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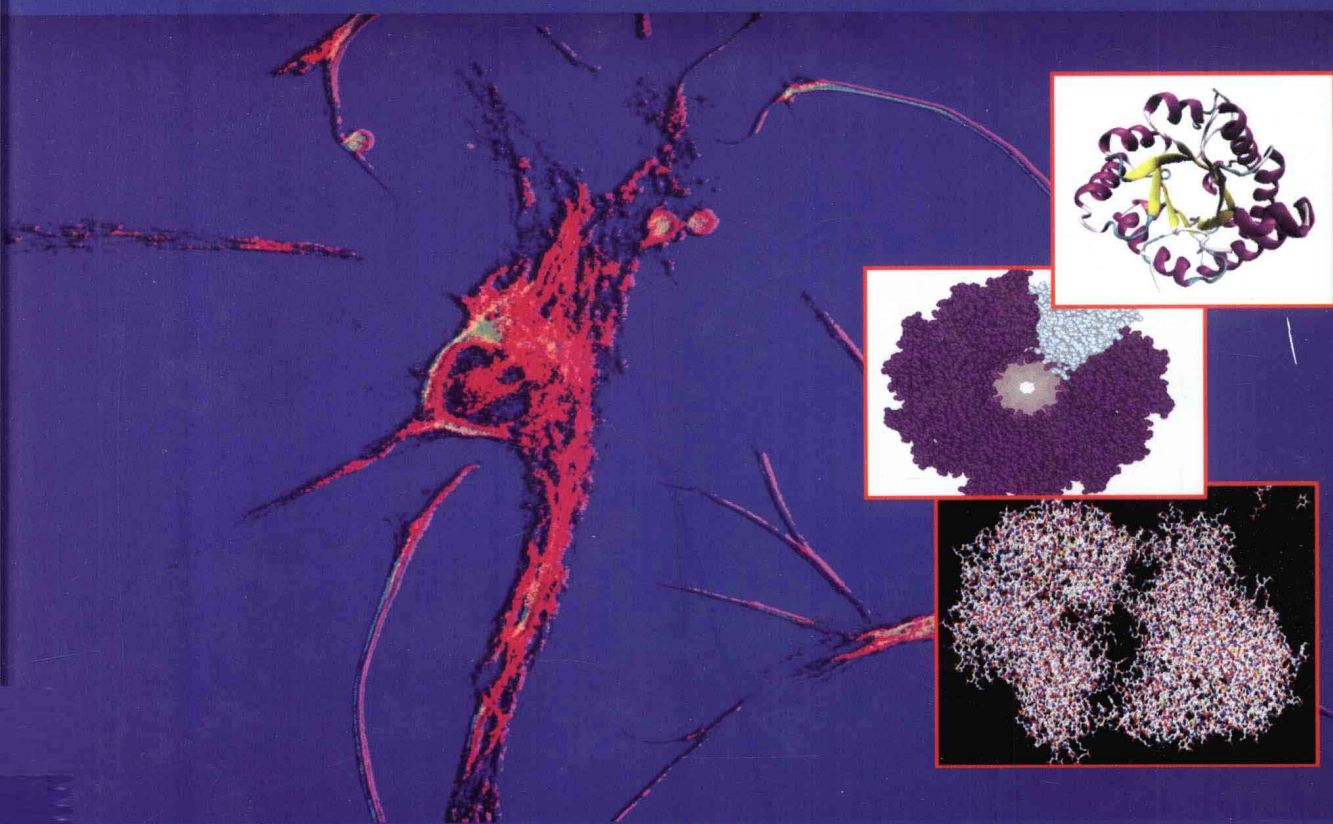
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细胞生物学实验：蛋白质

Cell Biology Assays: Proteins

Geri Kreitzer, Fanny Jaulin and Cedric Espenel



实验室解决方案

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导 读

近年来,现代生物学的发展有诸多亮点,例如干细胞技术、纳米生物技术,以及包括基因组学、蛋白质组学在内的系统生物学技术等。技术方法的迅速扩展有助于解决复杂的生物学问题,同时也提高了对现代生物学家的要求。

美国和英国科学家于2006年5月18日在英国《自然》杂志网络版上发表了人类最后一个染色体——1号染色体的基因测序。至此,历时16年的人类基因组计划书写完了最后一个章节。然而,科学家们仍然不能很好地对复杂的生命现象进行解释,这本“生命之书”几乎还是“四字天书”。蛋白质是生命活动的执行者,生物大分子之间的相互作用构成了生命活动的基础,因此,只有探索蛋白质是如何工作的才能够最终对各种生命现象做出解释。2001年,国际人类蛋白质组组织(Human Proteome Organisation, HUPO)宣告成立,从此开启了一系列蛋白质组研究计划。蛋白质组学的兴起,极大地推动了生命科学的发展,从“钓鱼”式的单个蛋白质分子的研究到“捕鱼”式的整体水平的蛋白质组研究,“鸟枪换炮”的高效使得越来越多的实验室迫切地希望了解和尝试运用这一工具。十几年来,蛋白质组学从发现阶段迅速地进入到解决具体生物学问题的阶段,对描述复杂的生命活动做出了初步贡献,如对细胞生物学中信号转导网络的研究等。

