Advances in
PROTEIN CHEMISTRY

VOLUME 63

Membrane Proteins

ACADEMIC PRESS

ADVANCES IN PROTEIN CHEMISTRY

EDITED BY

FREDERIC M. RICHARDS
Department of Molecular Biophysics
and Biochemistry
Yale University
New Haven, Connecticut

DAVID S. EISENBERG
Department of Chemistry and Biochemistry
University of California, Los Angeles
Los Angeles, California

JOHN KURIYAN
Department of Molecular Biophysics
Howard Hughes Medical Institute
Rockefeller University
New York, New York

VOLUME 63

Membrane Proteins

EDITED BY

DOUGLAS C. REES
Division of Chemistry and Chemical Engineering
Howard Hughes Medical Institute
California Institute of Technology
Pasadena, California

ACADEMIC PRESS
An imprint of Elsevier Science

Amsterdam Boston London New York Oxford Paris
San Diego San Francisco Singapore Sydney Tokyo

This book is printed on acid-free paper. ♾

Academic Press
An Elsevier Science Imprint.
525 B Street, Suite 1900, San Diego, California 92101-4495, USA
http://www.academicpress.com

Academic Press
84 Theobald's Road, London WC1X 8RR, UK
http://www.academicpress.com

International Standard Book Number: 0-12-034263-4

PRINTED IN THE UNITED STATES OF AMERICA
03 04 05 06 07 08 09 9 8 7 6 5 4 3 2 1

ADVANCES IN PROTEIN CHEMISTRY

Volume 63

Membrane Proteins

PREFACE

The first glimpse of the three-dimensional structure of a membrane protein was revealed in 1975, when Henderson and Unwin published their landmark analysis of bacteriorhodopsin at 7 Å resolution (Henderson and Unwin, 1975). This study established the presence of seven rodlike features, identified as α-helices, in the membrane spanning region of bacteriorhodopsin, consistent with expectations based on the behavior of polypeptides in nonaqueous solvents (see Singer, 1962). To place this development in context with the overall status of protein structure determinations at that time, X-ray crystal structures were available for ~80 water-soluble proteins (Matthews, 1976) when Henderson and Unwin's work appeared. During the intervening quarter century since this initial peek at bacteriorhodopsin, the structures of ~60 membrane proteins have been determined (see http://www.mpibp-frankfurt.mpg.de/michel/public/memprotstruct.html). Correcting for the ~25-year offset, the rate of membrane protein structure determinations (Figure 1) over the 15-year period following the first high resolution structure of the photosynthetic reaction center (Deisenhofer *et al.*, 1985) closely parallels the progress observed for water-soluble proteins after the myoglobin structure (Kendrew *et al.*, 1960). As we approach +20 years since the solution of the reaction center structure, it is not a very bold extrapolation to predict that the number of solved membrane protein structures is poised to explode, much as the number of water-soluble protein structures did in the 1980s, ~20 years after the myoglobin structure. For example, since the chapters for this review were commissioned, new structures have appeared for the chloride channel (Dutzler *et al.*, 2002), formate dehydrogenase (Jormakka *et al.*, 2002), the vitamin B_{12} transporter (Locher *et al.*, 2002), the multidrug efflux transporter AcrB

