will be adopted. Autophagy is, strictly speaking, a membrane trafficking process. It describes the *de novo* assembly and maturation of novel double-membrane organelles that expand and close to sequester cytoplasm, including whole organelles, in order to efficiently deliver surplus or redundant cellular cargo to the lysosomes for degradation and recycling. Driving this complex process of membrane assembly/remodelling, trafficking and heterotypic organelle fusion is a set of essential autophagy-specific proteins. These include protein and lipid kinases, membrane targeting and trafficking factors, endopeptidases, and protein and lipid ubiquitin-like modifiers. In this volume of *Essays in Biochemistry*, our current understanding of the events that control autophagosome assembly and maturation will be described (Chapters 1–7), before the influence of autophagy during ageing, infection and disease is examined (Chapters 9–12). Separating these, are two chapters (Chapters 7 and 8) that discuss how autophagy can become highly selective to degrade specific cytoplasmic cargoes, a key facet of cellular protein and organelle quality control.

In Chapter 1, "Early signalling events of autophagy", Laura Gallagher and Ed Chan explain how the autophagy-specific protein kinase, ATG1 (or ULK1 in mammalian cells) responds to changing nutrient levels via mTOR (mammalian/mechanistic target of rapamycin) signalling to trigger the cascade of downstream events that drive autophagosome biogenesis. Chapter 2 by Rebecca Roberts and Nicholas Ktistakis, "Omegasomes: PI3P platforms that manufacture autophagosomes", focuses on a key step during autophagosome assembly, the activation of a class III phosphoinositide 3-kinase that generates phosphatidylinositol 3-phosphate (PI3P) at the ER (endoplasmic reticulum) to enable recruitment of downstream effectors. These PI3P-rich membranes sub-domains expand to adopt an idiosyncratic Ω shape, and are thus described as 'omegasomes'. In Chapter 3, Sharon Tooze reviews current theories on the origins of membranes for autophagosome assembly, still a controversial area in the autophagy field. Chapters 4 and 5 focus on the mechanistic pathways that control the assembly of the autophagosome. In Chapter 4, "Two ubiquitin-like conjugation systems that mediate membrane formation during autophagy", Hitoshi Nakatogawa explains how conjugation of ATG12 to ATG5 and LC3/ATG8 to the lipid, phosphatidylethanolamine, controls the expansion and fusion of the nascent autophagosome. In Chapter 5, "The ATG8 family: multifunctional ubiquitin-like key regulators of autophagy", Moran Slobodkin and Zvulun Elazar focus on the only stably integrated protein constituents of the autophagosomal limiting membrane, the ATG8 family of ubiquitin-like lipid modifiers. They discuss how cleavage of ATG8 family members at conserved C-terminal sites by the ATG4 family of endopeptidases is crucial for ATG8 function. To understand the physiological significance of autophagy, and to appreciate how failures in autophagy can contribute to disease, it is essential that the efficiency of the lysosomal fusion step be considered. In Chapter 6, "Autophagosome maturation and lysosomal fusion", Ian Ganley examines the events that take place during autophagosome maturation, how autophagosomes merge with the endocytic compartment en route to the lysosome, and the roles played by the cytoskeleton, Rabs, and membrane tethers and SNAREs.

The next two chapters are dedicated to selective autophagy. In Chapter 7, "Selective autophagy", Steingrim Svenning and Terje Johansen discuss how the regulated binding of adaptor molecules to ATG8 family members allows the efficient incorporation of diverse cellular cargoes into the nascent autophagosome. They describe how this process enables cells to degrade protein aggregates, redundant/damaged organelles and invading microorganisms, a topic that is revisited in the final chapter. In Chapter 8, Thomas MacVicar describes the varied pathways that allow the selective degradation of mitochondria by autophagy. This process is described as "Mitophagy", and is essential for mitochondrial quality control and for mitochondrial removal during erythroid differentiation.

The final four chapters in the book examine how autophagy influences cell viability in health and disease. In Chapter 9, "Autophagy and cell death", Tohru Yonekawa and Andrew Thorburn discuss the ways in which autophagy can both contribute to and protect against cell death in a tissue- and context-specific manner.

