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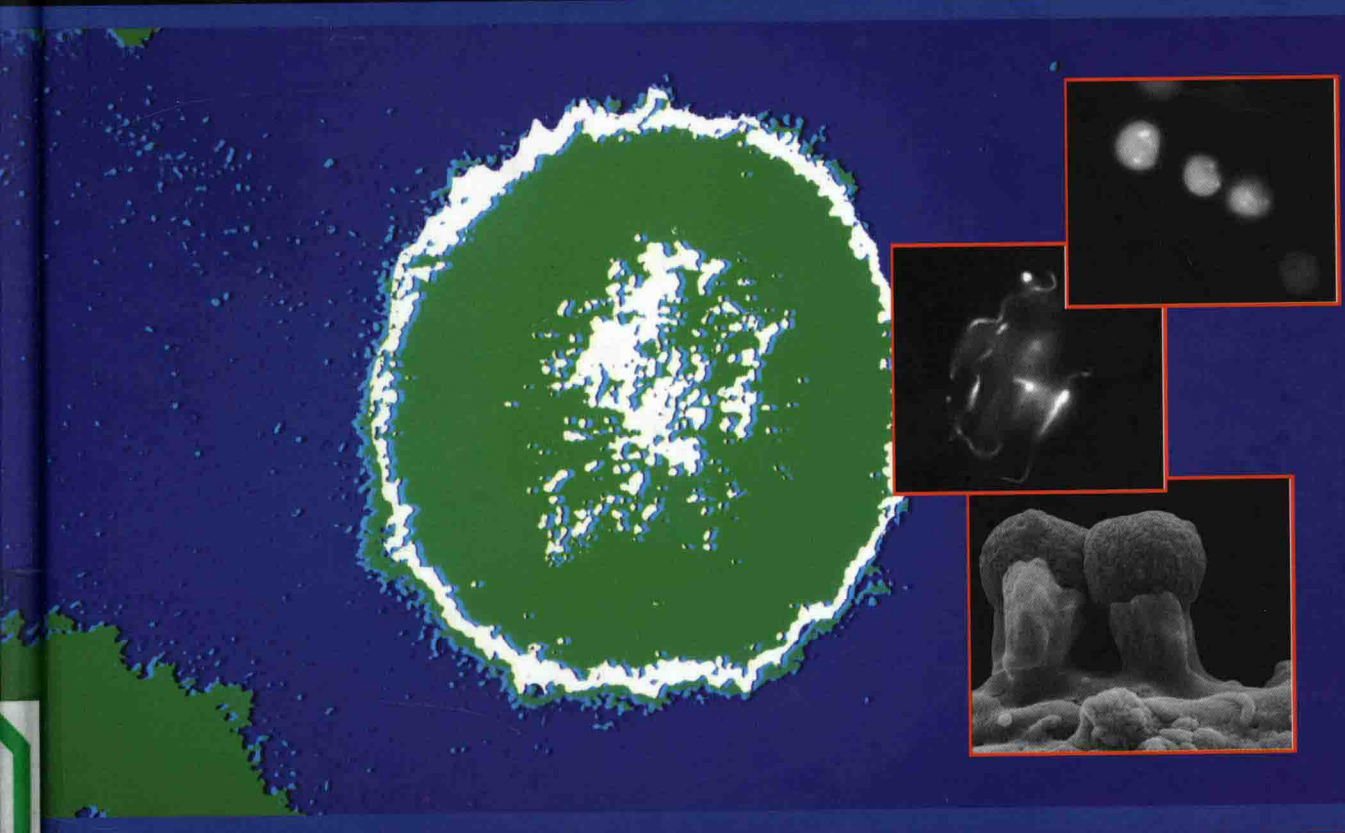
细胞生物学实验：方法精要

Cell Biology Assays: Essential Methods

Geri Kreitzer, Fanny Jaulin and Cedric Espenel



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导　读

科学的研究就是一个逻辑加实证的过程，在科学高度发展的今天，所谓的实证过程已经变得高度复杂，需要一系列设计精巧的科学实验来完成。科学的新发现是在实验技术革新的基础上实现的。我国既往科学研究的落后在很大程度上是实验技术的落后。国外先进的技术方法通常要经过一个很长的周期才能进入我国。现在随着信息技术的革命，科学实验技术的交流和推广变得越来越容易和迅速。我们很高兴地看到，2010 年才出版的《细胞生物学实验：方法精要》（*Cell Biology Assays*：*Essential Methods*）现在就可以和读者见面了。