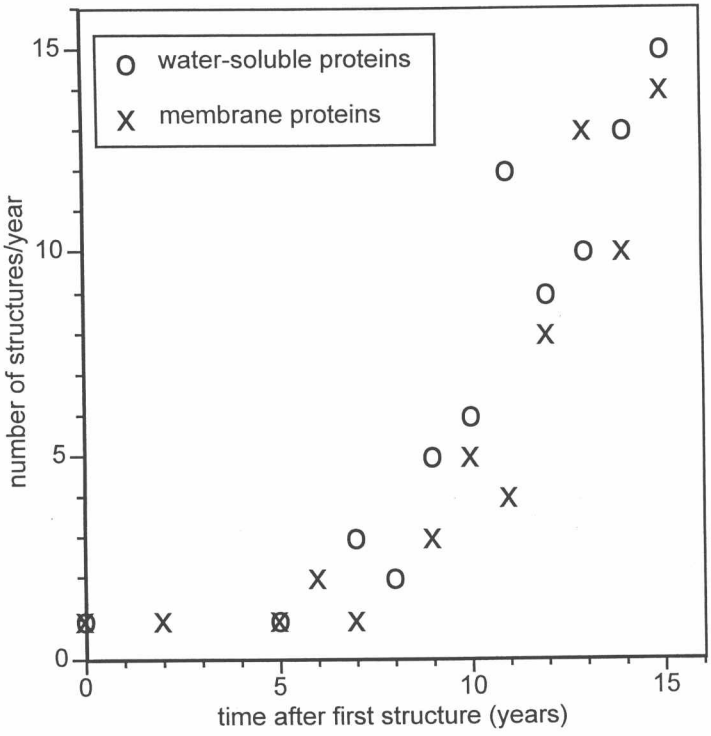

FIG. 1. The number of water soluble (o) and membrane proteins (x) solved per year, relative to the date of the first structure determination in each class, myoglobin (Kendrew *et al.*, 1960), and the photosynthetic reaction center (Deisenhofer *et al.*, 1985), respectively. The data for water-soluble proteins are from Matthews (1976), while those for membrane proteins are from the website http://www.mpibp-frankfurt.mpg.de/michel/public/memprostruct.html. Adjusting for the ~25 year difference, it is evident that progress in the structure determination of membrane proteins mirrors that experienced for water-soluble proteins. For perspective, there were 18,838 total available structures in the Protein Data Bank as of October 3, 2002, with 3298 structures deposited in 2001 (see http://www.rcsb.org/pdb/holdings_table.html).

(Murakami *et al.*, 2002), and the MscS mechanosensitive channel (Bass *et al.*, 2002), among others.

Although membrane protein structure will not likely become a mature field for another decade or two, one consequence of this recent surge in activity is that systematic coverage of all known structures is no longer possible. The eleven chapters in this volume review recent developments for selected membrane proteins from a variety of perspectives that emphasize the blending of structural and functional approaches, with the

objective of establishing a comprehensive mechanistic framework for a particular system.

The first three chapters address issues of general relevance to all membrane proteins, namely, their biosynthesis and the basic structural principles underlying their construction. Gunnar von Heijne describes the cellular mechanisms responsible for "functionalization" of the lipid bilayer with proteins and the possible constraints imposed on membrane protein structure by this process. A detailed understanding of these mechanisms is not only of biological interest, but also of practical significance since the overexpression of eukaryotic membrane proteins looms as one of the greatest challenges to their structural characterization. James Bowie and co-workers address the energetic principles, including lipid–protein interactions, that drive the formation and stability of helix-bundle membrane proteins. As described in this chapter, the thermodynamic stability of membrane proteins and the energetic consequences of protein folding in a nonaqueous environment represent some of the challenging and outstanding problems in this field. While many membrane proteins consist of α-helices, those found in the outer membranes of bacteria are based on β-barrels of remarkable diversity. Georg Schulz provides an analysis of their construction principles, highlighting the implications of these observations for the engineering of channels with novel properties.

Due to their high natural abundance, it is no coincidence that significant progress has been made in the characterization of membrane protein complexes that mediate energy transduction processes such as photosynthesis and respiration. P. Leslie Dutton and coauthors develop a comprehensive framework for analyzing energy and electron transfers in photosystems centered around the spatial organization of cofactors into chains. These considerations again have significant implications for the design principles of both biological and engineered systems. The "other" photosynthetic system, bacteriorhodopsin, has not only played a central role in the structural analysis of membrane proteins, but also in deciphering the basic features of energy transduction processes. Hartmut Luecke and Janos Lanyi describe the exciting recent progress and the challenges in establishing the mechanism of proton pumping by bacteriorhodopsin in molecular detail. The next two chapters describe the structure and mechanism of quinone binding respiratory complexes. Quinones play a central role in membrane bioenergetics, serving as diffusible, lipid-soluble carriers of electrons and protons that link many of the photosynthetic and respiratory systems in electron transfer chains. C. R. D. Lancaster reviews the *Wolinella* fumarate quinol reductase that, with the structure of the *Escherichia coli* enzyme (Iverson *et al.*, 1999), has

