
Methods in Cell Biology

VOLUME 39

Motility Assays
for Motor Proteins

Edited by

Jonathan M. Scholey

Methods in Cell Biology

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Motility Assays for Motor Proteins

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Jonathan M. Scholey

Section of Molecular and Cellular Biology

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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 immunofluorescence. 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.

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VOLUME 39

Motility Assays for Motor Proteins



Series Editors

Leslie Wilson

Department of Biological Sciences
University of California
Santa Barbara, California

Paul Matsudaira

Whitehead Institute for Biomedical Research and
Department of Biology
Massachusetts Institute of Technology
Cambridge, Massachusetts

CONTRIBUTORS

Numbers in parentheses indicate the pages on which the authors' contributions begin.

- Viki J. Allan** (203), Structural Studies Division, Medical Research Council Laboratory of Molecular Biology, Cambridge, CB2 2QH, United Kingdom
- Sung Baek** (137), Department of Physiology and Biophysics, University of Washington, Seattle, Washington 98195
- Scott T. Brady** (191), Department of Cell Biology and Neuroscience, University of Texas Southwestern Medical Center, Dallas, Texas 75235, and Marine Biological Laboratory, Woods Hole, Massachusetts 02543
- W. Zacheus Cande** (277), Departments of Molecular and Cell Biology, University of California, Berkeley, Berkeley, California 94720
- Rashmi Chandra** (115), Department of Microbiology, Duke University Medical Center, Durham, North Carolina 27710
- Steven Chu** (1), Departments of Physics and Applied Physics, Stanford University, Stanford, California 94305
- S. A. Cohn** (75), Department of Biological Sciences, DePaul University, Chicago, Illinois 60614
- Martine Coue**¹ (149), Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, Colorado 80309
- Giovanni Cuda** (23), Laboratory of Molecular Cardiology, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland 20892
- Sandra Dabora** (227), Department of Cell Biology, Washington University, St. Louis, Missouri 63130
- Sharyn A. Endow** (115), Department of Microbiology, Duke University Medical Center, Durham, North Carolina 27710
- Jeffrey T. Finer** (1), Departments of Biochemistry and Developmental Biology, Beckman Center, Stanford University School of Medicine, Stanford, California 94305
- Laura A. Fox** (89), Department of Anatomy and Cell Biology, Emory University School of Medicine, Atlanta, Georgia 30322
- Neal R. Glikzman**² (237), Department of Biology, University of North Carolina, Chapel Hill, North Carolina 27599
- Christopher J. Hogan** (277), Departments of Molecular and Cell Biology, University of California, Berkeley, Berkeley, California 94720
- Earl Homsher** (23), Department of Physiology, School of Medicine, Center for Health Sciences, University of California, Los Angeles, Los Angeles, California 90024

¹ *Present address:* Institute Jacques Monod, University of Paris, Paris, France.

² *Present address:* Department of Cell Biology, Duke University Medical Center, Durham, North Carolina 27710.

- Jonathon Howard** (105, 137), Department of Physiology and Biophysics, University of Washington, Seattle, Washington 98195
- Alan J. Hunt** (137), Department of Physiology and Biophysics, University of Washington, Seattle, Washington 98195
- A. A. Hyman** (105, 267), Department of Pharmacology, University of California, San Francisco, San Francisco, California 94143
- Bechara Kachar** (179, 253), Laboratory of Cellular Biology, National Institute for Deafness and Other Communication Disorders, National Institutes of Health, Bethesda, Maryland 20892
- Scot C. Kuo** (129), Department of Cell Biology, Duke University Medical Center, Durham, North Carolina 27710
- Carilee Lamb** (227), Department of Cell Biology, Duke University Medical Center, Durham, North Carolina 27710
- Manlin Lee** (277), Institute of Life Science, National Tsing Hua University, Hsingchu, Taiwan 30043, Republic of China
- Philip L. Leopold**³ (191), Department of Cell Biology and Neuroscience, University of Texas Southwestern Medical Center, Dallas, Texas 75235, and Marine Biological Laboratory, Woods Hole, Massachusetts 02543
- Vivian A. Lombillo** (149), Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, Colorado 80309
- R. J. Lye** (75), Department of Genetics, Washington University School of Medicine, St. Louis, Missouri 63110
- James M. McIlvain, Jr.** (227), Department of Cell Biology, Duke University Medical Center, Durham, North Carolina 27710
- J. Richard McIntosh** (149), Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, Colorado 80309
- Mark A. McNiven** (179, 253), Center for Basic Research in Digestive Diseases, Mayo Clinic, Rochester, Minnesota 55905
- T. J. Mitchison** (267), Department of Pharmacology, University of California, San Francisco, San Francisco, California 94143
- Douglas B. Murphy** (253), Department of Cell Biology and Anatomy, The Johns Hopkins Medical School, Baltimore, Maryland 21205
- Patrick J. Neale**⁴ (277), Department of Plant Biology, University of California, Berkeley, Berkeley, California 94720
- Stephen F. Parsons** (237), Department of Biology, University of North Carolina, Chapel Hill, North Carolina 27599
- Bryce M. Paschal** (65), Cell Biology Group, Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts 01545, and Department of Cell Biology, University of Massachusetts Medical School, Worcester, Massachusetts 01605

