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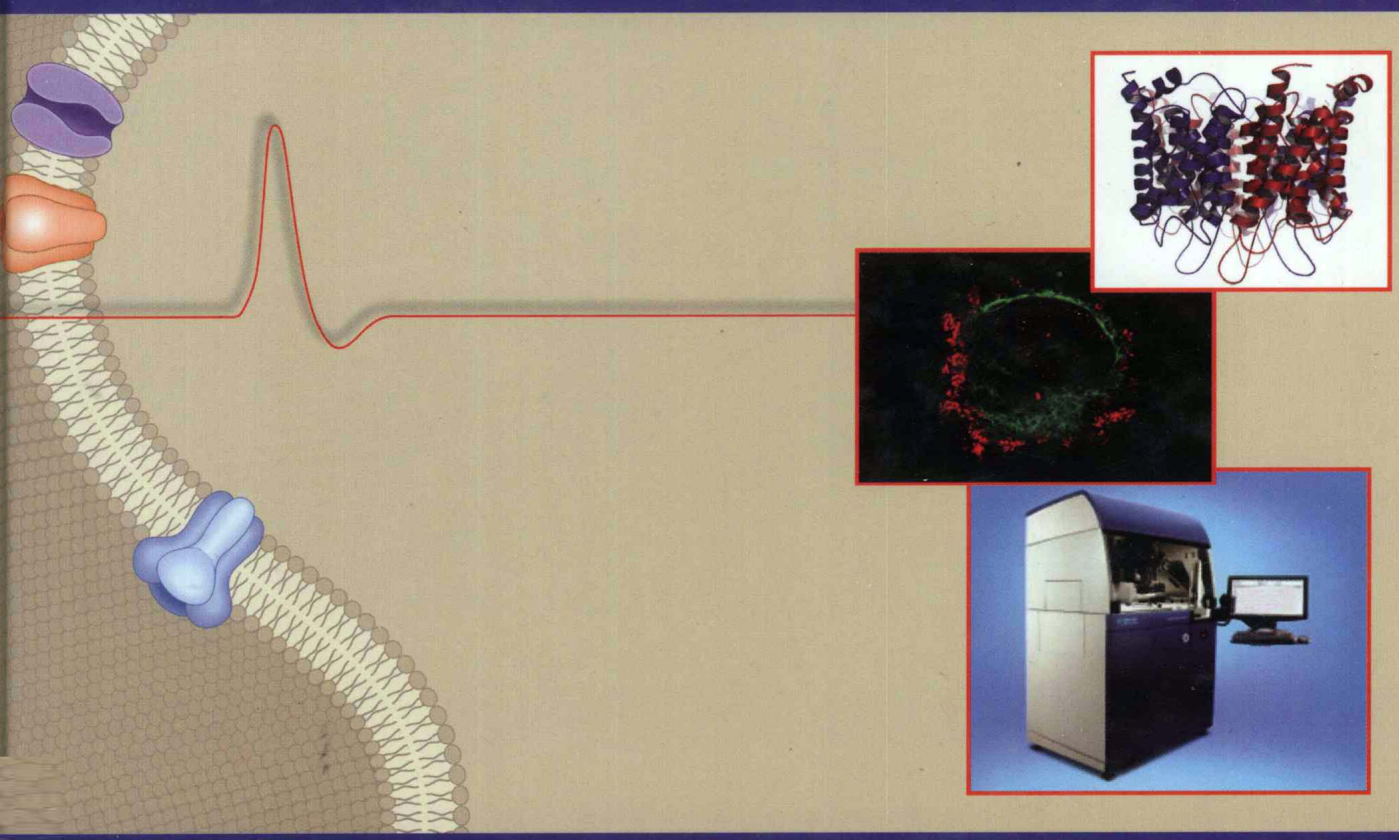
离子通道研究方法精要

Essential Ion Channel Methods

P. Michael Conn



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Essential Ion Channel Methods

离子通道研究方法精要

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导　读

作为生命最基本的载体，所有的活细胞都被细胞膜所包围，细胞膜把生命的化学过程有序地分隔在一个个的单元内进行，同时也制造出一道屏障，使得细胞内外物质的交流、细胞之间信息的传递成为一个生命体必须有效解决的问题。大自然用一系列嵌在细胞膜上的结构和功能各异的膜蛋白来完成这些重要的生理功能。在这些膜蛋白中，离子通道存在于从最简单的细菌到人类的几乎所有的生物中，它们通过带电离子的移动来改变细胞膜内外电压，使细胞对外界刺激产生瞬间（毫秒）反应，因此在神经兴奋传导、神经递质释放、肌肉运动以及控制细胞分化等各种生命活动中起着关键作用。