structurally defined the family of succinate:quinone oxidoreductases. This family is of central importance to the energy metabolism of many organisms, as various members can function in aerobic respiration (as complex II), the Krebs cycle, and as terminal acceptors during anaerobic respiration. So Iwata and co-workers describe the structure and function of two respiratory complexes, cytochrome bc_1 (or complex III) and cytochrome bo_3 (or ubiquinol oxidase) that can couple electron transfer to proton translocation at the heart of energy conservation during respiration. These structures, together with the structures of cytochrome *c* oxidase or complex IV (Iwata *et al.*, 1995; Tsukihara *et al.*, 1995), ATP synthase (Abrahams *et al.*, 1994; Stock *et al.*, 1999), and photosystem I (Jordan *et al.*, 2001), provide a nearly complete structural characterization of the major photosynthetic and aerobic respiratory complexes, missing only the structure of complex I (NADH dehydrogenase) and the extension of the photosystem II structure (Zouni *et al.*, 2001) to high resolution.

Channels and receptors mediate the flow of matter and information across the membrane bilayer that are fundamental to many biological processes. The last four chapters of this volume address systems that highlight the rich functional diversity of membrane proteins in these capacities. Microorganisms must be able to adapt to rapid changes in their environment, such as sudden drops in external osmolarity that can lead to swelling and lysis. To protect against this, stretch-activated (mechanosensitive) channels of large conductance, first identified by Kung and co-workers (Martinac *et al.*, 1987), are present in the cell membrane that appear to serve as safety valves to reduce the possibility of cell rupture under these conditions. Advances in the structure and mechanism of these mechanosensitive channels are reviewed in the chapter by my group. In eukaryotic organisms, voltage-gated channels mediate signaling processes that are of tremendous physiological and pharmacological significance. Francisco Bezanilla and Eduardo Perozo present an analysis of structural and dynamic properties of the voltage sensor derived from exquisite biophysical and biochemical studies. An important development will be the high resolution structure of a voltage-gated channel to define the structural organization of the voltage sensor, to complement the studies of MacKinnon in establishing the structural basis of ion selectivity (Doyle *et al.*, 1998). G-Protein-coupled receptors are key elements of signal transduction pathways in eukaryotes and represent important pharmacological targets. Rhodopsin, that initiates the visual response, is the paradigm of this receptor family. Wayne Hubbell and coauthors describe the properties of rhodopsin as established from crystallographic and biophysical studies, particularly the site-directed spin labeling

approach developed by his group. Of particular interest is the analysis of the light-mediated changes in the structure and dynamics of rhodopsin. In the final chapter, Robert Stroud and co-workers detail the structure and selectivity mechanism of the glycerol facilitator GlpF that is a member of the aquaporin family of channels. By mediating the flow of water and a few other small solutes such as glycerol across the relatively impermeable cell membrane, aquaporins are critical to the maintenance of the appropriate osmotic pressure balance in prokaryotic and eukaryotic cells.

At the start of the twenty-first century, the pace of membrane protein structure determinations is clearly accelerating (Figure 1). With the exceptions of rhodopsin (Palczewski *et al.,* 2000) and the calcium ATPase (Toyoshima *et al.,* 2000), however, eukaryotic channels, transporters, and receptors are conspicuously absent from the list of known membrane protein structures. These two exceptions, as proteins of naturally high abundance, highlight the current reality that no structure has been determined for an overexpressed eukaryotic membrane protein. This situation reflects the present difficulties in the reliable overexpression of membrane proteins, particularly those of eukaryotic organisms. Just as the development ~20 years ago of overexpression systems for water-soluble proteins revolutionized the structure determinations of this class of proteins, advances in membrane protein expression will be essential to successful realization of the goal of routine structural analysis of membrane proteins.

In this era of proliferating reviews, investigators have many opportunities to satisfy such urges, particularly in a field such as membrane protein structure and function. Consequently, I would particularly like to thank the authors of this volume for the time commitment and effort required to prepare their contributions.

