Calcium Techniques

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A Laboratory Manual

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Cold Spring Harbor Laboratory Press
Cold Spring Harbor, New York • www.cshlpress.org

CALCIUM TECHNIQUES
A LABORATORY MANUAL

Publisher	John Inglis
Acquisition Editor	Richard Sever
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Developmental Editors	Heather Cerne, David Hatton, Lori Martin, Christin Munkittrick, Catriona Tonks
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Director of Product Development & Marketing	Wayne Manos
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Front cover artwork: Representative Ca^{2+} signals generated in zebrafish embryos during (*top*) cytokinesis of the third cell division cycle; (*center*) in the slow muscles, which are developing in the trunk; and (*bottom*) in the developing head (see Chapter 10). Images courtesy of Sarah E. Webb and Andrew L. Miller, The Hong Kong University of Science and Technology.

Library of Congress Cataloging-in-Publication Data

Calcium techniques : a laboratory manual/[edited by] Jan B. Parys, Martin D. Bootman, David I. Yule, Geert Bultynck.

p. ; cm
Includes bibliographical references and index.
ISBN 978-1-62182-078-9 (cloth) -- ISBN 978-1-936113-58-3 (pbk.)
I. Parys, Jan B., 1958- editor of compilation. II. Bootman, Martin D., editor of compilation. III. Yule, David I., 1964- editor of compilation. IV. Bultynck, Geert, editor of compilation.

[DNLM: 1. Calcium--physiology--Laboratory Manuals. 2. Calcium Channels--Laboratory Manuals. 3. Calcium Signaling--Laboratory Manuals. 4. Fluorometry--methods--Laboratory Manuals. QV 25]

QP535.C2
572'.516--dc23

2013024333

10 9 8 7 6 5 4 3 2 1

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General Safety and Hazardous Material Information

This manual should be used by laboratory personnel with experience in laboratory and chemical safety or students under the supervision of such trained personnel. The procedures, chemicals, and equipment referenced in this manual are hazardous and can cause serious injury unless performed, handled, and used with care and in a manner consistent with safe laboratory practices. Students and researchers using the procedures in this manual do so at their own risk. It is essential for your safety that you consult the appropriate Material Safety Data Sheets, the manufacturers' manuals accompanying equipment, and your institution's Environmental Health and Safety Office, as well as the General Safety and Disposal Cautions in this appendix for proper handling of hazardous materials in this manual. Cold Spring Harbor Laboratory makes no representations or warranties with respect to the material set forth in this manual and has no liability in connection with the use of these materials.

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Appropriate sources for obtaining safety information and general guidelines for laboratory safety are provided in General Safety and Hazardous Material Information (Appendix 1 of this manual).

Preface

No other intracellular messenger rivals Ca^{2+} for complexity. It has long been established that cells use a variety of sources, buffers, and transporters to generate Ca^{2+} signals that span considerable temporal and spatial ranges. Some of the fastest Ca^{2+} signals occur within neurons, and underlie synaptic communication. These microsecond events provide nanoscopic Ca^{2+} transients that trigger the release of neurotransmitter-containing vesicles. At the other end of the temporal and spatial scale are signals such as the Ca^{2+} waves that persist for many tens of seconds and propagate within, and between, cells of various tissues. The rhythmic peristaltic contraction of gut and airway smooth muscle is driven by such repetitive, propagating Ca^{2+} waves. Given that cellular Ca^{2+} signals are hugely diverse, it is not surprising that cells express a wide range of effector molecules to interpret Ca^{2+} changes. Typically, these effector molecules bind Ca^{2+} ions directly, or via an intermediate/adaptor protein, and then allosterically regulate the activity of other proteins. The ability of an effector molecule to sense and respond to Ca^{2+} depends on factors such as its affinity and location. By mixing different Ca^{2+} sources with specific effectors, cells are able to utilize Ca^{2+} in a manner that uniquely suits their physiological functions.

The rich diversity of intra- and intercellular Ca^{2+} signaling mechanisms requires that a range of technical approaches are required to understand how cells generate and interpret particular Ca^{2+} transients. Consequently, over the past decades, researchers within the Ca^{2+} signaling field have been hugely inventive in driving perpetual methodological and technical advances to develop ever more sensitive and sophisticated means of detecting Ca^{2+} signals and their significance. This volume seeks to provide a resource for both novice and expert Ca^{2+} signaling researchers who are seeking to develop, or refine, the techniques they use. Several chapters within this volume are dedicated to Ca^{2+}-sensitive fluorescent reporters, and the different fluorescence imaging modalities (e.g., wide-field, confocal, FRET, two-photon) that are used in conjunction with these reporters. The reason for this emphasis is obvious: since the first introduction of Quin2 by Roger Tsien and colleagues in the early 1980s, the Ca^{2+} signaling field has been substantially propelled by findings obtained using fluorescence imaging. Indeed, fluorescent reporters enable visualization of Ca^{2+} signals in real time within subcellular domains, and the multiplexing of Ca^{2+} recordings with physiological readouts. However, whereas fluorescence is by far the most commonly used method for characterizing Ca^{2+} signals, it was predated by a number of techniques—electrophysiology of channels in situ or in planar lipid bilayers, radioactive $^{45}Ca^{2+}$ flux assays, and the use of Ca^{2+}-sensitive photoproteins—that are still vital in answering specific questions. Correspondingly, these topics are presented in detailed chapters. The majority of techniques described in this volume were developed to monitor Ca^{2+} signaling in mammalian cells. Nevertheless, they can be equally applied to other eukaryotic cells, and in some cases, prokaryotic cells too. A number of chapters have been devoted to measuring Ca^{2+} in specific cell types—oocytes, zebrafish embryos, neurons, cardiac myocytes, plants, and yeast—that require particular technical approaches. Predominant plasma membrane and intracellular Ca^{2+} transport mechanisms are also described within this volume, as are techniques for characterizing the relatively new Ca^{2+}-release pathways involving nicotinic acid adenine dinucleotide phosphate (NAADP) and cyclic adenosine diphosphate ribose (cADPR), methods for modulating intra- and intercellular Ca^{2+} signals using photolytic uncaging or electroporation, techniques for assessing Ca^{2+}-binding kinetics, and insights into developing mathematical models of Ca^{2+} signaling from empirical data.

From the outset, our aim was to compile a resource that was historically reflective while being forward-looking, and technically detailed but also readable. We hope these goals have been achieved. For some, the experimental protocols should encompass complete solutions for implementing new techniques. For others, the protocols may provide suggestions or ideas that the reader can incorporate and adapt to extend their technical abilities. In either case, we hope for yet more fascinating insights into Ca^{2+} signaling.

Our sincere thanks go to all the friends and colleagues that took time out of their busy schedules to share their expertise by contributing to this volume. We are indebted to our coworkers, who over the years have participated in the development and implementation of techniques in our respective laboratories. In addition, we are very grateful to the members of Cold Spring Harbor Laboratory Press who have assisted us so professionally, promptly, and with enduring enthusiasm. Particular mention goes to Maryliz Dickerson at CSHLP for keeping both editors and authors in order with her timely reminders of progress and things still to do. Maryliz ensured that we completed this project instead of wandering back to the lab!

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May 2013