ADVANCES IN EXPERIMENTAL MEDICINE AND BIOLOGY

Volume 677

Proteins

Membrane Binding and Pore Formation

Edited by
Gregor Anderluh
and Jeremy Lakey

Proteins: Membrane Binding and Pore Formation

ADVANCES IN EXPERIMENTAL MEDICINE AND BIOLOGY

Recent Volumes in this Series

Volume 669
NEW FRONTIERS IN RESPIRATORY CONTROL
Edited by Ikuo Homma and Hiroshi Onimaru

Volume 670
THERAPEUTIC APPLICATIONS OF CELL MICROENCAPSULATION
Edited by José Luis Pedraz and Gorka Orive

Volume 671
FRONTIERS IN BRAIN REPAIR
Edited by Rahul Jandial

Volume 672
BIOSURFACTANTS
Edited by Ramkrishna Sen

Volume 673
MODELLING PARASITE TRANSMISSION AND CONTROL
Edited by Edwin Michael and Robert C. Spear

Volume 674
INTEGRINS AND ION CHANNELS: MOLECULAR COMPLEXES AND SIGNALING
Edited by Andrea Becchetti and Annarosa Arcangeli

Volume 675
RECENT ADVANCES IN PHOTOTROPHIC PROKARYOTES
Edited by Patrick C. Hallenbeck

Volume 676
POLYPLOIDIZATION AND CANCER
Edited by Randy Y.C. Poon

Volume 677
PROTEINS: MEMBRANE BINDING AND PORE FORMATION
Edited by Gregor Anderluh and Jeremy Lakey

Proteins

Membrane Binding and Pore Formation

Edited by

Gregor Anderluh, PhD
Department of Biology, University of Ljubljana
Ljubljana, Slovenia

Jeremy Lakey, PhD
Institute for Cell and Molecular Biosciences, The Medical School
University of Newcastle upon Tyne
Newcastle upon Tyne, UK

Springer Science+Business Media, LLC

Landes Bioscience

Springer Science+Business Media, LLC
Landes Bioscience

Printed in the USA.

Springer Science+Business Media, LLC, 233 Spring Street, New York, New York 10013, USA
http://www.springer.com

Please address all inquiries to the publishers:
Landes Bioscience, 1002 West Avenue, Austin, Texas 78701, USA
Phone: 512/ 637 6050; FAX: 512/ 637 6079
http://www.landesbioscience.com

The chapters in this book are available in the Madame Curie Bioscience Database.
http://www.landesbioscience.com/curie

Proteins: Membrane Binding and Pore Formation, edited by Gregor Anderluh and Jeremy Lakey. Landes Bioscience / Springer Science+Business Media, LLC dual imprint / Springer series: Advances in Experimental Medicine and Biology.

ISBN: 978-1-4419-6326-0

Library of Congress Cataloging-in-Publication Data

Proteins : membrane binding and pore formation / edited by Gregor Anderluh, Jeremy Lakey.
p. ; cm. -- (Advances in experimental medicine and biology ; v. 677)
Includes bibliographical references and index.
ISBN 978-1-4419-6326-0
1. Membrane proteins. 2. Membrane lipids. I. Anderluh, Gregor, 1969- II. Lakey, Jeremy, 1958- III. Series: Advances in experimental medicine and biology ; v. 677.
[DNLM: 1. Membrane Proteins--physiology. 2. Cell Membrane Permeability. 3. Membrane Lipids--physiology. 4. Peptides--physiology. 5. Pore Forming Cytotoxic Proteins--physiology. 6. Protein Binding. W1 AD559 v.677 2010 / QU 55.7 P967 2010]
QP552.M44P757 2010
572'.69--dc22

