

Preface

Proteases are being recognized more and more as important players in a wide range of biological processes; for example, the cell cycle, blood clotting, angiogenesis, apoptosis, cell differentiation and growth, cell motility, lipid metabolism, antigen presentation and cell-fate determination are all dependent upon the controlled action of proteases. When the activity of proteases is not regulated appropriately, disease processes can result, as is seen in Alzheimer's disease, cancer metastasis and tumour progression, inflammation, pain, atherosclerosis and haemophilia. Thus, inhibition of proteases is being seen as a potential therapeutic strategy for these and other disorders. Probably the most successful examples of protease inhibitors in medicine today are the inhibitors of angiotensin-converting enzyme that are widely used to treat hypertension and congestive heart failure, and the HIV protease inhibitors that are at the forefront of the battle against this virus.

This volume covers many of these topics and highlights the role of proteases in biology and medicine. The first chapter introduces the reader to the basic terminology in the field of protease research and the classification systems used, and this is followed by a chapter by Guy Salvesen, who expertly introduces the caspases, the executioners of cell death (apoptosis). Yoshifumi Itoh and Hideaki Nagase then provide an excellent account of the large family of matrix metalloproteinases and their role in cancer metastasis, while Janelle Nunan and David Small outline the role of proteases (secretases) in the processing of the Alzheimer's amyloid precursor protein, and the potential sites of therapeutic intervention in this disease. In the subsequent chapter, John Mayer and colleagues describe the ubiquitin pathway of intracellular proteolysis and the central role played by 'the big mean proteolytic machine', the proteasome, in this process. The next three chapters cover the roles that proteases play in angiogenesis (Ralph Bradshaw and Elizabeth Yi), the processing of protein precursors (Nabil Seidah and Annik Prat), and the blood clotting cascade (Peter Walsh and Syed Ahmad). The two chapters that follow highlight the potential use of protease inhibitors as therapeutic agents, with a description of the HIV peptidase by Ben Dunn, and the use of angiotensin-converting enzyme inhibitors by Louise Burrell and colleagues. The latter chapter also introduces other peptidases as possible future drug targets in hypertension and heart disease. The next three chapters introduce topics that have only recently come to the fore. Marcia Moss and Millard Lambert provide an overview of membrane-protein shedding and the role of the ADAMs family of proteases in this process, Rob Rawson introduces the fascinating area of regulated intramembrane proteolysis, while Nigel Bunnett and colleagues review the

surprising role that protease-activated receptors have to play in cell signalling. The final chapter, by David Coates, covers the important place that bioinformatics and genome database screening has, and will continue to have, in the identification of novel proteases.

With any volume of this nature, it is inevitable that many proteases have been omitted and to those whose favourite protease is not covered, I apologize. I would like to thank all the authors for their scholarly contributions and for keeping them within strict length limits. My hope is that this volume will provide a taster to the exciting field of protease research for senior undergraduates, junior postgraduates and even seasoned researchers. Finally, my thanks are owing to Sophie Dilley and the other staff at Portland Press Ltd for their hard work in the production of this book.

Nigel M. Hooper
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Authors

Nigel Hooper is Professor of Biochemistry in the School of Biochemistry and Molecular Biology at the University of Leeds. He received his B.Sc. in Biochemistry in 1981 and his Ph.D. in 1984, both from the University of Leeds. His Ph.D. study on the 'Metabolism of neuropeptides by cell-surface peptidases' stimulated his interest in proteases. After a 2-year post-doctoral position at Leeds, he joined the academic staff as a lecturer in 1989. At present, he co-leads the Proteolysis Research Group with Tony Turner, and among other topics, he continues to study the structure and function of several cell-surface proteases, with a particular interest in their mode of attachment to the membrane.