³ *Present address:* Department of Pathology, Columbia University College of Physicians and Surgeons, New York, New York 10032.

⁴ *Present address:* Smithsonian Environmental Research Center, Edgewater, Maryland 21037.

- Thomas D. Pollard** (51), Department of Cell Biology and Anatomy, The Johns Hopkins Medical School, Baltimore, Maryland 21205
- Bruce W. Richards** (191), Department of Cell Biology and Neuroscience, University of Texas Southwestern Medical Center, Dallas, Texas 75235, and Marine Biological Laboratory, Woods Hole, Massachusetts 02543
- Marcelo N. Rivolta** (179), Laboratory of Cellular Biology, National Institute for Deafness and Other Communication Disorders, National Institutes of Health, Bethesda, Maryland 20892
- Winfield S. Sale** (89), Department of Anatomy and Cell Biology, Emory University School of Medicine, Atlanta, Georgia 30322
- E. D. Salmon** (237), Department of Biology, University of North Carolina, Chapel Hill, North Carolina 27599
- W. M. Saxton** (75), Department of Biology, Indiana University, Bloomington, Indiana 47405
- J. M. Scholey** (75), Section of Molecular and Cellular Biology, University of California, Davis, Davis, California 95616
- James R. Sellers** (23), Laboratory of Molecular Cardiology, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland 20892
- Michael P. Sheetz** (129, 227), Department of Cell Biology, Duke University Medical Center, Durham, North Carolina 27710
- Takashi Shimizu** (167), Research Institute of Bioscience and Human-Technology, Higashi, Tsukuba, Ibaraki 305, Japan
- Robert M. Simmons** (1), MRC Muscle and Cell Motility Unit, Randall Institute, Kings College London, London WC2B 5RL, England
- Elizabeth F. Smith** (89), Department of Anatomy and Cell Biology, Emory University School of Medicine, Atlanta, Georgia 30322
- James A. Spudich** (1), Departments of Biochemistry and Developmental Biology, Beckman Center, Stanford University School of Medicine, Stanford, California 94305
- Yoko Y. Toyoshima**⁵ (167), Department of Biology, Ochanomizu University, Ohtsuka, Bunkyo-ku, Tokyo 112, Japan
- Raul Urrutia** (179, 253), Laboratory of Cellular Biology, National Institute for Deafness and Other Communication Disorders, National Institutes of Health, Bethesda, Maryland 20892, and Center for Basic Research in Digestive Diseases, Mayo Clinic, Rochester, Minnesota 55905
- Taro Q. P. Uyeda** (1), Departments of Biochemistry and Developmental Biology, Beckman Center, Stanford University School of Medicine, Stanford, California 94305
- Ronald D. Vale** (167), Department of Pharmacology, University of California, San Francisco, San Francisco, California 94143
- Richard B. Vallee** (65), Cell Biology Group, Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts 01545

⁵ *Present address:* Department of Pure and Applied Sciences, College of Arts and Sciences, University of Tokyo, Meguro-ku, Tokyo 113, Japan.

Fei Wang (23), Laboratory of Molecular Cardiology, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland 20892

Hans M. Warrick (1), Departments of Biochemistry and Developmental Biology, Beckman Center, Stanford University School of Medicine, Stanford, California 94305

Henry G. Zot (51), Department of Physiology, University of Texas Southwestern Medical Center, Dallas, Texas 75235

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

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CHAPTER 1

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

**Hans M. Warrick,[★] Robert M. Simmons,[†] Jeffrey T. Finer,[★]
Taro Q. P. Uyeda,[★] Steven Chu,[‡] and James A. Spudich[★]**

[★]Departments of Biochemistry and Developmental Biology
Beckman Center, Stanford University School of Medicine
Stanford, California 94305

[†]MRC Muscle and Cell Motility Unit
Randall Institute, Kings College London
London WC2B 5RL, England

[‡]Departments of Physics and Applied Physics
Stanford University
Stanford, California 94305

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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