In doing so, they describe the key proteins that provide a mechanistic link between autophagy and apoptosis. In Chapter 10, "Autophagy and ageing: implications for age-related neurodegenerative diseases", Bernadette Carroll, Graeme Hewitt and Viktor Korolchuk describe the roles that autophagy play during the ageing process, focusing on how autophagy pathways (including CMA) prevent the accumulation of toxic protein aggregates that are known to contribute to neuronal cell death during neurodegenerative disease. In Chapter 11, "Role of autophagy in cancer prevention, development and therapy", G. Vignir Helgason, Tessa Holyoake and Kevin Ryan describe how the signalling pathways that are disrupted in cancer influence the autophagy pathway, and how autophagy can be both oncogenic and tumour suppressive, depending on context. The authors then go on to examine how autophagy might be targeted for cancer therapy. In Chapter 12, "Autophagy as a defence against intracellular pathogens", Tom Wileman describes the essential role played by autophagy in recognizing and degrading invading microorganisms, an important facet of host defences against pathogens.

Finally, I would like to acknowledge the people who have made this book possible. I am indebted to the Portland Press staff, in particular, Clare Curtis who has coordinated this process in a highly efficient and professional manner. I am grateful to the anonymous reviewers of the initial proposal and of the chapters themselves, and of course I thank the authors for writing such stimulating and high-quality reviews.

AUTHORS

Jon Lane is a Reader in Cell Biology at the University of Bristol, U.K. He obtained a Ph.D. at the University of Exeter in the mid-1990s, using insect and frog model systems to study the regulation of microtubule organization and stability through the cell cycle and during development. He then did postdoctoral research in Manchester, studying microtubule motor proteins and microtubule-based membrane movement using frog egg and embryo extracts. During this time, he became interested in cell death (apoptosis), and how dying cells re-organize their cytoskeletal and organelle systems to facilitate recognition and removal by cells of the innate immune system. Moving to Bristol in 2003 as a Wellcome Trust Career Development Fellow, then later as a Research Councils UK Fellow, he developed a strong interest in autophagy. His group is now focused on autophagy, with the aim of understanding how autophagy regulates cellular function during development and disease.

Laura Gallagher graduated with a first class honours Master of Pharmacology degree from the University of Bath in 2012. She is currently a Ph.D. student working with Dr Edmond Chan characterizing the role of autophagy during breast cancer metastasis.

Edmond Chan is a Lecturer of Biochemistry in the Faculty of Science, University of Strathclyde. Dr Chan has research interests in the signalling mechanisms that coordinate the multiple stages of autophagy. From 2003 to 2008, Dr Chan worked at the Cancer Research UK London Research Institute, where he screened siRNA libraries for autophagy factors and began focusing on nutrient-dependent kinases. Before that, he studied neurodegeneration using mouse models of Huntington's disease at the University of British Columbia, Canada. Dr Chan obtained his Ph.D. in Biochemistry (1999) from the University of Alberta, Canada.

Rebecca Roberts is a Research Associate in the Ktistakis laboratory in the signalling department at the Babraham Research Institute, Cambridge. For her Ph.D. from the University of East Anglia in Norwich, Rebecca studied the activation of autophagy with viral and non-viral particles. In her current research, Rebecca is searching for novel regulators of autophagy using an siRNA genome library.

Nicholas Ktistakis has been a group leader at the Signalling Programme of the Babraham Research Institute since 1996. He studies lipid signalling, with special emphasis on pathways regulated by phosphatidic acid and phosphatidylinositol 3-phosphate.

Sharon A. Tooze is a senior scientist at the London Research Institute, Cancer Research UK. Dr Tooze completed her Ph.D. and postdoctoral training at the European Molecular Biology Laboratory in Heidelberg, Germany, studying membrane trafficking in the Cell Biology Programme. Dr Tooze established her own laboratory at the London Research Institute in 1993 to pursue her interest in organelle biogenesis, first addressing secretory granule biogenesis. She began studying autophagy in 2006.