现代生物科学研究中,科学问题和需要使用的技术都具有复杂性。复杂的生物学问题往往不能通过单一的分子生物学或者生物化学的手段得到解决,而是需要不同领域的研究人员相互合作,或者研究人员变成多面手。但是,相比于技术,科学问题更为重要,科研人员极其需要一本实用的实验手册为他们破除技术壁垒。《细胞生物学实验:蛋白质》这本实验手册中所讲述的内容,恰好能够帮助研究人员快速地了解细胞生物学研究中与蛋白质研究相关的跨学科技术,找到合适的实验方案。

本书的章节摘自经典的实验手册《细胞生物学实验手册》(第三版),描述了标记、检测和分离蛋白质,确定蛋白质-蛋白质相互作用、蛋白质-小分子相互作用、蛋白质-DNA相互作用,以及应用质谱鉴定蛋白质和蛋白质修饰的多种实验方法。其中约三分之一的篇幅涉及蛋白质与蛋白质以及蛋白质与其他生物分子的相互作用。这是因为在具体的生物学问题中,蛋白质如何发挥功能往往需要通过蛋白质与其他生物分子的相互作用来说明。此外,现代细胞生物学对蛋白质的研究很多都离不开质谱技术,编者使用三分之一的篇幅描述了一整套用于鉴定蛋白质和蛋白质翻译后修饰的质谱方法。对生物大分子的质谱分析与测定溶液中生物大分子三维结构的核磁共振技术于2002年被授予诺贝尔化学奖,质谱技术解决了“看清”蛋白质“是谁”的问题,核磁共振技术解决了“看清”蛋白质“是什么样子”的问题。这两个化学领域的新技术对生物领域,特别是对蛋白质等生物大分子的研究具有革命性的意义,使得人类可以通过对蛋白质进行详细的分析而加深对生命过程的认识。

从内容编写来看,本书汇总了细胞生物学研究中与蛋白质相关的各种重要实验方法。每种实验方法中,背景介绍、材料设备、方法步骤、分析解释以及参考文献一应俱全。更周

到的是对每种实验方法举出了具体的实验案例和可能出现的问题。参考一本实验工具书,使用者通常期望不仅仅有实验材料和步骤的罗列,还要有对操作过程中需要注意的问题以及可能出现的状况的说明,这些说明往往是“老手”才拥有的宝贵经验,也是实验成败的关键。这本手册正是扮演了这样一个“老手”的角色,为使用者详细地指出各种方法的适用范围,将小窍门和经验倾囊相授。详细的解释、循序渐进的指导,使得这本实验手册成为研究人员的一份重要而实用的参考资料。

此外,本实验手册由来自世界各地的相关领域一线专家编写,内容具有专业性和前沿性。同时,手册把最可能用到的并且最重要的技术方法及相关经验技巧呈现给使用者,不仅对于本领域的研究人员具有可读性,而且能让不同学科和层次的研究人员很快地获得详细而完整的实验方案,极具实用性。例如,本手册为使用质谱技术解决特定的生物学问题提供了方案,这对于只是需要偶尔使用而不是经常使用质谱的研究人员尤其有帮助。

当然,与蛋白质研究相关的技术迅速发展,仅仅是蛋白质-蛋白质相互作用的实验方法就可以编写出一本书,并且不断有新技术更新,但评价一本实验手册是否经典,从来不以它能否替代最新文献作为标准。从内容、可操作性、系统性以及人性化各方面而言,《细胞生物学实验:蛋白质》一书完全可以称得上是一本优秀的实验手册,是研究人员了解跨学科技术的重要资源。

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前 言

在过去的几十年里,被生物学家们所使用的实验方法的范围和多样性急剧扩大。如今,基于细胞的生物学过程的分析往往与分子生物学、生物化学、蛋白质组学和基因组学的使用有密切联系。现代细胞生物学由于实验室技术的进步和富有想象力地应用各种实验方法而使得研究目标更易于达到。现在,这些方法相对容易地使用,导致了科学家们跨越不同学科,从专家到多面手的演变。在令人兴奋的同时,世界各地的研究人员提出日益增长且复杂的生物学问题,跨学科实验方法的发展也像期待的那样提高了水平。因此,熟练掌握或者至少是深入了解这些不同的方法已经成为现代生物学家的一個必要属性。本书的章节摘自《细胞生物学实验手册》(第三版),描述了鉴定感兴趣的蛋白质、检测和分析这些蛋白质,确定蛋白质-蛋白质相互作用以及 DNA-蛋白质相互作用的各种方法。与基于细胞的实验相结合,每种方法都可能成为旨在阐明关于细胞内部运作的复杂的分子生物学机制的研究中的关键因素。

