Preface

The importance of ion channels in the generation and transmission of signals in the nervous system has been well known for over forty years, since the classical work of Hodgkin, Huxley, and Katz. The more recent introduction of new electrophysiological methods for the study of ion channels, in particular, the development of the patch clamp technique by Neher and Sakmann, has led to an explosion of research on ion channels in many different systems. It is now thought that ion channels are present in most, if not all, cell types, and are found in all organisms, both eukaryotes and prokaryotes. Furthermore, although it was initially thought that there may be relatively few different ion channel types, it is becoming increasingly clear that ion channels constitute an extremely large group of heterogeneous proteins having one feature in common, the ability to form a pore for the passive movement of ions across membranes. Electrophysiological techniques allowing the study of ion movements with both high sensitivity and temporal resolution are still the methods of choice for the functional study of ion channels. However, transport of ions through ion channels occurs with such high efficiency that the channels themselves need only be present in minute quantities, thus making the biochemical study of ion channels exceedingly difficult. The recent application of molecular biological techniques has facilitated the study of these relatively rare membrane proteins by allowing one to predict the amino acid sequence of the channel protein directly from the nucleotide sequence of the cloned ion channel gene and by allowing one to express both normal and mutant channel proteins in heterologous systems.

This book was conceived with the idea in mind that it would be useful not only to membrane biophysicists, but also to the many cell biologists, biochemists, molecular biologists, pharmacologists, geneticists, and microbiologists who are finding the study of ion channels important in their work. Given their fundamental significance in all organisms, it is essential that an accessible source be made available to scientists which describes not only techniques for recording and analysis of ion channels, but also potential sources of artifacts that may arise during electrophysiological experimentation. In addition, updates on classical techniques, as well as improved methods for expressing cloned ion channel genes and purifying and reconstituting ion channel proteins, should be helpful to all investigators.

Many excellent papers have been published describing the most important electrophysiological methods used today in the study of ion channels. Rather than attempt to duplicate these papers, we have chosen, instead, to focus on the more recent applications and modifications of these standard methods (Section I). Original papers describing these methods are referred

to in chapters contained in this section and other sections of the volume. Many of the chapters in Section I address problems and sources of artifacts that one may encounter in the applications of electrophysiological techniques. We hope both newcomers to the field, as well as experienced membrane biophysicists, will find these chapters useful. A second fundamental aspect of electrophysiological experimentation emphasized in the volume is that of data analysis (Section VI). Here lies the power of electrophysiological study of ion channels. These methods of analysis allow the distinction between different channel types, the identification and elimination of potential common artifacts, and permit detailed analysis of ion channel function. Additional chapters throughout the volume also emphasize these two aspects of electrophysiological study of ion channels.

Methods to reconstitute ion channels in lipid bilayers are described in Section III. Although reconstitution methods are not as widely applied today as the patch-clamp technique, they continue to provide invaluable contributions to our present knowledge of ion channels. For example, the recent discovery that the ryanodine receptor is a calcium release channel was obtained from studies incorporating purified receptors into lipid bilayers and played a crucial role in our present understanding of excitation–contraction coupling. Reconstitution methods will undoubtedly continue to have wide application in the future discovery of new channels in membranes that remain inaccessible to other electrophysiological techniques and in functional studies of proteins isolated from native tissue or expressed from cloned ion channel genes. Furthermore, reconstitution methods are still the best means of examining the influence of lipid composition on ion channel function.

We did not include many standard molecular biological techniques because the application of these techniques to the study of ion channel genes is essentially no different than for any other gene, and many excellent books and papers describing these methods already exist and are referred to in chapters contained in Sections II and IV. Similarly, although we feel strongly that some of the most recent breakthroughs in the field are a direct result of years of research in *Drosophila* neurogenetics, we have chosen not to include chapters on these methods since their adequate description would require an entire volume. A few applications of molecular biological techniques particularly useful in the study of ion channel genes, for example, hybrid arrest and expression cloning, are described in Section IV. This section also contains chapters describing methods to purify some selected ion channel proteins. Although methods for purification of other channel proteins will, obviously, not be identical to those described here, these examples will be useful for the development of new purification protocols. Pharmacological tools have been, and will continue

to be, extremely useful in the identification and examination of many distinct ion channel types. Two chapters in Section IV give overviews of drugs and toxins that interact with ion channels. In addition, an excellent chapter on the analysis of drug action at the single channel level can be found in Section VI. Section V deals with exciting applications of electrophysiological methods for studying ion channels in cellular organelles and single celled organisms including protozoa, bacteria, and yeast. Microbiologists should find these methods helpful in determining the functional role of ion channels in the physiology and pathology of microorganisms.

Section II deals with the expression of ion channels in heterologous systems. These methods serve as the bridge between the molecular biology and electrophysiology of ion channels. We have, therefore, elected to make this the most extensive and comprehensive section of the volume. Considerable focus is directed toward the description of methods for the expression of ion channels from RNA injected into *Xenopus* oocytes. The simplicity and utility of this system to express ion channels, demonstrated by the work of Miledi and colleagues, have resulted in it becoming the standard method for the heterologous expression of ion channel proteins. This section includes chapters describing sources and methods for handling frogs, preparation of oocytes, preparation of RNA from tissue, and *in vitro* synthesis of RNA from cloned cDNAs, as well as methods for recording from oocytes. The second part of this section includes chapters describing methods developed for the heterologous expression of ion channels in other cell types that have proved extremely useful. Using these heterologous expression systems, researchers are now able to address detailed questions concerning which channel structures are involved in determining or influencing specific channel properties such as kinetics, ion selectivity, voltage dependence, ligand binding sites, subunit composition, and modulation via second messenger systems, as well as the identification and isolation of new ion channel genes.

We are extremely grateful to our colleagues for their excellent contributions and the care and quality of their work. It is sometimes easy to forget how difficult the task of explaining subjects to those outside the field is. This is particularly true in the case of ion channels because many of the techniques used to analyze these molecules cannot be applied as recipes. Considerable effort was required on the part of the authors to describe complex methods and concepts to a broad audience. Without their dedication this volume would not have been possible. In an attempt to contain these chapters within a single *Methods in Enzymology* volume, many difficult and somewhat arbitrary decisions were necessary in the selection of topics. Inevitably, omissions have occurred due to oversight on our part, to several potential authors already being overcommitted, and to the rate

of progress in this rapidly expanding area of research. We apologize to all those individuals who have made significant contributions to the field but whose work is not represented in this volume.

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