Hitoshi Nakatogawa received his Ph.D. from Kyoto University, Kyoto, Japan, in 2002, where he studied the regulation of protein synthesis and secretion in bacteria. Then, he joined Yoshinori Ohsumi's group at the National Institute for Basic Biology, Okazaki, as a postdoc in 2004, and the group moved to the Tokyo Institute of Technology, Yokohama, in 2009. He is currently an associate professor in the same group and is working on the molecular mechanisms of autophagy in yeast with a special focus on ubiquitin-like conjugation systems.

Moran Rawet Slobodkin is currently a postdoctoral fellow in the Elazar laboratory at the Weizmann Institute of Science. She completed her Ph.D. studies at the Faculty of Biology, Technion, Haifa in Professor Cassel's laboratory where she studied the mechanism of action of ARF-GAPs in membrane trafficking. Her present research focuses on the regulation of autophagy under different growth conditions.

Zvulun Elazar obtained his Ph.D. at the Weizmann Institute of Science in Rehovot, Israel. Currently, he is the Harold Korda Professor at the Department of Biological Chemistry of the Weizmann Institute of Science where he studies different mechanistic aspects of autophagy in yeast and mammalian systems. He is particularly interested in the role of the Atg8s, unique ubiquitin-like proteins that are part of the core autophagic machinery. The current focus of his laboratory is on the identification of factors that regulate these processes and are defective in pathophysiological conditions such as neurodegeneration and cancer.

Ian Ganley obtained his Ph.D. from the University of Cambridge working on the enzyme phospholipase D with Dr Nicholas Ktistakis. This was followed up with postdoctoral work in the laboratories of Professor Suzanne Pfeffer and Dr Xuejun Jiang at Stanford University and Memorial Sloan-Kettering Cancer Center respectively. It was there that Ian developed a passion for intracellular transport and how this relates to autophagy. In 2010, Ian relocated to Dundee to start his own laboratory focusing on the mechanisms of autophagy regulation.

Steingrim Svenning is currently working on his Ph.D. thesis as a member of Terje Johansen's group. His main work lies in characterizing the role of the selective autophagy receptor protein NBR1.

Terje Johansen is a Professor and group leader at the Institute of Medical Biology at the University of Tromsø, Norway. During work on cell signalling on atypical protein kinase C observations on the behaviour of an interacting protein p62/SQSTM1 led to the discovery of p62/SQSTM1 as the first selective autophagy receptor. Since then his focus has been set on the autophagy receptor proteins, as well as other aspects of selective autophagy.

Thomas MacVicar graduated with a B.Sc. in Biochemistry from the University of Bristol in 2009. He is currently studying for a Ph.D. in Jon Lane's laboratory (School of Biochemistry, University of Bristol) and is conducting research on mitophagy regulatory mechanisms.

Tohru Yonekawa is a postdoctoral fellow at the University of Colorado. His current research focuses on understanding signalling pathways that differentially regulate autophagy.

Andrew Thorburn is Professor and Chair of the Pharmacology Department and Deputy Director of the Cancer Center at the University of Colorado, School of Medicine. His research focuses on the role of autophagy and its interplay with apoptosis in cancer progression and treatment.

Bernadette Carroll recently completed her Ph.D. at Imperial College London. She is now working as a postdoctoral associate at Newcastle University investigating cellular signalling in response to nutrients.

Graeme Hewitt completed his undergraduate and Master's studies in Biomedicine with a focus on ageing. He is currently studying for his Ph.D. at Newcastle University investigating DNA damage and its associations with autophagy and ageing.

Viktor Korolchuk obtained his Ph.D. in Kiev, Ukraine, followed by postdoctoral training at Bristol and Cambridge Universities. He is currently a Lecturer at Newcastle University, studying nutrient-dependent signalling and trafficking pathways and their relevance to ageing.

