METHODS IN MOLECULAR BIOLOGY

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Potassium Channels

Methods and Protocols

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ISSN 1064-3745 ISSN 1940-6029 (electronic)
Methods in Molecular Biology
ISBN 978-1-4939-7361-3 ISBN 978-1-4939-7362-0 (eBook)
DOI 10.1007/978-1-4939-7362-0

Library of Congress Control Number: 2017954932

Printed on acid-free paper

This Humana Press imprint is published by Springer Nature
The registered company is Springer Science+Business Media LLC
The registered company address is: 233 Spring Street, New York, NY 10013, U.S.A.

Foreword

The past 70 years have witnessed unprecedented advances in our knowledge of ion channels. In 1952, Hodgkin and Huxley derived a mathematical model to describe how action potentials are initiated and propagated in the squid giant axon. This conductance-based model required only voltage-gated Na^+ and K^+ channels, leak channels, and ion pumps, and did not require an understanding of the molecules themselves or how they function. Yet, even in the absence of molecular knowledge of the channels, there were remarkably accurate depictions of how the channels function. For example, in 1973 Armstrong proposed a model of the Na^+ channel in which ion permeation through an aqueous pore was regulated by a "ball and chain" in which the ball portion of the ion channel protein occluded the pore from the inside of the cell. Indeed, using protease treatment he showed that the ball could be "cut off" and leave the channel continuously open. Then, with the discovery of the patch clamp by Sakmann and Neher in the early 1980s, the activities of single ion channels could be studied and suggested a much wider array of individual ion channels than had been previously considered. In 1982, the recombinant DNA revolution, started in the 1970s, allowed Noda and colleagues to present the first predicted amino acid sequence of an ion channel protein, the Na^+ channel from the electric ray, Torpedo. They had biochemically purified channel protein from the electric organ where it is very highly expressed. This purified protein was used to generate antibodies. In addition, the amino acid sequences of tryptic digest peptides were determined. Immunological screening of a cDNA library identified several candidate clones that were rescreened with degenerate oligonucleotide pools containing all possible coding combinations of the tryptic peptide sequences. Using clones that contained the coding sequences of the tryptic peptides, and subsequently, larger cDNA probes, they deduced the entire predicted coding sequence of the Na^+ channel protein. These studies marked the dawn of the cloning era of ion channel proteins.

Studies of native K^+ channels had indicated a more diverse molecular family than for Na^+ channels. Yet no highly enriched tissue source was available to facilitate biochemical purification. However, Jan and colleagues devised an alternative strategy using *Drosophila* genetics. Electrophysiological studies of larval muscle showed that *Shaker* mutants, flies that shake their legs when anesthetized with ether, selectively affect an A-type K^+ current. Therefore, based on cytological localization they used a chromosomal walk and then fine structure gene mapping to isolate clones encoding portions of the *Shaker* protein. These were used, in turn, to isolate cDNA clones that ultimately yielded the entire predicted protein coding sequence of a K^+ channel. Notably, they identified several alternatively spliced variants. Finally, they expressed the *Shaker* cDNA clones in *Xenopus* oocytes and used two-electrode voltage clamp recordings to present the first heterologous expression of functional K^+ channels. They showed that *Shaker* encoded an A-type current and that the alternatively spliced mRNAs gave rise to functionally distinct K^+ channels. Importantly, a single mRNA species directed expression of functional K^+ channels.

These pioneering studies opened the "gates". Low stringency homology screens of cDNA libraries captured an unexpected expanse of voltage-gated K^+ channels from flies and mammals that presented subtle yet important functional distinctions when expressed in heterologous systems such as oocytes or transfected cell lines.

While homology screens were capable of identifying the members of the Kv families, other strategies were required to isolate clones encoding different K^+ channel families. Thus, the Hebert and Jan groups independently fractionated mRNA isolated from two different sources—outer medulla of rat kidney and a mouse macrophage cell line, respectively—and identified fractions that directed expression of a K^+ current following injection into *Xenopus* oocytes. Expression screening the corresponding cDNA libraries yielded the first two inward rectifier clones, ROMK1 and IRK1. Once again, low stringency homology screens identified a large family of related but distinct inward rectifier channels. Among these, in addition to a host of classic inward rectifiers, were the G-protein coupled inward rectifier channels (GIRKs) and the K_{ATP} channels.

The availability of the primary amino acid sequences for multiple subfamilies of K^+ channels focused attention on the question of what structural components mediate the universal properties of exquisite ion selectivity and permeation. Based upon sequence conservation and mutagenesis studies, and the nascent sequence databases, the selectivity sequence within the pore of the channels was identified. Lazdunski and colleagues then searched the databases for predicted proteins with the K^+ channel signature sequence and identified a yeast protein with a tandem of inward rectifier like motifs, the first K2P channel, YORK1, and then a mammalian counterpart, TWIK1. Once again, low stringency screens revealed a large family of K2P channels that vary particularly in their sensitivities to modulatory effectors.