Guy Salvesen is Professor and Director of the Program in Apoptosis and Cell Death Research at the Burnham Institute, La Jolla, CA, and Adjunct Professor of Molecular Pathology at the University of California, San Diego, CA. He received his Ph.D. after studying the regulation of proteolysis under the supervision of Alan Barrett at Cambridge University. After post-doctoral training on inflammatory proteases with James Travis at the University of Georgia, he moved to Duke University, Durham, NC, as Assistant Research Professor, where he started his work on caspases. He moved to the Burnham Institute in 1996.

Yoshifumi Itoh is a Senior Lecturer of Matrix Biology at the Kennedy Institute of Rheumatology, Faculty of Medicine, Imperial College of Science, Technology and Medicine, London. He received his B.Sc. in Pharmacy in 1989 and his M.Sc. in Clinical Pharmacy in 1991 from Tokyo University of Pharmacy and Life Science. He then became a Research Associate working on matrix metalloproteinases in Hideaki Nagase's laboratory at the University of Kansas Medical Center, Kansas City, KS, and was awarded a Ph.D. in Pharmaceutical Science in 1996 by Tokyo University of Pharmacy and Life Science. In 1997, he moved back to Tokyo and became a *Joshu* (Lecturer) of the Department of Cancer Cell Research in Motoharu Seiki's laboratory at the Institute of Medical Science, University of Tokyo. He assumed his present position in 2001 and his research interests involve investigating the role of pericellular proteolysis in cell migration. **Hideaki Nagase** is Professor of Matrix Biology at the Kennedy Institute of Rheumatology Division, Imperial College of Science, Technology and Medicine, London. He received his B.Sc. in Pharmacy from Tokyo University of Pharmacy and Life Science in 1971, an M.Sc. in Physiological Chemistry from the Science University of Tokyo in 1973 and his Ph.D. in Biochemistry from the University of Miami in 1977. He was an Assistant Professor of Medicine at Robert Wood Johnson Medical

School, University of Medicine and Dentistry of New Jersey, and a Professor of Biochemistry and Molecular Biology at the University of Kansas Medical Center. He assumed his present position in 1999 and his research interests involve investigating the structure, function and pathophysiological roles of matrix metalloproteinases and their inhibitors.

Janelle Nunan is a Ph.D. student in the Laboratory of Molecular Neurobiology at the University of Melbourne, Australia. Her doctoral studies focus on amyloid- β protein precursor secretases. **David H. Small** is Head of the Laboratory of Molecular Neurobiology at the University of Melbourne, Australia. After postdoctoral work in the early 1980s at Massachusetts Institute of Technology and Flinders University, Adelaide, he moved to the University of Melbourne. His work has focused on the role of proteases in the trafficking of proteins in the central nervous system, and on the biochemistry and cell biology of cholinesterases.

Fergus Doherty is a Lecturer in Biochemistry in the School of Biomedical Sciences at Nottingham University, and his research interests include the role of the ubiquitin-like protein UCRP (ubiquitin cross-reactive protein) in cells of the immune system and the human endometrium. **Simon Dawson** is a Lecturer in Molecular Biology in the School of Biomedical Sciences at Nottingham University, and during the last few years he has been involved in employing the yeast two-hybrid technique to study the molecular interactions of proteasomal subunits. **John Mayer** is Professor of Molecular Cell Biology and head of the Intracellular Proteolysis Laboratory in the School of Biomedical Sciences at Nottingham University. Professor Mayer's current research interests include the molecular interactions of proteasome subunits with each other and with non-proteasomal proteins.

Ralph A. Bradshaw is a Professor in the Department of Physiology and Biophysics at the University of California at Irvine. He holds degrees from Colby College, Waterville, ME, and Duke University, and has been a researcher/faculty member at Indiana University, University of Washington, Seattle, WA, and Washington University in St. Louis, MO. He is a member of several learned societies, including the American Society for Biochemistry and Molecular Biology and the Protein Society, and has served as an Associate Editor of the *Journal of Biological Chemistry* and *Protein Science*. He is also the founding editor of *Molecular and Cellular Proteomics*. He has a long-term interest in protein structure and the function of proteases, and in polypeptide growth factors and their receptors. **Elizabeth Yi** is a staff research associate in the Department of Physiology and Biophysics at the University of California at Irvine. She holds a B.S. degree from Long Beach State University, CA.