G. Vignir Helgason trained for a Ph.D. degree in Professor Ryan's laboratory at the Beatson Institute and graduated from the University of Glasgow in October 2007. Since 2007, Dr Helgason held postdoctoral positions in Professor Holyoake's laboratory at the Paul O'Gorman Leukaemia Research Centre and then became Kay Kendall Leukaemia Fund Intermediate Research Fellow in 2013. Dr Helgason has also held a Lord Kelvin Adam Smith Leadership Fellowship from University of Glasgow since 2013. His research currently focuses on the role of autophagy in disease persistence and drug resistance in chronic myeloid leukaemia.

Tessa Holyoake is Director of the Paul O'Gorman Leukaemia Research Centre, University of Glasgow and a Consultant Haematologist at the West of Scotland Cancer Centre. Her hypothesis, driven translational research on cancer stem cells in chronic myeloid leukaemia, is of international standing and she is widely recognized as one of the key players in this field. This research focus developed from her Ph.D. at the Beatson Institute, Glasgow, on haemopoietic stem cell expansion for therapeutic use, through a 2-year postdoctoral fellowship in the Terry Fox Laboratories, British Columbia, to the current cancer stem cell focus. Professor Holyoake was made a fellow of the Royal Society of Edinburgh in 2008, was awarded the Scottish Health Award in Cancer in 2009 and the Lord Provost's award for Health in 2010.

Kevin Ryan received his Ph.D. from the Beatson Institute, University of Glasgow in 1996. Following postdoctoral work at the US National Cancer Institute, he was awarded a Cancer Research UK Senior Cancer Research Fellowship in 2002 and returned to the Beatson Institute to establish his own research group. In 2007, he was promoted to Senior Group Leader at the Beatson Institute and later that year was appointed Professor of Molecular Cell Biology at the University of Glasgow. Kevin's studies focus on the identification of cell death regulators involved in tumour development and tumour therapy with particular focus on how the apoptotic and autophagic pathways integrate to determine cell fate. In recognition of his work, he

was awarded the 2010 European Association for Cancer Research (EACR) 'Cancer Researcher Award' and the 2012 Tenovus Medal.

Tom Wileman is Professor of Molecular Virology at the Norwich Medical School at the University of East Anglia. He trained in cell biology and immunology at Washington University and Harvard Medical Schools in the U.S.A. between 1982 and 1994. During this period he studied receptor-mediated endocytosis and endoplasmic reticulum-related protein degradation. In 1994 he moved to the Institute for Animal Health Pirbright Laboratories in the U.K. and continued his interests in membrane traffic by studying the role played by membrane compartments in the control of virus infection and replication. His current interests at Norwich Medical School focus on understanding the role played by autophagy in controlling the replication of viruses, particularly how viral proteins activate autophagy, and the consequences this has for the survival of viruses within cells.

ABBREVIATIONS

AIM	ATG8 family-interacting motif
ALFY	autophagy-linked FYVE
AMBRA1	activating molecule in BECN1-regulated autophagy 1
AMPK	AMP-activated protein kinase
Asn*	invariable asparagine residue
ATF4	activating transcription factor 4
ATG/Atg	autophagy gene product/autophagy-related protein
BAD	Bcl-2/Bcl-x-L antagonist, causing cell death
BH	Bcl-2 homology
BIK	Bcl-2-interacting killer
BIM	Bcl-2-interacting mediator of cell death
CMA	chaperone-mediated autophagy
CML	chronic myeloid leukaemia
COPI	coatomer protein I
CQ	chloroquine
CR	caloric restriction
Cvt	cytoplasm-to-vacuole targeting
DAG	diacylglycerol
DAMP	damage-associated molecular pattern
dBRUCE	*Drosophila* baculovirus inhibitor of apoptosis repeat-containing ubiquitin-conjugating enzyme
DFCP1	double FYVE domain-containing protein 1
DISC	death-inducing signalling complex
DRAM-1	damage-regulated autophagy modulator 1
DUB	de-ubiquitinating enzyme
EAT	early autophagy targeting/tethering
EM	electron microscopy
ER	endoplasmic reticulum
ESCRT	endosomal sorting complex required for transport
FADD	Fas-associated death domain
FIP200	focal adhesion kinase family-interacting protein 200 kDa
FMDV	Foot and Mouth Disease virus
FYCO1	FYVE and coiled-coil domain containing 1
GABARAP	γ-aminobutyric acid receptor-associated protein
GAP	GTPase-activating protein
GATE-16	Golgi-associated ATPase enhancer of 16 kDa
GBM	glioblastoma multiforme
GEF	guanine nucleotide-exchange factor
gp78	glycoprotein 78
GSK3	gycogen synthase kinase 3