Douglas C. Rees

References

Abrahams, J. P., Leslie, A. G., Lutter, R., and Walker, J. E. (1994). *Nature* **370,** 621–628.

Bass, R. B., Strop, P., Barclay, M., and Rees, D. C. (2002). *Science* **298,** 1582–1587.

Deisenhofer, J., Epp, O., Miki, K., Huber, R., and Michel, H. (1985). *Nature* **318,** 618–624.

Doyle, D. A., Cabral, J. M., Pfuetzner, R. A., Kuo, A., Gulbis, J. M., Cohen, S. L., Chait, B. T., and MacKinnon, R. (1998). *Science* **280,** 69–77.

Dutzler, R., Campbell, E. B., Cadene, M., Chait, B. T., and MacKinnon, R. (2002). *Nature* **415,** 287–294.

Henderson, R. and Unwin, P. N. T. (1975). *Nature* **257,** 28–32.

Iverson, T. M., Luna-Chavez, C., Cecchini, G., and Rees, D. C. (1999). *Science* **284,** 1961–1966.

Iwata, S., Ostermeier, C., Ludwig, B., and Michel, H. (1995). *Nature* **376,** 660–643.
Jordan, P., Fromme, P., Witt, H. T., Klukas, O., Saenger, W., and Krauß, N. (2001). *Nature* **411,** 909–917.
Jormakka, M., Törnroth, S., Byrne, B., and Iwata, S. (2002). *Science* **295,** 1863–1868.
Kendrew, J. C., Dickerson, R. E., Strandberg, B. E., Hart, R. B., Davies, D. R., Phillips, D. C., and Shore, V. C. (1960). *Nature* **185,** 422–427.
Locher, K. P., Lee, A. T., and Ress, D. C. (2002). *Science* **296,** 1091–1098.
Martinac, B., Buechner, M., Delcour, A. H., Adler, J., and Kung, C. (1987). *Proc. Natl. Acad. Sci. USA* **84,** 2297–2301.
Matthews, B. W. (1976). *Ann. Rev. Phys. Chem.* **27,** 493–523.
Murakami, S., Nakashima, R., Yamashita, E., and Yamaguchi, A. (2002). *Nature* **419,** 587–593.
Palczewski, K., Kumasake, T., Hori, T., Behnke, C. A., Motoshima, H., Fox, B. A., Trong, I. L., Teller, D. C., Okada, T., Stenkamp, R. E., Yamaoto, M., and Miyano, M. (2000). *Science* **289,** 739–745.
Singer, S. J. (1962). *Adv. Prot. Chem.* **17,** 1–68.
Stock, D., Leslie, A. G. W., and Walker, J. E. (1999). *Science* **286,** 1700–1705.
Toyoshima, C., Nakasako, M., Nomura, H., and Ogawa, H. (2000). *Nature* **405,** 647–655.
Tsukihara, T., Aoyama, H., Yamashita, E., Tomizaki, T., Yamaguchi, H., Shinazawa-Itoh, K., Nakashima, R., Yaono, R., and Yoshikawa, S. (1995). *Science* **69,** 1069–1074.
Zouni, A., Witt, H.-T., Kern, J., Fromme, P., Krauß, N., Saenger, W., and Orth, P. (2001). *Nature* **409,** 739–743.

CONTENTS

Transmembrane β-Barrel Proteins

GEORG E. SCHULZ

Length, Time, and Energy Scales of Photosystems

CHRISTOPHER C. MOSER, CHRISTOPHER C. PAGE, RICHARD J. COGDELL, JAMES BARBER, COLIN A. WRAIGHT, AND P. LESLIE DUTTON

Structural Clues to the Mechanism of Ion Pumping in Bacteriorhodopsin

HARTMUT LUECKE AND JANOS K. LANYI

The Structure of *Wolinella succinogenes* Quinol:Fumarate Reductase and Its Relevance to the Superfamily of Succinate:Quinone Oxidoreductases