Nabil Seidah received his Ph.D. degree in biophysics and physical chemistry from Georgetown University, Washington, DC, in 1973. After a brief post-doctoral training, he joined Michel Chrétien at the Clinical Research Institute of Montreal in 1974, where he has been ever since. From 1974 to

1989, his research dealt with the characterization of various polypeptide hormones, including β -endorphin and atrial natriuretic factor, as well as the definition of their biosynthetic pathways. In 1987, he spent a sabbatical year at the Pasteur Institute, Paris, to study renin processing and activation. On his return to Montreal, he cloned and characterized the proprotein convertases PC1 and PC2. This led him to identify the other members of the family, such as PC4, PC7 and SKI-1. He is now continuing his work on the structure–function of the convertases and is actively exploring their implication in proliferative and neurodegenerative diseases. **Annik Prat** received her Ph.D. in Cellular and Molecular Biology from the Pierre and Marie Curie University, Paris in 1988. After a 2.5-years of post-doctoral training in Patrick Linder's laboratory at the Biozentrum in Basel, Switzerland, she joined Paul Cohen's group in Paris in 1990. Between 1990 and 1997, she cloned and studied a newly characterized enzyme, the *N*-arginine dibasic converter (NRDc). After 1 year in Guy Boileau's laboratory at the University of Montreal, she joined Nabil Seidah's team where she is pursuing her work on NRDc.

Peter N. Walsh, M.D., Ph.D., is a physician/scientist in the Departments of Medicine and Biochemistry at Temple University School of Medicine, and at the Sol Sherry Thrombosis Research Center, Philadelphia, PA. He received his undergraduate degree at Amherst College, Amherst, MA, in 1957, an M.D. degree from Washington University School of Medicine in 1961, and the D.Phil. from the University of Oxford in 1972. His research interests include the interactions of coagulation proteins with platelets, and the biochemistry of blood coagulation proteins, especially factors XI, IX, VIII and X. **Syed S. Ahmad**, M.D., Ph.D., is a Research Professor of Biochemistry and the Sol Sherry Thrombosis Research Center at Temple University School of Medicine. He received his Ph.D. from the University of Karachi, Pakistan, in 1979, and his M.D. degree from University of Ciudad. Juarez, Mexico, in 1983. His research interests include the structural and functional relationships of coagulation proteins and their interaction with platelets.

Ben Dunn is Distinguished Professor of Biochemistry and Molecular Biology at the University of Florida College of Medicine. He received a B.S. degree in Chemistry from the University of Delaware in 1967, and a Ph.D. from the University of California, Santa Barbara, in 1971. From 1971 to 1974, he was a post-doctoral fellow and staff fellow at the National Institutes of Health, Bethesda, MD. His research interests are in the structure and function of enzymes, particularly the peptidases. He has contributed to studies of the substrate and inhibitor specificity of the aspartic peptidase family of enzymes, including those from HIV-1 and feline immunodeficiency virus, the malarial parasite *Plasmodium falciparum*, fungi and humans.

Eiji Kubota is a Visiting Fellow in the Department of Medicine, University of Melbourne. He undertook his medical training in Japan and qualified as a specialist in Internal Medicine in 1995. His research interests include the role of

