Measurement and Manipulation of Intracellular Ions

Edited by

Jacob Kraicer

S. Jeffrey Dixon

Methods in Neurosciences

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Volume 27

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Front cover photograph: Fura-2 stained neuron. Neuron from the nucleus accumbens in cell culture stained with fura-2. The image was obtained using Axon Imaging Workbench by digitizing an integrated frame (500 msec) from a CCD camera (Cohu). The cell was illuminated at 380 nm. Courtesy of J. Plant and B. A. MacVicar, University of Calgary.

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Methods in Neurosciences

Volume 27

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Editor-in-Chief

P. Michael Conn

To my mentors, John Logothetopoulos,
Claude Fortier, and Don Hatcher

JACOB KRAICER

To my parents, Michael and Helen

S. JEFFREY DIXON

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Preface

Neuroscientists, as do other biologists, recognize the crucial importance of intracellular ions in cellular physiology and pathology. Under resting conditions, cytosolic ion concentrations are closely regulated by intracellular buffers and membrane transport systems. Changes in ion concentrations contribute to the regulation of numerous cell functions, including metabolism, secretion, motility, membrane permeability, cell proliferation, and apoptosis. Phenomena such as long-term potentiation and depression and excitotoxic cell death are thought to be mediated through changes in intracellular ion concentrations. To understand these processes, it is important that experimental biologists monitor changes in intracellular ion levels in response to various physiological and pathological stimuli. Similarly, it is crucial to experimentally manipulate intracellular ion concentrations and observe the resulting effects on cell function.

The purpose of this volume is to provide the reader with expert guidance on practical methods for measuring and manipulating cytosolic ionic activities. The first four chapters provide an overview of the use of optical probes, ion-selective microelectrodes and magnetic resonance spectroscopy for measuring ion levels, and the use of ionophores for manipulating intracellular ion concentrations. These are followed by chapters presenting relevant techniques, grouped according to the ion of interest (Ca^{2+}, H^+, Na^+, Mg^{2+}, and Cl^-). We have not attempted to include chapters on all available techniques or even all biologically relevant ions. Emphasis has been placed on widely studied ions, such as Ca^{2+}, and techniques that can be easily modified to measure related ionic species. The concluding chapter presents methods for the measurement of cell volume, the control of which is intimately linked to regulation of intracellular ion levels. In many cases, the techniques presented can be applied only to *in vitro* preparations; however, where possible, techniques for *in vivo* measurement of ion levels have also been included. Although many of the biological preparations described in this volume are of neural origin, most techniques are readily applicable to other tissues. Therefore, we hope that this volume will be of use to all cell biologists with interests in the role of cytosolic ions in cellular function, regulation, and pathology.

We thank the authors for their excellent contributions. Their enthusiasm and cooperation have made our role as editors a pleasant task. We especially thank Drs. Mary E. Morris and Stephen J. Karlik for their invaluable assis-

tance in editing the chapters on ion-selective microelectrodes and NMR spectroscopy, respectively. Last, we thank Dr. P. Michael Conn, Editor-in-Chief of *Methods in Neurosciences,* and Shirley Light of Academic Press for their assistance and encouragement.

JACOB KRAICER
S. JEFFREY DIXON

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[1] Light-Optical-Based Reagents for the Measurement and Manipulation of Ions, Metabolites, and Macromolecules in Living Cells

Kenneth A. Giuliano and D. Lansing Taylor

The chemical processes that comprise living organisms are the result of the precise orchestration of ions, metabolites, macromolecules, macromolecular assemblies, and organelles in time and space. Regulation of free ion concentration is crucial to processes that include maintenance of membrane potential, control of biochemical energetics, and mediation of signal transduction. Hence, we begin this survey by describing the progress made in the design and use of fluorescent indicators of the major intracellular free ion concentrations. Next, the development of fluorescent analogs of macromolecules, fluorogenic substrates, and protein-based optical biosensors is discussed. Then, we describe how free ion indicators are coupled with other fluorescence-based reagents in the same living cell to provide an understanding of the complex temporal and spatial relationships between molecular processes. Finally, we discuss the progress and the prospects of what we believe to be an exciting application of optical-based reagents—the manipulation of specific molecular events using photomodulated ions, metabolites, and macromolecules. The use of photomodulated reagents to change the chemical and molecular properties of cells coupled with the use of reagents that detect specific chemical and molecular events in living cells will propel us toward the dissection of the myriad of chemical reactions that define cellular physiology. Figure 1 portrays in graphical form how cells can be used as living cuvettes for the measurement and manipulation of ions, metabolites, macromolecules, macromolecular assemblies, and organelles.