细胞学说的提出曾被恩格斯认为是 19 世纪的三大科学发现之一，该学说认定细胞是生物体的基本组成成分和一切生命现象的基础。美国细胞生物学家威尔逊（E. B. Wilson）在 1925 年出版的《细胞——在发育和遗传中》(第三版）一书中对这一观点作了进一步的发挥："每一个生物科学问题的答案都必须在细胞中寻找。"这些理论强调细胞这一生命层次在整个生命体系中占有独特地位。细胞是生物体的基本构件和完整生命过程的最小单位，也是遗传和生命延续的基本单元，细胞内部结构的复杂性足以使其担当此任。

早期的细胞学研究偏重纯结构性的观察，以此为基础逐渐赋予亚细胞结构的功能和动态变化的意义，并与其他学科相互影响而扩大其研究范围。例如，通过染色体的动态改变与遗传现象的关系而发展出的细胞遗传学；对细胞组分分离纯化并进行化学结构和过程分析的细胞生物化学和分子生物学等，由此综合形成了细胞生物学科。可以看出，现代生命科学的发展已经使不同学科之间的界限日趋模糊。像细胞生物学、生物化学和分子生物学的研究对象已经融为一体，难以区分。因为业已认识到，重要的生物功能，比如 DNA 的复制和转录、RNA 的加工、蛋白质的合成和修饰、酶促反应和代谢的变化等并非发生在非结构的均一环境中，而是在包括各种细胞器和细胞骨架在内的细胞内复杂的分隔区域内完成，其间涉及各区域间的物质转运和信号传导。各种生物化学或分子生物学实验所得到的结果必须联系到各种亚细胞结构中才能产生意义。

生命科学在 20 世纪的大发展源自物理学和化学等相对成熟的科学向生物世界研究领域的迈进，细胞生物学的每一项成果亦得益于大量物理和化学的研究方法被引进和更新。在过去的 20 年时间里，细胞生物学进入了一个技术高度发展的时期，由此带来对各细胞器的化学结构、功能和动态过程的深入了解，并对细胞的整体调控和细胞之间的信息传递有了较明确的认识。我们看到，每一种新技术的发明和引进都带来生物学观念的更新。现代生物学不是一个空洞的理论体系，而是通过大量实验结果支撑起来的实证科学。全部生物学的知识系统实际上是一系列实验结果的总结的外推。在细胞生物学发展的历程中，显微影像技术、生物大分子示踪技术、细胞体外培养技术和细胞组分的分离技术的发展尤为重要，这广泛依赖于分子生物学、免疫学、生理学、生物物理学和生

物化学技术的引入和综合运用。

《细胞生物学实验：方法精要》精选自 Elsevier 出版集团出版的包罗万象的《细胞生物学实验手册》(第三版)。原手册分为 4 个分卷，主要内容分别是，第 1 卷：细胞和组织培养相关技术，还涉及病毒、抗体和免疫细胞化学等；第 2 卷：细胞器和细胞结构，以及细胞生物学检测技术；第 3 卷：各种成像技术、显微技术、组织矩阵、细胞遗传学和原位杂交、基因工程和基因组学；第 4 卷：大分子的转移、表达系统和基因表达模型等技术。本书内容精选自第 2 卷，并将各实验方法进行重新归类，内容涉及细胞生理过程中的几个关键环节的检测，包括生命分子在细胞内不同区域之间以及细胞内外的转移、细胞信号传导和细胞及细胞内容物的运动性等，由 24 个相对独立的实验组成。由于每个实验的新颖性和专业性，其中涉及到许多专门研究某些问题而设计的实验模型，故由不同的专家分别撰写。这些实验设计精巧，常综合运用不同的细胞生物学实验手段解决具体问题，如果读者善于思考，举一反三，综合运用这些实验模型，可以解决内容广泛的细胞结构与功能问题。

本书的第 1 部分涉及生物膜特性，以及与跨膜物质的转运和分选相关的实验。细胞功能的区域化是细胞进化的一个重要方向，而细胞内容物在区域之间的转移过程和调控是细胞生理的基本研究方面。一些实验方法常有独到之处，例如使用转染的病毒产生的蛋白质进行蛋白质转移和分选的研究；使用温度敏感的病毒 VSV 的糖蛋白作为报告分子，通过在敏感温度下该蛋白折叠障碍来研究蛋白质的动态转运过程等。