the renin–angiotensin system and bradykinin in hypertension and renal disease, as well as the pharmacology of vaso-peptidase inhibitors and calcium antagonists. He will be returning to Japan in 2002 to work at the Shizuoka Red Cross Hospital Department of Medicine, Shizuoka-ken. **Rachael G. Dean** is a post-doctoral fellow in Department of Medicine, University of Melbourne. She obtained her Ph.D. in 1997 and her research interests include the roles of vaso-active peptides in the kidney and heart and of growth factors involved in cardiac remodelling. **Leanne Balding** undertook her medical training at Monash University, Melbourne, and her cardiology training at the Royal Melbourne Hospital. She currently works as a clinical cardiologist at the Royal Melbourne and Freemasons' Hospitals and is completing her Doctor of Medicine in the Department of Medicine, University of Melbourne. Her research has focused on the use of vaso-peptidase inhibitors and vasopressin receptor antagonists in heart failure. She is the recipient of the Viola Edith Reid Bequest Scholarship, a Cardiac Society of Australia and New Zealand Research Grant, an Austin Research Medical Foundation Grant and a Pfizer CVL Research Grant. **Louise M. Burrell** is an Associate Professor of Medicine in the Department of Medicine, University of Melbourne, and a General Physician/Endocrinologist. She obtained a British–Australian Heart Foundation Fellowship in 1991 and now runs a Cardiovascular Endocrinology Group that investigates cardiac and renal aspects of hypertension, heart failure and diabetes.

Mill Lambert is a computational chemist at GlaxoSmithKline in Research Triangle Park, NC. He received his B.A. in Physics at the University of Virginia in 1982, and Ph.D. in Physics at Cornell University, NY, in 1988. During his doctoral and post-doctoral work with Harold Scheraga, he developed the Molecular Viewing Program for protein modelling, molecular docking and structure-based drug design. Dr Lambert has worked on a number of drug discovery projects at Glaxo, GlaxoWellcome and GlaxoSmithKline, including those for phospholipase A₂, collagenase, tumour necrosis factor- α -converting enzyme, and peroxisome-proliferator-activated receptor- α , - γ and - δ . **Marcia L. Moss** received her B.S. in chemistry from the University of Michigan, Ann Arbor, in 1982 and her Ph.D. in biochemistry from the University of Wisconsin, Madison, in 1989. After two post-doctoral positions, she began working at Glaxo Inc. During her time at Glaxo, she headed the work that purified TNF- α -converting enzyme (TACE). She later led a project that studied matrix metalloproteinases in cancer. She retired from Glaxo Wellcome owing to complications from multiple sclerosis. At present, she works part-time at Cognosci Inc., a company that is researching anti-inflammatory treatments for neurological and other diseases.

Rob Rawson studied botany and history at the University of California, Berkeley, and graduated in 1982. After a few years in the computer business, he earned a Master's degree from the California State University, Hayward, in 1987. He received a Ph.D. from the University of Texas Southwestern Graduate

School of Biomedical Science in 1993. He then did a postdoctoral fellowship in the laboratory of Michael Brown and Joseph Goldstein in the Department of Molecular Genetics at University of Texas Southwestern Medical Center. In 1999, he joined that Department as a faculty member, where he continues research into the control of lipid metabolism in vertebrates and insects.

Nigel Bunnett is a Professor of Surgery and Physiology at the University of California, San Francisco and is the Director of the Gastrointestinal Research Center. He obtained a Ph.D. degree at Cambridge and completed post-doctoral training at University of California, Los Angeles. **Graeme Cottrell** and **Anne-Marie Coelho** are post-doctoral fellows in Nigel Bunnett's research laboratory at the University of California, San Francisco. Graeme received a Ph.D. degree from the University of Leeds, where he studied aminopeptidases with Nigel Hooper and Tony Turner in the School of Biochemistry and Molecular Biology. Anne-Marie obtained her Ph.D. degree in the Neuro-Gastroenterology Unit at the University of Toulouse, where she studied the role of proteases and their receptors in hyperalgesia and inflammation with Dr Bueno.

David Coates originally trained as a plant molecular biologist at the John Innes Institute, Norwich, and Purdue University, West Lafayette, IN, before moving to Leeds via Oxford to pursue his interests in the evolution of duplications and small gene families using the classic molecular model systems of *Caenorhabditis elegans* and *Drosophila melanogaster*, as well as a broad set of genomes that include the plant RNA viruses. The enormous changes in the amount of information available to biologists is now driving a deep ambition to understand how to program a computer!