2010014176

PREFACE

Formation of transmembrane pores is a very effective way of killing cells. It is thus not surprising that many bacterial and eukaryotic toxic agents are pore-forming proteins. Pore formation in a target membrane is a complex process composed of several steps; proteins need to attach to the lipid membrane, possibly aggregate in the plane of the membrane and finally form a pore by inserting part of the polypeptide chain across the lipid bilayer. Structural information about toxins at each stage is indispensible for the biochemical and molecular biological studies that aim to understand how pores are formed at the molecular level. There are currently only two structures of pores available, of α-toxin from *Staphylococcus aureus* and hemolysin E from *Escherichia coli*. Therefore, what we know about these proteins was obtained over many years of intense experimentation. We have nevertheless, in the last couple of years, witnessed a significant rise in structural information on the soluble forms of pore-forming proteins. Surprisingly, many unexpected similarities with other proteins were noted, despite extremely low or insignificant sequence similarity. It appears that lipid membrane binding and formation of transmembrane channels is achieved in many cases by a limited repertoire of structures. This book describes how several of the important pore forming toxin families achieve membrane binding and which structural elements are used for formation of transmembrane pores. Our contributors have thus provided the means for a comparative analysis of several unrelated families.

The introductory chapter by Mike Parker and colleagues gives a comprehensive overview of what we know about these proteins and highlights their general structural properties. The succeeding chapter by William Wimley sets up the stage upon which pore forming toxins act by describing the properties of the lipid membrane and the thermodynamics of membrane binding and insertion. Pore formation may be effectively achieved by simple structures, and the succeeding chapter by Burkhard Bechinger describes the structural requirements for efficient membrane binding and insertion of single alpha helices. The role of lipids was undervalued for a long time, especially in the process of protein insertion and the structural role they may play in the final pore. In recent years it became clear that their role is significant, and the next chapter from Jesús Salgado's group discusses the role of the bilayer

lipids in the pore forming process. After these general chapters all of the important protein toxins families are discussed, specifically cholesterol dependant cytolysins from Gram positive bacteria (Robert Gilbert), the aerolysin protein family (José Miguel Mancheño et al), colicins from *Escherichia coli* and related proteins that act as apoptotic regulators (Ana J. García-Sáez et al), actinoporins from sea anemones, Hemolysin E and related toxins (Peter Artymuik and colleagues), Cry toxins from *Bacillus thuringiensis* (Alejandra Bravo and colleagues) and cardiotoxins from cobra venom (Wen-guey Wu and colleagues). The final chapter by Bruce Kagan and Jyothi Thundimadathil provides a comparison of the properties of membrane channels formed by amyloid proteins and pore forming toxins and discusses the role that amyloid channels may have in disease.

Although in recent years the focus of some studies on pore forming toxins may have changed from structure-function relationships to measuring their effects on cell biology, comparative structural biology still has a lot to teach us. As highlighted in this book, novel structures and biophysical studies upon these proteins define the common threads of how proteins interact with lipid membranes and thus inform us of the rules of the game. We hope that by assembling this book we have helped to define where we currently are and where the science may go in the future. Our work as editors was made fun and interesting by the excellent contributors who have made this volume an engaging and fresh insight to the subject, we thank them for their hard work and our families for their patience.

Gregor Anderluh, PhD
Department of Biology, University of Ljubljana
Ljubljana, Slovenia

Jeremy Lakey, PhD
Institute for Cell and Molecular Biosciences, The Medical School
University of Newcastle upon Tyne
Newcastle upon Tyne, UK

ABOUT THE EDITORS...

GREGOR ANDERLUH is an Associate Professor of Biochemistry at the Department of Biology, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia. He and his coworkers are studying protein-membrane interactions and how cellular membranes are damaged by proteins. He is the director of the Infrastuctural Centre for Surface Plasmon Resonance at the University of Ljubljana, where they study molecular interactions and are developing novel approaches on how to study protein binding to membranes. He received his PhD in Biology from University of Ljubljana and completed his Postdoctoral training at University of Newcastle, UK.

ABOUT THE EDITORS...

JEREMY LAKEY is a Professor of Structural Biochemistry at the Institute for Cell and Molecular Biosciences, University of Newcastle, UK and runs an academic research group based loosely on the theme of protein biophysical chemistry with interests in protein toxins, membranes and bionanotechnology. Following a first degree in Zoology, Jeremy completed a PhD in Membrane Biophysics at the University of East Anglia UK, followed by periods at the Centre de Biophysique Moléculaire, Orléans, France; EMBL, Heidelberg, Germany and the EPFL, Lausanne Switzerland. He is currently an editor of the *Biochemical Journal* and member of the facility access panel for the ISIS pulsed neutron source, UK.