第 2 部分是为研究不同环境条件对细胞膜功能的影响而设计的实验。其基本原理是利用液体剪切压将标记大分子导入细胞，可检测不同因素对导入量的影响。

第 3 部分的实验涉及蛋白质在细胞内不同区域之间的转运，包括进入线粒体，进出细胞核的过程等。有些实验设计很有特点，比如通过异核体细胞进行细胞核-质的穿梭转运的实验，就是利用细胞内两个细胞核内物质成分的不同来鉴定核定位物质由细胞质转移到另一个细胞核的过程。

第 4 部分介绍了几种新的钙离子报告蛋白，并使用荧光显微镜进行检测。

第 5 部分介绍了对贴壁细胞实施电穿孔，由此将外源信号分子导入细胞的方法，这是对既往只对悬浮细胞进行电穿孔的方法的革新。

第 6 部分介绍了几种分析细胞骨架的方法。包括对微丝和微管运动性的测定，微丝和微管组装或重排的测定，细胞收缩力的测定等。细胞骨架的活动与许多细胞活动有关，包括内生细菌的感染和细菌在细胞内的运动等。这里有几个实验涉及李斯特菌诱发的微丝骨架的重排。这些实验既是研究内生细菌感染细胞过程的模型，也是研究细胞骨架动力学的良好模型。

本书作为实验指导手册，涉及理论的部分不多，只是在每个实验前面有一个简要的背景介绍；而涉及操作的部分则非常翔实具体，条理清楚，附有必要的插图，易于理解。对于文中未尽的内容都给出了参考文献以供查阅。在每个实验介绍的最后附有简短的评论，使读者对实验有较明确的认识；还附有一个实验中可能出现的差错及解决方案的说明。因而，本书给人以便于使用的感觉。

本书所选的实验方法新颖独特，富于启发，很多内容在其他的细胞生物学实验指导

中很难见到，绝少过剩信息，因此本书物有所值。本书不但可作为在研究中随时参阅的手册，平时翻阅也可能对本以为不相干的其他研究产生有益的启发和借鉴。

谭　信

2011 年 4 月于北京理工大学

前　言

生物学的基本目标之一就是理解基因和蛋白质的表达、激活和失活过程是如何相互协调配合，并以此形成种类繁多的细胞、器官和组织，最终构成活的有机体的。对控制这些过程的调节网络的阐明将会使我们从机制和分子层面上对正常发育过程有更深入的了解，并且有可能导致某些细胞因子的发现。这些细胞因子或可成为治疗损害细胞与组织的结构和功能的众多疾病的靶点。不管工作从哪里开始，在特定阶段研究细胞内特异性基因和蛋白质是实现这一目标的关键所在。

本书包含的各章选自《细胞生物学实验手册》(第三版)(*Cell Biology: A Laboratory Handbook, 3rd edition*)，介绍了一系列细胞生物学实验。这些实验可以实际阐明负责调节不同类型细胞功能的关键性事件。概括地讲，包括了对蛋白质在亚细胞区室之间的转移，细胞间的信号传送和细胞骨架构建的分析方法。这些过程对于确定细胞形态、功能和对细胞内外信号的应答都非常关键。

这些章节所述的实验方法可以归于以下三个基本类别。第一组实验列出了对半完整的或完整的细胞进行离体实验，检测其蛋白质在亚细胞区室之间的转移，或跨膜转移的基本原理和技术。这些实验有助于深入了解出胞和入胞过程的调节，核质之间的转移，以及蛋白质在亚细胞区域移位的机制（包括与翻译同步进行的内质网的转移机制）。总之，这些技术为研究者提供了充分的工具，对细胞内蛋白质转运进行确认、归类，并认识其精细机制。第二组实验详细提供了在单一活细胞内信号转导和蛋白质活性定位的研究方法。这些实验利用关键的荧光传感器测量细胞内钙的局部变化、蛋白质构象、蛋白质磷酸化和三磷酸核苷的占位、所有蛋白质活性解读等。此外，这些实验综合了不同的方法，运用强大的时空分辨力对细胞中的信号过程进行控制和分析。第三组实验详述各种体外的实验和细胞内的实验，用于了解在环境的扰动作用下特定蛋白质、特定细胞质、信号级联传导和细菌的反应，肌动蛋白和微管网络的调节与构建。这些实验也帮助研究者鉴定细胞骨架的组装和动态变化的分子效应物，这些效应物在细胞响应不同的生理刺激中起到重要作用。从任何一组实验中得到的结果都可以有效地运用于使用其他实验方法的综合实验中，从而更透彻地洞察个体细胞活动的调控机制。比如显示马达蛋白沿肌动蛋白和微管移动的体外实验已经成功地与蛋白质转运和信号测定相结合，确定了细胞骨架马达对细胞内众多蛋白质和细胞器的转运事件负责。

