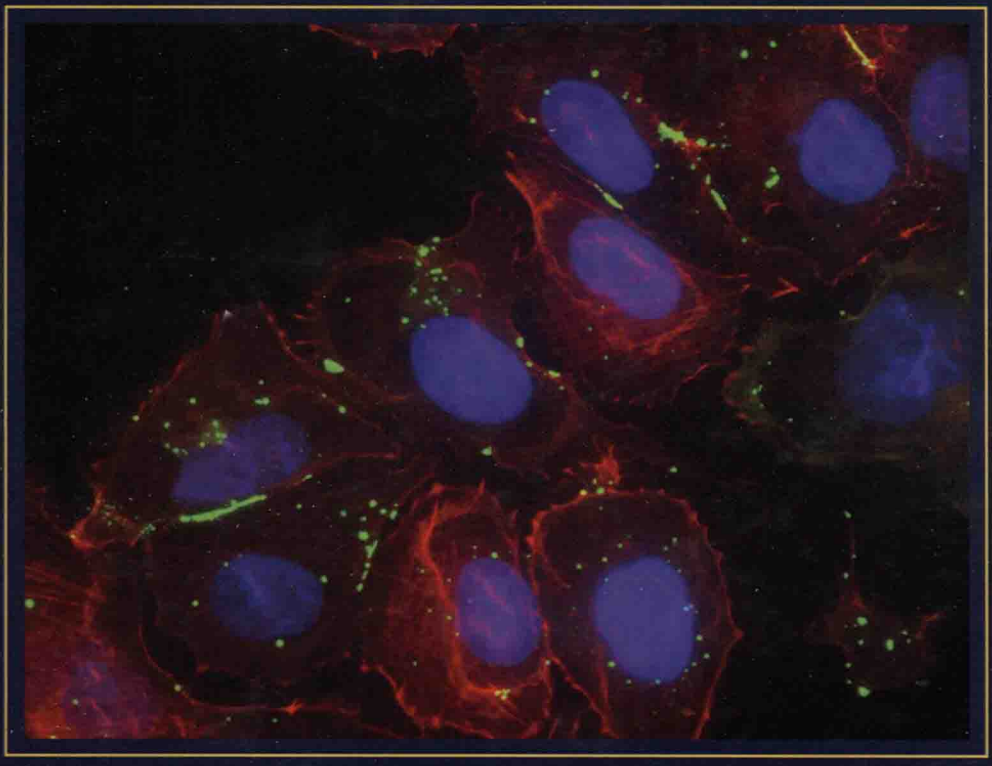
Gap Junction Channels and Hemichannels



Edited by Donglin Bai and Juan C. Sáez

METHODS IN SIGNAL TRANSDUCTION

METHODS IN SIGNAL TRANSDUCTION SERIES

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Gap Junction Channels and Hemichannels

Edited by **Donglin Bai and Juan C. Sáez**

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Gap Junction Channels and Hemichannels

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Joseph Eichberg, Jr. and Michael X. Zhu
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Series Preface

The concept of signal transduction is now long established as a central tenet of biological sciences. Since the inception of the field close to 50 years ago, the number and the varieties of signal transduction pathways, cascades, and networks have steadily increased and now constitute what is often regarded as a bewildering array of mechanisms by which cells sense and respond to extracellular and intracellular environmental stimuli. It is not an exaggeration to state that virtually every cell function is dependent on the detection, amplification, and integration of these signals. Moreover, there is increasing appreciation that in many disease states, aspects of signal transduction are critically perturbed.

Our knowledge of how information is conveyed and processed through these cellular molecular circuits and biochemical switches has increased enormously in scope and complexity since this series was initiated 15 years ago. Such advances would not have been possible without the supplementation of older technologies, drawn chiefly from cell and molecular biology, biochemistry, physiology, and pharmacology, with newer methods that make use of sophisticated genetic approaches as well as structural biology, imaging, bioinformatics, and systems biology analysis.

The overall theme of this series continues to be the presentation of the wealth of up-to-date research methods applied to the many facets of signal transduction. Each volume is assembled by one or more editors who are preeminent in their specialty. In turn, the guiding principle for editors is to recruit chapter authors who will describe procedures and protocols with which they are intimately familiar in a reader-friendly format. The intent is to assure that each volume will be of maximum practical value to a broad audience, including students and researchers just entering this area, as well as seasoned investigators.

It is hoped that the information contained in the books of this series will constitute a useful resource to the life sciences research community well into the future.

Joseph Eichberg
Michael Xi Zhu
Series Editors

Preface

Gap junction channels are a group of intercellular channels ubiquitously expressed in tissues and organs to synchronize many physiological processes. Hemichannels were discovered about two decades ago (a hemichannel is half of a gap junction channel) as a relevant cell membrane pathway for the release and uptake of ions and small molecules, many of which are autocrine and paracrine signal molecules. Mutations in 14 out of 21 genes encoding gap junction protein subunits, called connexins, have been found to be associated with inherited human disorders and diseases, including hearing loss, skin diseases, peripheral and central neuropathic disorders, lens cataracts, cardiac arrhythmias, and developmental disorders. The prevalence of connexin 26 (Cx26) gene mutation-linked congenital sensorineural hearing loss is so high that complete DNA sequencing of the coding region of *GJB2* (the gene encoding Cx26) has become routine for newborns or during pregnancy. Because of the important physiological role of connexins and the linkage of the connexin gene mutations with many diseases, the field of connexin research has recently exploded and has become one of the most active areas of research. Numerous novel approaches and techniques have been developed, but there is no single book dedicated on the unique techniques and protocols for the research on these large pore channels. The last book on gap junction methods and protocols was published 15 years ago, which cannot meet the current need of researchers and trainees in the fields. To fill this gap, we recruited expert researchers in the field to share their state-of-the-art techniques, protocols, and thoughts/reviews on various approaches commonly used in the studies of gap junction channels and hemichannels.