离子通道所具备的重要生理功能使得它的研究始终处于现代生命科学前沿，然而离子通道的研究严重依赖于方法学上的突破或者与其他学科的交叉融合。1939 年，霍奇金（Hodgkin）与赫胥黎（Huxley）用微电极插入枪乌贼巨神经纤维中，直接测量到膜内外电位差。在随后一系列工作基础上，霍奇金和赫胥黎于 1952 年提出动作电位产生的离子本质理论，并首次提出了离子通道的概念，然而在此后的二十多年里，离子通道的物质基础，即离子通道是什么一直争论不清。直到 20 世纪 80 年代初，随着蛋白质分离纯化技术的成熟，研究者们先后从细胞膜上分离和纯化了一些运输离子的功能性蛋白质，并在人工膜上重组通道功能，才平息了争论，明确了离子通道实体就是膜上一些特殊蛋白质分子或其复合物。1976 年，内尔（Neher）和萨克曼（Sakmann）合作发明了膜片钳（patch clamp）技术，这个技术对神经科学、生理学及细胞生物学产生了革命性的影响，它能够精确测量到细胞膜上单一或多个离子通道的电流变化，为科学工作者提供了了解单一通道变化特点的手段。通过观测单个通道开放和关闭的电流变化，可直接得到各种离子通道开放的电流幅值分布、开放几率、开放寿命分布等功能参量，并分析它们与膜电位、离子浓度等之间的关系。此技术的出现使得细胞膜上大量的离子通道被一一鉴定出来进行单通道分析，并将细胞水平和分子水平的生理学研究联系在一起。分子生物学技术的成熟从另一个角度为离子通道的研究带来了巨大的前进动力。自从 1982 年第一个离子通道——N 型 Ach 受体的氨基酸序列被测定出来，越来越多的离子通道的基因被克隆测序，通过基因重组技术，科学工作者在原先单通道研究的基础之上可以进一步通过改变离子通道的一级序列来分析这些变化如何影响通道的功能。这些研究一方面给离子通道的研究带来了更多信息，另一方面也暴露出明显的缺陷：都是基于通道的一级序列，并不知其空间构象，所以很难对离子通道的功能与工作机制进行深入地了解。这个缺陷的存在不可避免地激发了科学工作者获得离子通道原子分辨率三维结构的欲望。20 世纪 90 年代，随着结构生物学方法开始成熟，麦金农（MacKinnon）引入结构生物学方法，结合电生理学技术，在一系列研究中通过清晰的三维结构结合电生理学分析阐明了钾离子通道的功能机制。

离子通道的研究在我国日渐成为生命科学领域的热点，但国内在相关领域一直缺乏较为权威和前沿的离子通道方法学方面的参考书。本书以细胞膜上离子通道为主要对

象，以介绍离子通道相关研究的主要方法和实验技术，提供实证性数据为目的，以不同的实验手段为切入点，从离子通道的组装、遗传、电生理、表达系统、模型模拟、物理、纯化和重建、第二信使和生化手段、特殊通道等多个方面对离子通道的特点进行了较为详尽的解释和阐述，既展示给读者最前沿的理论，也提供了多种可行和改进的实验方法和数据，并比较了不同方法间的优势所在。

本书作者均为在离子通道相关领域中卓有建树的科学家，汇集他们在各自研究中的丰富经验和大量的数据，内容几乎涵盖了多种离子通道的各个研究方面，针对离子通道研究领域的研究人员，既提供给读者从多个方面理解和思考离子通道的广阔视野，又介绍和展示了切实可行且高效的研究方法，使读者能迅速获取需要的信息。因此，本书既能作为了解离子通道相关知识的权威读本，也是研究人员不可多得的专业工具书。

衷心希望此书能为我国离子通道研究领域的发展提供与国际接轨的桥梁，带给国内科研工作者前沿的方法和技术，使中国的离子通道研究水平日益提高，并为人类生命科学研究作出更大贡献。

叶升

2011 年春于浙江大学紫金港

前　言

科研工作者在过去几年对离子通道越来越感兴趣，离子通道的研究快速增长，这一切都彰显了离子通道在生命活动中的重要性。本书的主旨在于提供对离子通道研究具有重大价值和意义的分子和物理方法。

本书的作者均是《酶学方法》（*Methods in Enzymology*）系列丛书中的撰稿者，之所以选择他们来阐述不同的主题，是因为他们在其领域内有卓越的研究贡献。我们鼓励他们多利用图表，将某种方法和其他方法作对比，并提供有用的技巧和实用的途径，以期能简单地应用于其他体系。

我们鼓励本书的作者采取一种既容易被这个领域的入门者所模仿，又能为经验丰富的研究者提供有价值的信息的方式来介绍这些研究方法。

本人由衷感谢撰稿者对稿件的修订，以及 Academic Press 的工作人员的帮助和为保持卓越的产品标准所付出的努力。

（董巍　叶升　译校）

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PREFACE

The rapid growth of interest and research activity in ion channels is indicative of their fundamental importance in the maintenance of the living state. This volume was prepared with a view to providing a sampling of the range of molecular and physical methods that are significant for the study of ion channels.

The authors were selected from the contributors of previous Methods in Enzymology volumes on that topic on the basis of their significant research contributions in the area about which they have written. They have been encouraged to make use of graphics and comparisons with other methods, and to provide tricks and approaches that make it possible to adapt methods to other systems.

The authors were encouraged to present these methods in a fashion that allows their replication by individuals new to the field, yet providing valuable information for seasoned investigators.

I express my appreciation to the contributors for revising their contributions and to the staff of Academic Press for helpful input and maintaining outstanding production standards.

P. Michael Conn

目　　录