通过对实验例证的详细讲解，本书各章中的大量技术和方法得到证明，可应用于多数实验室。尽管某些方法有一定的技术要求，多数方法都可以利用一般研究机构常用的设备和工具完成，这些方法也能适用不同科学家的特殊要求。在当前细胞生物学领域，研究者如果能采用多样的技术，就可以在分子水平上解决与复杂生物事件有关的各种问题。总之，对每一种技术的详细介绍和讨论使这本实验室手册成为理想的实验参考。

（谭信　译）

Preface

One of the primary aims of biology is to understand how expression, activation and inactivation of genes and proteins is orchestrated to generate the wide variety of cells, organs and tissues that comprise a living organism. Ultimately, elucidation of regulatory networks controlling these processes will provide key mechanistic and molecular insight into normal development and have the potential to lead to the discovery of cellular factors that can be targeted in attempts to combat numerous diseases that affect cell and tissue architecture and function. Regardless of where the work starts, at some point the need to study specific genes and proteins within the cellular milieu, becomes central toward achieving this goal.

The chapters included in this volume, taken from *Cell Biology: A Laboratory Handbook, 3rd edition*, describe an array of cell biological assays that can be used to define and characterize experimentally the machinery that regulates events critical to the function of diverse cell types. Broadly defined, these encompass methods to analyze translocation of proteins between sub-cellular compartments, intracellular signaling and cytoskeletal organization. Each of these processes is key to the definition of cell shape, function and responsiveness to both intracellular and extracellular cues.

The methods described in these chapters can be separated into three general categories. The first group of assays lays out the rationale and techniques to measure protein transport between sub-cellular compartments or the plasma membrane *in vitro*, in semi-intact cells and in intact cells. Each of these assays have been used to gain fundamental insight into the mechanisms regulating exocytosis and endocytosis, nuclear-cytoplasmic shuttling and translocation of proteins (including co-translational transport into the endoplasmic reticulum) between intracellular compartments. In combination, these techniques provide researchers with a full arsenal of approaches with which to identify, characterize and determine the minimal essential machinery for protein transport in cells. The second group of assays provides in depth methods to study intracellular signaling and localized protein activity within single, living cells. These assays take advantage of vital fluorescent sensors to measure local changes in intracellular calcium, protein conformation, protein phosphorylation and nucleotide triphosphate occupancy, all read-outs of protein activity. In addition, these assays incorporate methods to control and analyze signaling events with tremendous spatial and temporal resolution in cells. The third group of methods detail *in vitro* and cell-based assays to evaluate the regulation and organization of actin and microtubule networks in response to individual proteins, defined cytosol, signaling cascades and environmental insult by bacteria. These assays also provide researchers with the ability to identify molecular effectors of cytoskeletal dynamics and organization that play key roles in how cells respond physically to a variety of physiological stimuli. Results obtained in any one groups of assays can be fruitfully incorporated into experiments using the others to gain more complete

insight into regulation individual cellular processes. Indeed, *in vitro* experiments showing that motor proteins move along actin and microtubules have been combined successfully with protein transport and signaling assays to identify cytoskeletal motors responsible for numerous protein and organelle transport events in cells.

Using detailed experimental examples, the chapters in this volume examine a wide range of techniques and assays that can be applied in most laboratories. While several of these methods are technically demanding, most are straight-forward, utilize equipment, tools and supplies commonly available at research institutions, and can be adapted to suit specific questions of individual scientists. In this era of cell biology, the ability to apply diverse technical approaches enables researchers to address, at a molecular level, the many questions associated with complex biological events. Together, the step-by-step instructions with detailed discussion of each technique make this laboratory handbook an essential resource.

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目　录

（谭信　译）

Contents

IV

MEASURING INTRACELLULAR SIGNALING EVENTS USING FLUORESCENCE MICROSCOPY

V

IN SITU CELL ELECTROPORATION TO STUDY OF SIGNALING PATHWAYS

VI

CYTOSKELETON ASSAYS

SECTION I

MEMBRANE PROTEIN TRANSLOCATION ASSAYS: EXOCYTOSIS, ENDOCYTOSIS, ER-GOLGI, TRANSLOCATION THROUGH THE ER