Chapters 1 to 6 in this book are focused on the protocols on, approaches to, and reviews of gap junction channels, and Chapters 7 to 12 are on connexin hemichannels. We hope that this book will be a very useful reference book for graduate students, postdoctoral fellows, and researchers with an interest in gap junction channels and hemichannels. It should be noted that we have covered many common approaches in studying these channels, but some areas/approaches may not be discussed in detail in this book because of the difficulties in finding appropriate contributors.

We would like to thank all the contributors for taking their valuable time to put their chapters together to share their detailed experimental protocols and their views on different approaches. Without their willingness to help, consistent efforts in developing and revising their chapters, and their contribution in helping to reviewing some chapters, we would not be able to complete this book.

A video that relates to Chapter 3 is available from the CRC Press Web site. Interested readers should click on the Downloads/Updates tab at the following URL: https://www.crcpress.com/Gap-Junction-Channels-and-Hemichannels/Bai-Sez/9781498738620.

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Editors

Dr. Donglin Bai received his PhD from the University of Cambridge, United Kingdom, in 1994. He then moved to Canada and worked on neurotransmitter receptors at the Loeb Research Institute, Ottawa, in the Department of Physiology of the University of Toronto, and at the Samuel Lunenfeld Research Institute, Toronto, as a postdoctoral fellow. In 2002 he was recruited as an assistant professor in the Department of Physiology and Pharmacology of the University of Western Ontario, London, Ontario, Canada. He is currently a tenured associate professor at the same university. His current research interests are the physiology of gap junction channels, including gap junction channel docking, single channel conductance, and gating properties. He is also interested in revealing how gap junction gene mutations are linked to human diseases (cardiac arrhythmias, hypomyelination, cataracts, deafness, and skin diseases) and developing strategies for rescuing the mutants. He received grant support from the following Canadian funding agencies: Canadian Institutes of Health Research, Canada Research Chairs, Heart and Stroke Foundation of Canada, Natural Sciences and Engineering Research Council, Canada Foundation for Innovation, and Early Researcher Award.

Dr. Juan C. Sáez received his PhD in neuroscience from Albert Einstein College of Medicine (AECOM), New York, New York, in 1986. He stayed for one year as an instructor in the Department of Neuroscience of AECOM, and then, he became an assistant professor in the same department. In 1993, he joined the Physiology Department of the Pontificia Universidad Católica de Chile, Santiago, Chile, where he has been a professor since 2003. His current research interest is understanding the regulation and the function of connexin- and pannexin-based channels in different cell types, including the cells of the nervous system, the immune system, and the gastrointestinal system and, more recently, on skeletal muscles. He has also characterized biophysical features of the mentioned channels. Recently, he has also used protocols for identifying highly selective inhibitors of connexin hemichannels without an effect on gap junction channels and with potent anti-inflammatory activity to treat chronic diseases. He has been continuously funded through the National Institutes of Health and different foundations of the Chilean government.

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Contents

1 Immunofluorescence: Applications for Analysis of Connexin Distribution and Trafficking

Sandra A. Murray and T. I. Shakespeare

CONTENTS

1.1 SUMMARY

Immunofluorescence methods and techniques for visualizing the subcellular location of gap junction proteins (connexins) and their associated proteins will be presented in this chapter. Specifically, a step-by-step guide for localizing connexin in cell cultures and tissues with immunofluorescence will be presented. The concepts, rationale, and advantages of using particular protocols will be discussed. The methods of preparing and processing cells and tissues for immunofluorescence will be provided. This chapter will serve as a guide for researchers interested in the morphological analysis of gap junctions.

1.2 INTRODUCTION

Gap junctions, which are membrane channels composed of proteins called connexins (Goodenough et al. 1996), permit intercellular communication of regulatory molecules between contacting cells (Decker et al. 1978; Munari-Silem et al. 1995; Oyoyo et al. 1997; Shah and Murray 2001). Information on the assembly of connexins into functional channels and the removal of these channels from the plasma membrane (reviewed in the schematic seen in Figure 1.1) is critical to understanding gap junction turnover, the regulation of cell–cell communication, and the possible role of connexins as anchors for scaffold molecules. To study gap junction assembly and turnover, it is necessary to be able to identify the localization of gap junction proteins at the cell surface and within the cytoplasm (as seen in Figure 1.2). While biochemical methods are powerful tools for detecting the presence, abundance, and types of connexin found in cell populations, immunofluorescence provides information on the specific compartments and cellular locations of connexin and associated proteins needed to study gap junction trafficking and turnover.

Immunofluorescence is a relatively straightforward and inexpensive method for detecting the presence and the subcellular location of connexins within cells. Variations in fluorescent stain intensity and location can be used to gain insight into connexin function and cell–cell communication in cell populations. By using a specific primary antibody that binds to connexin and a secondary antibody that has been conjugated to a fluorophore, connexins can be visualized with a fluorescence microscope (Oyoyo et al. 1997). Colocalization procedures for detecting the