Methods in Cell Biology

VOLUME 39

Motility Assays
for Motor Proteins

Edited by

Jonathan M. Scholey

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ACADEMIC PRESS, INC.

A Division of Harcourt Brace & Company

San Diego New York Boston London Sydney Tokyo Toronto

Cover photograph (paperback edition only): Immunofluorescent micrograph of a multipolar spindle formed in a cell-free lysate of oocytes from the clam *Spisula solidissima*. Lysate prepared from unfertilized oocytes arrested in prophase of meiosis I was mixed with an equal volume of lysate prepared from partenogenetically activated oocytes to induce spindle formation. This mixture was incubated at room temperature for 15 min and processed for immunofluorescense. Microtubules revealed by antitubulin antibody are shown in red and chromosomes revealed by DNA staining with DAPI are shown in blue. Courtesy of Eugeni A. Vaisberg and Robert E. Palazzo.

This book is printed on acid-free paper. ♾

Academic Press, Inc.
1250 Sixth Avenue, San Diego, California 92101-4311

United Kingdom Edition published by
Academic Press Limited
24–28 Oval Road, London NW1 7DX

International Standard Serial Number: 0091-679X

International Standard Book Number: 0-12-564139-7 (Hardcover)
International Standard Book Number: 0-12-638920-4 (Paperback)

PRINTED IN THE UNITED STATES OF AMERICA
93 94 95 96 97 98 EB 9 8 7 6 5 4 3 2 1

Methods in Cell Biology

VOLUME 39
Motility Assays for Motor Proteins

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PREFACE

Motor proteins that hydrolyze ATP to generate force for movement along cytoskeletal fibers are now thought to have a variety of important cellular and developmental functions in eukaryotes, such as controlling the steady-state structural organization of cytoplasm, moving and positioning intracellular macromolecules or organelles, as well as driving cell movement, cell division, flagellar movement, and muscular contraction.

It would be difficult to overstate the importance of the development, during the mid-1980s, of light microscopic "motility assays" for monitoring the activity of these motor proteins; the application of such assays has led to the identification, purification, and characterization of many novel motor proteins, and is illuminating the precise molecular mechanisms by which motor proteins generate force and motion.

Thus, the editors of *Methods in Cell Biology* recognize that a volume in this series should contain a comprehensive sample of methods for performing microscopic motility assays on purified myosins, dyneins, and kinesins (Chapters 1–12) and on crude cell extracts capable of supporting organelle transport and mitotic movements (Chapters 13–20). Each chapter represents a practical guide for any researcher who may wish to perform a particular type of motility assay. Consequently it is hoped that the volume will prove useful for a large number of investigators of the cytoskeleton and related areas of cell biology.

Jonathan M. Scholey

CONTENTS

CHAPTER 1

In Vitro Methods for Measuring Force and Velocity of the Actin–Myosin Interaction Using Purified Proteins

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I. Introduction

Myosin is a class of molecular motor that causes unidirectional movement of actin filaments, using the chemical energy obtained from the hydrolysis of ATP. In both muscle and nonmuscle cells, myosins play critical roles in various forms of cellular movement and shape changes. Cytokinesis, directed cell migration by chemotaxis, capping of ligand-bound cell surface receptors, developmental changes in cell shape, and muscle contraction are only a few examples of events in which molecular motors of the myosin class are involved. In spite of decades of investigation, the molecular basis of the conversion of the chemical energy into mechanical work remains an enigma. Studies with muscle fibers have been important in defining many aspects of the contractile process; however, such studies are complicated by problems associated with the large number of motor molecules working simultaneously and asynchronously, as well as by the presence of a large number of components whose functions are in many cases unknown. *In vitro* motility assays provide an important approach to investigate myosin function using only a small number of purified components. Several *in vitro* motility assays have been used to quantitate velocity as a parameter of myosin function, and recently assays have been developed that measure force production. In the future, additional *in vitro* assays will need to be developed to probe other aspects of motor function (e.g., cooperativity, efficiency).

A number of *in vitro* movement experiments with extracts containing actin and myosin were reported in the 1970s, which formed the foundation for all subsequent work in this area (for review, see Kamiya, 1986). Early reports that purified actin and myosin can produce directional movement *in vitro* involved measuring the streaming of an actin- and myosin-containing solution in glass capillaries (Oplatka and Tirosh, 1973), movement of bundles of actin as measured by dark-field microscopy (Higashi-Fujime, 1985), and rotation of cylinders or pinwheels coated with actin in a solution of myosin (Yano, 1978; Yano *et al.*, 1982). A quantitative assay was later developed that used the oriented polar cables of actin filaments that are found in the giant internodal cells of the alga *Nitella axillaris*. Polystyrene beads coated with purified myosin were observed