C. Roy D. Lancaster

Structure and Function of Quinone Binding Membrane Proteins

Momi Iwata, Jeff Abramson, Bernadette Byrne, and So Iwata

Prokaryotic Mechanosensitive Channels

PAVEL STROP, RANDAL BASS, AND DOUGLAS C. REES

The Voltage Sensor and the Gate in Ion Channels

FRANCISCO BEZANILLA AND EDUARDO PEROZO

Rhodopsin Structure, Dynamics, and Activation: A Perspective from Crystallography, Site-Directed Spin Labeling, Sulfhydryl Reactivity, and Disulfide Cross-Linking

WAYNE L. HUBBELL, CHRISTIAN ALTENBACH, CHERYL M. HUBBELL, AND H. GOBIND KHORANA

The Glycerol Facilitator GlpF, Its Aquaporin Family of Channels, and Their Selectivity

ROBERT M. STROUD, PETER NOLLERT, AND LARRY MIERCKE

MEMBRANE PROTEIN ASSEMBLY *IN VIVO*

By GUNNAR VON HEIJNE

Department of Biochemistry and Biophysics, Stockholm University,
SE-10691 Stockholm, Sweden

I. Introduction

Although much current interest is focused on the structure–function relationships of membrane proteins, a good understanding of the cellular processes responsible for the insertion of proteins into lipid bilayer membranes is a necessary prerequisite for assessing how much the accessibly "structure space" is constrained not by the bilayer per se but rather by the idiosyncrasies of the machineries that have evolved to guide the insertion process. In most cases, membrane proteins use the same targeting and insertion mechanisms used to sort soluble proteins between different cellular compartments but add an extra level of complexity: How are membrane proteins recognized as proteins that should be only partly translocated, leaving the transmembrane segments of the polypeptide chain spanning the bilayer? Is the basis for this recognition the same in different translocation machineries, and, if not, how are such differences translated into different constraints on the allowable structures in any given cellular membrane?

In this article, I will review the current knowledge of how membrane proteins are handled by different targeting–translocation machineries from the perspective of possible structural constraints imposed by these machineries. As will become clear, this point of view has not been much elaborated up to now, and there are not many clear examples of structural constraints that go beyond those imposed by the lipid bilayer itself. Nevertheless, I will argue that such constraints do exist and that they cannot be ignored if we wish to fully understand the principles underlying

ADVANCES IN PROTEIN CHEMISTRY, Vol. 63

0065-3233/03 $35.00

membrane protein structure. For in-depth reviews of protein targeting mechanisms in general, a good up-to-date source is Dalbey and von Heijne (2002).

II. Overview of Membrane Protein Assembly Pathways in Prokaryotic and Eukaryotic Cells

Over the past 30 years, the study of intracellular protein sorting has grown to a large and diversified field. A host of different sorting pathways have been found in both prokaryotic and eukaryotic cells, and most of these can handle both soluble and membrane-bound proteins.

The most well understood pathway is the one that delivers secretory and membrane proteins to the endoplasmic reticulum (ER) membrane in eukaryotic cells and to the inner membrane in bacteria. In both kinds of cells, the pivotal role is played by the so-called Sec61 (in eukaryotes) or SecYEG (in prokaryotes) translocon, a multisubunit translocation channel that provides a conduit for soluble proteins to cross the membrane. The same translocon also serves to integrate membrane proteins into the lipid bilayer.

Bacteria harbor additional inner membrane translocation machineries, such as the "twin-arginine translocation" (Tat) system, the YidC system, and the so-called type II translocation systems that are dedicated to one or a small number of substrates and ensure their delivery into the extracellular medium.

The Tat and YidC systems are also found in certain organelles in eukaryotic cells. The former is present in the thylakoid membrane in plant cells, and the latter is found both in the thylakoid membrane (where it is called the Albino3 system) and in the inner mitochondrial membrane (where it is called the Oxa1p system). Both chloroplasts and mitochondria appear to have unique systems for importing proteins across their outer and inner membranes, and these systems also handle membrane proteins.

Finally, peroxisomes contain another unique machinery for protein import and membrane protein assembly.

From this list of targeting–translocation machineries it is clear that membrane proteins from different subcellular compartments are not handled in the same way and thus may be expected to be under different constraints as concerns the requirements for membrane insertion. However, very few comparative data are available and the only system for which we have a detailed understanding of the insertion process and