Abbreviations

A β	amyloid- β protein
ACE	angiotensin-converting enzyme
AD	Alzheimer's disease
ADAM	a disintegrin and metalloproteinase
Ang	angiotensin
ANP	atrial natriuretic peptide
APC	anaphase-promoting complex
APP	amyloid- β protein precursor
ATF	activating transcription factor
BACE	β -site APP-cleaving enzyme
BDNF	brain-derived neurotrophic factor
BIR	baculovirus IAP repeat
CA	capsid protein
Cbz	benzyloxycarbonyl
CARD	caspase recruitment domain
Cdk	cyclin-dependent kinase
CGRP	calcitonin gene related peptide
CHIP	C-terminal Hsp70-interacting protein
DED	death effector domain
DUB	de-ubiquitylating enzyme
E1	ubiquitin-activating enzyme
E2	ubiquitin-conjugating enzyme
E3	ubiquitin-protein ligase
ECM	extracellular matrix
EGF	epidermal growth factor
EIAV	equine infectious anemic virus
eIF	eukaryotic initiation factor
EPR-1	effector cell protease receptor-1
ER	endoplasmic reticulum
FAD	familial Alzheimer's disease
FGF	fibroblast growth factor
FIV	feline immunodeficiency virus
FLIP	Flice-like inhibitory protein
GP-C	76-kDa precursor glycoprotein of LAV
GPCR	G-protein-coupled receptor
GPI	glycosylphosphatidylinositol
HB-EGF	heparin-binding EGF-like growth factor

HK	high molecular mass kininogen
HMM	hidden Markov model
Hpx domain	hemopexin-like domain
IAP	inhibitor of apoptosis protein
I κ B	inhibitor of NF- κ B
IDE	insulin-degrading enzyme
IGF	insulin-like growth factor
IGFBP	insulin-like growth factor-binding protein
LAV	Lassa virus
LCMV	lymphocytic choriomeningitis virus
MAP kinase	mitogen-activated protein kinase
MetAP	methionine aminopeptidase
MHC I	Major Histocompatibility Complex Class I
MMP	matrix metalloproteinase
MT-MMP	membrane-type matrix metalloproteinase
NC	nucleocapsid protein
NEP	neutral endopeptidase
NF κ B	nuclear factor κ B
NK1R	neurokinin ₁ receptor
NRDc	<i>N</i> -arginine dibasic convertase
PACE	paired basic amino acid converting enzyme
PAI	plasminogen activator inhibitor
PAR	protease-activated receptor
PC	proprotein convertase
α 1-PDX	α 1-antitrypsin Portland
PK	prekallikrein
PKC	protein kinase C
PN-2	protease nexin 2
PS	presenilin
RA	rheumatoid arthritis
Rip	regulated intramembrane proteolysis
RSV	Rous sarcoma virus
RUP	regulated ubiquitin/proteasome-dependent processing
sAPP α	soluble N-terminal fragment of APP
SCAP	SREBP cleavage activating protein
SCF	Skip-cullin-F-box
SERPIN	serine protease inhibitor
SET domain	serine-, glutamine- and threonine-rich domain
SG	secretory granule
SIV	simian immunodeficiency virus
SKI-1	subtilisin/kexin-like isozyme 1
SP	substance P

S1P	site-1 protease
S2P	site-2 protease
SREBP	sterol regulatory element binding protein
STAT	signal transducer and activator of transcription
SUMO	small ubiquitin-like modifier
SVMP	snake venom metalloprotease
TACE	tumour necrosis factor- α -converting enzyme
TFPI	tissue factor pathway inhibitor
TGF	transforming growth factor
TGN	<i>trans</i> Golgi network
TIMP	tissue inhibitor of metalloproteinases
TNF	tumour necrosis factor
t-PA	tissue plasminogen activator
TRAF	tumour necrosis factor-receptor-associated factor
UBP	ubiquitin-specific protease
VEGF	vascular endothelial cell growth factor
XIAP	X-linked IAP
ZPI	protein Z-dependent protease inhibitor