HCQ	hydroxychloroquine
HDAC6	histone deacetylase 6
HOPS	homotypic fusion and vacuolar protein sorting
HSC	haemopoietic stem cell
Hsc70	heat-shock cognate 70 stress protein
Hsp	heat-shock protein
IM	imatinib
IMM	inner mitochondrial membrane
KEAP1	Kelch-like ECH (erythroid cell-derived protein with cap 'n' collar homology)-associated protein 1
LC3	light-chain 3
LIR	LC3-interacting region
LLR	leucine rich
MAM	mitochondria-associated endoplasmic reticulum membrane
MCL-1	myeloid cell leukaemia sequence 1
MER	minimal essential region
Mfn	mitofusin
MTM	myotubularin
mTOR	mammalian/mechanistic target of rapamycin
mTORC	mammalian/mechanistic target of rapamycin complex
NBR1	neighbour of BRCA1 (breast cancer early-onset 1) gene 1
NDP52	nuclear dot protein 52
NIX	NIP3-like protein X
NOD	nucleotide-binding and oligomerization domain
NSF	*N*-ethylmaleimide-sensitive factor
OMM	outer mitochondrial membrane
OPA1	optic atrophy 1
PAMP	pathogen-associated molecular pattern
PAS	phagophore assembly site/pre-autophagosomal structure
PB1	Phox and Bem1p
PD	Parkinson's disease
PE	phosphatidylethanolamine
PI3K	phosphoinositide 3-kinase
PI3P	phosphatidylinositol 3-phosphate
PINK1	PTEN (phosphatase and tensin homologue deleted on chromosome 10)-induced putative kinase 1
PIP_3	phosphatidylinositol 3,4,5-trisphosphate
proAPI	precursor aminopeptidase-I
PUMA	p53 up-regulated modulator of apoptosis
raptor	regulatory associated protein of mTOR
Rheb	Ras homologue enriched in brain
RILP	Rab-interacting lysosomal protein
ROS	reactive oxygen species

SCV	*Salmonella*-containing vacuole
SINV	Sindbis virus
SLR	sequestosome 1-like receptor
SMURF1	SMAD-specific E3 ubiquitin ligase 1
SNAP-25	25 kDa synaptosome-associated protein
SNARE	soluble *N*-ethylmaleimide-sensitive factor-attachment protein receptor/soluble *N*-ethylmaleimide-sensitive fusion protein-attachment protein receptor
SQSTM1	sequestosome 1
Stbd1	starch-binding-domain-containing protein 1
TBK	TANK (tumour-necrosis-factor-receptor-associated factor-associated nuclear factor-κB activator)-binding kinase
TECPR	tectonin β-propeller repeat-containing protein 1
Tip60	HIV-1 Tat (transactivator of transcription)-interactive protein 60 kDa
TIRF	total internal reflection fluorescence
TLR	Toll-like receptor
TOR	target of rapamycin
TRAIL	tumour-necrosis-factor-related apoptosis-inducing ligand
TSC	tuberous sclerosis complex
UBA	ubiquitin-associated
Ubl	ubiquitin-like
ULK	uncoordinated-51-like kinase
UPS	ubiquitin–proteasome system
UVRAG	UV radiation resistance-associated gene
VMP1	vacuolar membrane protein 1
v-SNARE	vesicle-associated *N*-ethylmaleimidesensitive factor attachment protein receptor
WIPI	WD-repeat protein interacting with phosphoinositides