Fluorescent Indicators of Intracellular Ion Concentration: Powerful Tools for Describing Cellular Physiology

Rationale for Using Fluorescent Indicators in Living Cells

Cells contain endogenous fluorescent compounds such as the nicotinamide adenine dinucleotides, the flavins, and the fluorophoric amino acids trypto-

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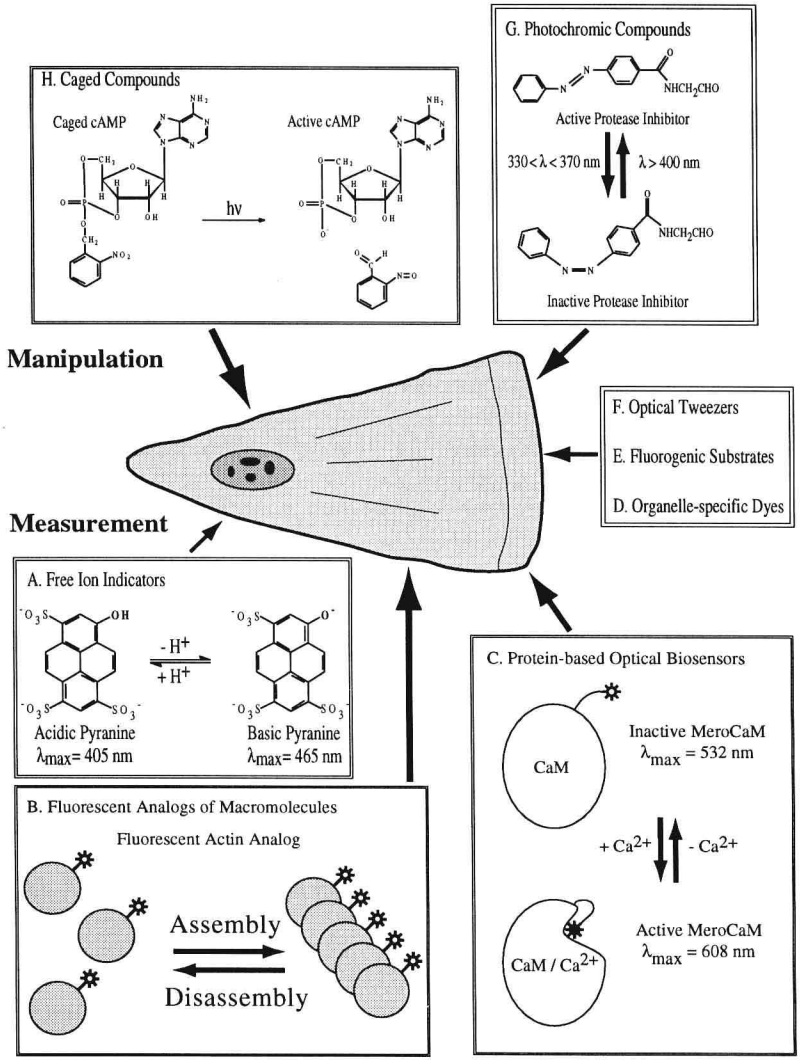

FIG. 1 Cells as living microcuvettes: Measurement and manipulation of chemical and molecular activities in single living cells. The potential to measure and manipulate several specific molecular processes in the same cell is being realized. Optical-based methods for the measurement (A–E) and manipulation (F–H) of cellular components are depicted surrounding a living cell. Examples of several classes of optical reagents are shown. These include (A) pyranine as a fluorescent indicator of pH_i [K. A. Giuliano and R. J. Gillies, *Anal. Biochem.* **167,** 362 (1987)]; (B) fluorescent analogs of actin to measure the dynamics of the actin cytoskeleton [Y. L. Wang and D. L.