Ganetsky and colleagues used *Slowpoke* mutant flies, lacking a Ca^{2+}-activated K^+ current in larval muscle, and a molecular genetic strategy similar to that used for *Shaker* to isolate genomic and cDNA clones encoding the BK large conductance voltage and Ca^{2+}-activated K^+ channels, *Slo* channels. Slo2.1 (*Slack*), a Na^+ and voltage activated K^+ channel, was identified using a pore sequence as an in silico probe, and Slo2.2 (*Slick*), a Na^+ and Cl^- activated K^+ channel, was isolated based on homology to *Slack*. We used an anchored PCR strategy and degenerate pools of oligonucleotides based upon the K^+ channel selectivity sequence, to isolate the SK, small conductance Ca^{2+} activated K^+ channels.

The number of K^+ channel genes was far more than had been anticipated; the mammalian genome harbors over 80. Yet the molecular—and functional—diversity goes much farther. First, unlike Na^+ or Ca^{2+} channels for which a single polypeptide with four homologous repeat units comprises a functional channel, K^+ channels assemble from four (or two; K2P) independent subunits, such that the functional channel always contains four pore domains. Within a given subfamily these subunits may be the same or different, dramatically expanding channel diversity. Second, each of the K^+ channel genes undergoes alternative splicing to further expand functional diversity. Indeed, the single BK channel gene can generate more than 1000 possible exon combinations. Third, channel activity and/or subcellular trafficking and localization are modulated by nonhomologous subunits that may co-assemble with the channels. Thus, the combinatorial K^+ channel repertoire is the most diverse among the ion channel families.

The cloning of the K^+ channel superfamily permitted detailed investigations into the structure and function of individual K^+ channels. Cloned channels could be functionally expressed and their activities, in virtual purity and isolation, could be measured accurately. This was combined with site-directed mutagenesis to reveal the molecular bases of essential attributes including gating, permeation, and pharmacology. With the historic presentation of the crystal structure of KCSA, a new level of resolution was obtained that revealed the chemistry of K^+ selectivity and permeation.

Today, structure-function studies that incorporate a diverse toolbox of approaches continue to yield profound insights into the molecular mechanisms underlying potassium channel function. This book, edited by Show-Ling Shyng, Francis Valiyaveetil, and Matt Whorton, provides a timely update on techniques and approaches used to study these channels, with a wide range of topics including channel structure, gating, cell biology, pharmacology, physiological functions, and channelopathies. This volume will be a welcome addition to the Methods in Molecular Biology book series collection and serve those who are interested in studying potassium channels.

Portland, OR, USA *John P. Adelman*

Preface

Potassium is nature's ion of choice for regulating cellular excitability. This is underscored by the fact that potassium channels are the most widely expressed type of ion channel and are found in virtually all organisms. In fact, there are over 90 K^+ channel genes in humans, which is more than the number of Na^+, Ca^{2+}, and Cl– channel genes combined. Potassium channel diversity is further expanded by differential splicing, heterotetrameric channel assembly, and association with accessory proteins. This breadth of diversity allows K^+ channels to respond to and integrate a variety of chemical, electrical, and mechanical signals. This has necessitated the development of a wide range of methods to study K^+ channels and their roles in physiology and disease.

Over the past few decades, advances in structural biology have produced atomic-resolution structures of many representative examples of K^+ channels. While these structures have offered unprecedented insight into the mechanisms of ion selectivity, gating, and ligand regulation, many questions remain regarding channel dynamics which are best addressed through other methods. These complementary approaches are covered in this book and include the use of NMR, EPR, single-molecule FRET, and molecular dynamics simulations.

As new modalities of K^+ channel regulation are discovered, increasingly sophisticated methods are needed to characterize the biophysical properties of certain K^+ channels. Several such methods are presented in this book, including photo-crosslinking, patch clamp and mechanical manipulation of channels reconstituted into lipid vesicles, stopped-flow spectroscopy, and incorporation of unnatural amino acids.

The identification and cloning of K^+ channels was only the beginning in understanding their roles in physiology and disease. This book includes a number of chapters dedicated to this topic, including ways to modulate and monitor protein expression levels in vivo, real-time correlation of channel activity and physiology, as well as optogenetic control of K^+ channel activity. As K^+ channels have so many important physiological roles, it is no surprise that they have great potential as therapeutic drug targets which is addressed in this book with a chapter describing a thallium-based high-throughput drug screen.

The methods used to study K^+ channels continue to improve and expand. This book is intended to provide an update on techniques and approaches that have been developed since the last Methods in Molecular Biology book on Potassium Channels published in 2009. With the wide range of topics covered including channel structure, gating, cell biology, pharmacology, physiological functions, and channelopathies, it is our hope that this book will serve as a practical guide for investigators wishing to study potassium channels.

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