PARTICIPANTS

Gregor Anderluh
Department of Biology
University of Ljubljana
Ljubljana
Slovenia

Peter J. Artymiuk
The Krebs Institute
Department of Molecular Biology and Biotechnology
University of Sheffield
Sheffield
UK

Biserka Bakrač
Department of Biology
University of Ljubljana
Ljubljana
Slovenia

Burkhard Bechinger
Institut de chimie
CNRS
Université de Strasbourg
Strasbourg
France

Alejandra Bravo
Departamento de Microbiología Molecular
Instituto de Biotecnología
Universidad Nacional Autónoma de México
Mexico City
Mexico

Santi Esteban-Martín
Instituto de Ciencia Molecular
University of Valencia
Pol. La Coma
Paterna, Valencia
Spain

Susanne C. Feil
St. Vincent's Institute of Medical Research
Fitzroy, Victoria
Australia

Gustavo Fuertes
Instituto de Ciencia Molecular
University of Valencia
Paterna, Valencia
Spain

Ana J. García-Sáez
Biotechnologisches Zentrum der TU Dresden
Tatzberg, Dresden
Germany

Robert J.C. Gilbert
Division of Structural Biology
Wellcome Trust Centre for Human Genetics
University of Oxford
Oxford
UK

Diana Giménez
Instituto de Ciencia Molecular
University of Valencia
Pol. La Coma
Paterna, Valencia
Spain

Irwin J. Goldstein
Grupo de Cristalografía Macromolecular y Biología Estructural
Instituto de Quimica Fisica Rocasolano
CSIC
Madrid
Spain

Michael A. Gorman
St. Vincent's Institute of Medical Research
Fitzroy, Victoria
Australia

Isabel Gómez
Departamento de Microbiología Molecular
Instituto de Biotecnología
Universidad Nacional Autónoma de México

Jeffrey Green
The Krebs Institute
Department of Molecular Biology and Biotechnology
University of Sheffield
Sheffield
UK

Stuart Hunt
The Krebs Institute
Department of Molecular Biology and Biotechnology
University of Sheffield
Sheffield
UK

Christopher L. Johnson
Institute for Cell and Molecular Biosciences
University of Newcastle upon Tyne
Newcastle upon Tyne
UK

Bruce L. Kagan
Department of Psychiatry and Biobehavioral Sciences
Semel Institute for Neuroscience and Human Behavior
David Geffen School of Medicine at UCLA
University of California
Los Angeles, California
USA

Je-hung Kuo
National Synchrotron Radiation Research Center
Department of Life Science
National Tsing Hua University
Hsinchu
Taiwan

Jeremy H. Lakey
Institute for Cell and Molecular Biosciences
The Medical School
University of Newcastle upon Tyne
Newcastle upon Tyne
UK

José Miguel Mancheño
Grupo de Cristalografía Macromolecular y Biología Estructural
Instituto de Quimica Fisica Rocasolano
CSIC
Madrid
Spain

Carlos Muñóz-Garay
Departamento de Microbiología Molecular
Instituto de Biotecnología
Universidad Nacional Autónoma de México
Mexico City
Mexico

Liliana Pardo
Departamento de Microbiología Molecular
Instituto de Biotecnología
Universidad Nacional Autónoma de México
Mexico City
Mexico

Michael W. Parker
St. Vincent's Institute of Medical Research
Fitzroy, Victoria
Australia

Galina Polekhina
St. Vincent's Institute of Medical Research
Fitzroy, Victoria
Australia

Helena Porta
Departamento de Microbiología Molecular
Instituto de Biotecnología
Universidad Nacional Autónoma de México
Mexico City
Mexico

Helen Ridley
Institute for Cell and Molecular Biosciences
University of Newcastle upon Tyne
Newcastle upon Tyne
UK

Jesús Salgado
Instituto de Ciencia Molecular
University of Valencia
Pol. La Coma
Paterna, Valencia
Spain

Jorge Sánchez
Departamento de Microbiología Molecular
Instituto de Biotecnología
Universidad Nacional Autónoma de México
Mexico City
Mexico

Orlando Sánchez
Instituto de Ciencia Molecular
University of Valencia
Pol. La Coma
Paterna, Valencia
Spain