这些章节中描述的方法大致可分为五个部分。第一部分对用放射性氨基酸、离子和磷酸盐或荧光基团化学修饰来标记已知或未知的蛋白质进行了概述。还描述了银染和荧光染色的灵敏的蛋白质检测方法。第二部分编排了用于蛋白质组学分析的通过二维凝胶中等电聚焦和迁移率来分离和检测蛋白质的方法步骤。第三部分收录的章节描述了免疫沉淀,有、无化学交联,可溶性蛋白与其他固定在固体支持物上的蛋白亲和结合,酵母双杂交,以及细胞内共转运法的使用来确定和描绘蛋白质-蛋白质相互作用。在第四部分的章节中,使用染色质免疫沉淀、电泳迁移率变动分析以及寡核苷酸捕获来确定蛋白质和 DNA 相互作用的方法得到描述。最后一部分则详细描述了一整套用于鉴定蛋白质和蛋白质翻译后修饰的质谱方法。

本书的各章节通过实验案例为多种可以在大多数实验室应用的实验技术提供了详细的方法。有的技术相对简单,几乎不需要特殊的设备并且可以被调整以适应于常用的工具和用品。描述质谱方法的章节也表现出对每种技术在评价涉及蛋白质鉴定和描述的特定问题时的有效性和适用性的重要考虑。这对于只需要偶尔而不是常常使用质谱的研究人员尤其有帮助。总之,对每种技术有详细讨论的逐步指导使得这本实验手册成为一项重要资源。在这个细胞生物学的时代,技术的多样使得研究人员能够在分子水平上解决许多与复杂的生物学事件有关的问题。

(朱莉思 高友鹤 译)

Preface

Over the past several decades the range and diversity of experimental approaches used by biologists has expanded dramatically. Today, cell-based analyses of biological processes are often linked intimately with the use of molecular biology, biochemistry, proteomics and genomics. The research goals of modern cell biology have been facilitated tremendously by advances in laboratory techniques and the imaginative application of a broad spectrum of experimental approaches. The relative ease with which these approaches can now be used has led to an evolution of scientists across varied disciplines from being specialists to jacks-of-all-trades. While extremely exciting, this has also raised the bar with respect to cross-disciplinary approaches that can be used, and are often expected, when addressing the ever-growing and complex biological questions posed by researchers around the globe. Thus proficiency in, or at least a thorough understanding of, such diverse approaches has become an attribute necessary to the modern biologist. The chapters included in this volume, taken from *Cell Biology: A Laboratory Handbook, 3rd edition*, describe a variety of methods for identification of proteins of interest, detection and analysis of these proteins, determination of protein-protein interactions and DNA-protein interactions. In combination with cell-based experiments, each may factor critically in studies aimed at elucidating detailed molecular mechanisms as to the inner-workings of cells.

The methods described in these chapters can be broadly separated into five groups. In the first, incorporation of radioactive amino acids,

ions and phosphates or chemical modification with fluorophores to label known or unknown proteins are outlined. Sensitive methods for detecting proteins with silver and fluorescent stains are also described. The second group of methods lays out procedures for the separation and detection of proteins by isoelectric focusing and mobility in 2D gels for proteomic analysis. The third section includes chapters describing the use of immunoprecipitation, with and without chemical cross-linking, affinity binding of soluble proteins to others immobilized on solid supports, yeast-2-hybrid, and co-transport assays in cells to identify and characterize protein-protein interactions. In the fourth group of chapters, methods to identify interactions of proteins with DNA using chromatin immunoprecipitation, electrophoretic mobility shift assays and oligonucleotide trapping are described. And finally, an extensive set of mass spectrometry approaches are described in detail for the identification of proteins and protein post-translational modifications.

Using experimental examples, the chapters in this volume provide detailed methods for a wide range of techniques that can be applied in most laboratories. Some are relatively straightforward, require little special equipment and can be adapted to accommodate commonly available tools and supplies. The chapters describing mass spectrometry methods also enunciate important considerations regarding the usefulness and applicability of each technique for assessing specific questions related to protein identification and characterization. This is particularly helpful

for researchers that aim to use mass spectrometry on occasion, rather than routinely. Together, the step-by-step instructions with detailed discussion of each technique make this laboratory handbook an essential resource. In this era of

cell biology, technical breadth serves an enabling function, allowing researchers to address, at a molecular level, the many questions associated with complex biological events.

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(朱莉思 高友鹤 译)

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SECTION I

LABELING AND DETECTING
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