Daniel Sher
Grupo de Cristalografía Macromolecular y Biología Estructural
Instituto de Quimica Fisica Rocasolano
CSIC
Madrid
Spain

Mario Soberón
Departamento de Microbiología Molecular
Instituto de Biotecnología
Universidad Nacional Autónoma de México
Mexico City
Mexico

Jacob Suckale
Medizinisch Theoretisches Zeutrum der TU Dresden
Dresden
Germany

Hiroaki Tateno
Grupo de Cristalografía Macromolecular y Biología Estructural
Instituto de Quimica Fisica Rocasolano
CSIC
Madrid
Spain

Jyothi Thundimadathil
Research and Development Group Leader
Peptisyntha, Inc.
Torrance, California
USA

Siu-Cin Tjong
National Synchrotron Radiation Research Center
Department of Life Science
National Tsing Hua University
Hsinchu
Taiwan

William C. Wimley
Department of Biochemistry
Tulane University Health Sciences Center
New Orleans Louisiana
USA

Karen Wu
National Synchrotron Radiation Research Center
Department of Life Science
National Tsing Hua University
Hsinchu
Taiwan

Po-long Wu
National Synchrotron Radiation Research Center
Department of Life Science
National Tsing Hua University
Hsinchu
Taiwan

Wen-guey Wu
National Synchrotron Radiation Research Center
Department of Life Science
National Tsing Hua University
Hsinchu
Taiwan

CONTENTS

Chapter 1

Introduction

Susanne C. Feil, Galina Polekhina, Michael A. Gorman and Michael W. Parker*

Abstract

Pore-forming proteins (PFPs) possess the intriguing property that they can exist either in a stable water-soluble state or as an integral membrane pore. These molecules can undergo large conformational changes in converting between these two states. Much of what we know about how these proteins change their shape comes from work on bacterial toxins and increasingly, in more recent years, on toxins from other organisms. Surprisingly, a number of pore-forming proteins have recently been characterised that appear to have adopted similar stratagies to toxins for binding and inserting into biological membranes.

Introduction

Pore-forming peptides and proteins (PFPs) are produced by many, if not all, organisms. They are secreted as water-soluble proteins but once their target is reached can be transformed into membrane proteins for the purpose of inserting into or translocating across biological membranes. Many of these proteins are toxins where they can aid the digestion of prey or can protect the producing organism by killing invaders. At least a third of the more then 300 protein toxins characterized to date act by disrupting membranes.[1] Many pore-forming toxins (PFTs) appear to function simply by forming pores in cell membranes, disrupting the permeability barrier leading eventually to cell death. In recent years a number of proteins have been characterised that do not function as toxins but nevertheless bind and insert into biological membranes using similar strategies as seen for toxins.

The major steps involved in the generation of pores by PFPs are summarized in Figure 1. In the first step the PFP must be secreted from the host. Organisms have developed a number of ways of secreting PFPs from the less subtle (e.g., colicins are secreted with the help of lysis proteins that punch a hole in the outer membrane of the producing cell)[2] to the more complex (e.g., proaerolysin appears to be secreted through a complex protein secretion system)[3]. In order to avoid premature conversion to its membrane-active state, some PFPs protect themselves by being produced as a proprotein and/or as oligomers (generally dimers). The PFPs are then targeted to the correct cells by means of parasitizing a host cell surface feature such as a protein or other substance (e.g., lipids, sugars) and using them as a receptor. Most often the result of concentration is the formation of oligomers on the cell surface although some PFPs may oligomerize in the membrane. Membrane insertion follows leading to formation of the pore that varies in size from less than 10 Å to more than 150 Å in diameter. They tend to form nonspecific pores, not surprisingly since the role for many is to destroy target cells. The pores sometimes are voltage-gated with the channels opened under normal physiological conditions. In some cases the in vitro pore-forming activity observed is almost certainly not a biological activity but rather a reflection of their in vivo translocating capacity.

*Corresponding Author: Michael W. Parker—St. Vincent's Institute of Medical Research, 9 Princes Street, Fitzroy, Victoria 3065, Australia. Email: mparker@svi.edu.au

Proteins: Membrane Binding and Pore Formation, edited by Gregor Anderluh and Jeremy Lakey