

Methods and Achievements in Experimental Pathology

**The Cytoskeleton in Normal and
Pathologic Processes**

Cell Biology

8

**Volume Editor
G. Gabbiani, Geneva**

**Series Editors
G. Jasmin, Montreal, Que.
M. Cantin, Montreal, Que.**



**S. Karger
Basel · München · Paris · London · New York · Sydney**

ISBN 3-8055-2917-1

The Cytoskeleton in Normal and Pathologic Processes

Cell Biology

Volume Editor
G. Gabbiani, Geneva

32 figures and 2 tables, 1979



S. Karger · Basel · München · Paris · London · New York · Sydney

Methods and Achievements in Experimental Pathology

- Vol. 3: Ultrastructural, Histopathologic Chemical Approaches.
VIII + 392 p., 159 fig., 15 tab., 1967. ISBN 3-8055-0524-8
- Vol. 4: Examples of Descriptive and Functional Morphology.
VI + 256 p., 150 fig., 2 cpl., 1 tab., 1969. ISBN 3-8055-0525-6
- Vol. 5: Functional Morphology of the Heart.
VIII + 596 p., 273 fig., 4 cpl., 23 tab., 1971. ISBN 3-8055-1209-0
- Vol. 6: Nutritional Pathobiology.
VIII + 245 p., 84 fig., 18 tab., 1972. ISBN 3-8055-1343-7
- Vol. 7: Disease Patterns. Correlations between Human and Animal Diseases.
VIII + 229 p., 63 fig., 7 tab., 1975. ISBN 3-8055-2155-3
-

National Library of Medicine Cataloging in Publication

The cytoskeleton in normal and pathologic processes /
volume editor, G. Gabbiani. — Basel ; New York : Karger 1979.
2 v. (Methods and achievements in experimental pathology ; v. 8-9)
Contents: v. 1. Cell biology. — v. 2. Cell physiopathology.
1. Cells — physiology 2. Cells — physiopathology
I. Gabbiani, Giulio, ed. II. Title III. Series
W1 ME9613 v. 8-9 QH 631 C999
ISBN 3-8055-2917-1

All rights reserved.

No part of this publication may be translated into other languages, reproduced or utilized in any form or by any means, electronic or mechanical, including photocopying, recording, microcopying, or by any information storage and retrieval system, without permission in writing from the publisher.

- © Copyright 1979 by S. Karger AG, 4011 Basel (Switzerland), Arnold-Böcklin-Strasse 25
Printed in Switzerland by Thür AG Offsetdruck, Pratteln
ISBN 3-8055-2917-1

The Cytoskeleton in Normal and Pathologic Processes
Cell Biology

Methods and Achievements in Experimental Pathology

Vol. 8

Series Editors

G. Jasmin and M. Cantin, Montreal, Que.



S. Karger · Basel · München · Paris · London · New York · Sydney

Acknowledgments

This work was supported in part by the Swiss National Science Foundation, Grant No. 3.692-0.76. We thank Drs. *B. Bertolini, S.H. Blose, B. Bowers, U. Brunk, K.K. Buckley, K. Burrige, S. Chacko, K.T. Edds, H. Erickson, B.A. Fredriksson, R.C. Hunt, M. Kirschner, S. Lowey, S. Lux, N.S. McNutt, M.S. Mooseker, T.D. Pollard, R.V. Rice, M. Schliwa, J.A. Spudich, J.G. White* and *D. Zucker-Franklin*, *The American Journal of Pathology*, *Annals of the New York Academy of Sciences*, *Biochimica Biophysica Acta*, *Human Pathology*, *International Review of Cytology*, *Journal of Cell Biology*, *Journal of Clinical Investigations*, *Journal of Experimental Medicine*, *Journal of Molecular Biology*, *Journal of Submicroscopic Cytology*, *Journal of Supramolecular Structure*, *Journal of Ultrastructure Research*, *Progress in Hemostasis and Thrombosis*, *Virchows Archiv B Cell Pathology*, Academic Press, London; Cold Spring Harbor Laboratories, New York; Raven Press, New York and Wiley, New York; Elsevier/North Holland Biochemical Press, Amsterdam; Schwabe & Co. AG, Basel, and Williams and Wilkins Company, Baltimore, for granting permission to reproduce micrographs and tables in various articles. We gratefully acknowledge the secretarial help of Miss *M.M. Cuenat* without whom the assembly of this book would not have been possible.

Preface

During the last few years, a great deal of work has been devoted in many laboratories to the study of structures responsible for maintenance of cell shape, intracellular movement and cell locomotion. It has been shown that such structures are similar in a wide variety of cell types and consist of contractile proteins such as actin and myosin and cytoskeletal elements such as microtubules and intermediate (100-Å) filaments. A large literature has been published on the structure and function of contractile and skeletal cell elements including reviews and symposia. Clearly, the alterations of shape, cytoplasmic movements and locomotory activity in single cells must result in pathologic changes of the affected organs or tissues and, indeed, in few instances, a relationship between the presence of modifications at the level of contractile and/or skeletal elements and pathologic changes has been shown. Research on cytoskeleton-mediated pathologic changes is very young and it is easy to anticipate that in a not distant future, several pathologic manifestations will be related to alterations of contractile or skeletal cell elements. The purpose of this book is on the one hand to collect the most recent advances in the field of the function of cytoskeleton and, on the other hand, to emphasize some of the recent advances in the correlation between modifications of contractile and skeletal elements and morbid changes in animals or humans. We hope that this approach will stimulate further fruitful research on the role of contractile and skeletal structures in pathologic processes.

Giulio Gabbiani

The Cytoskeleton in Normal and Pathologic Processes

Cell Biology

The papers with emphasis on '*Cell Biology*', appearing under the title 'The Cytoskeleton in Normal and Pathologic Processes – Cell Biology' are published in this volume.

The papers with emphasis on '*Cell Physiopathology*', appearing under the title 'The Cytoskeleton in Normal and Pathologic Processes – Cell Physiopathology', are published in vol. 9 of the series (see table of contents on p. IX).

Contents

Acknowledgments	XIV
Preface	XV

The Cytoskeleton and Cell Movement: General Considerations

<i>R.S. Adelstein, S.P. Scordilis and J.A. Trotter, Bethesda, Md.</i>	1
Introduction	2
Actin and Myosin: Historical Perspective	3
Actin	4
General Considerations	4
Sites of Interaction	4
Polymerization	4
Critical Concentration	6
Interactions with Non-Contractile Proteins	6
Sequence	7
Isoelectric Points	7
Actin Filaments	8
Decoration	10
Polarity	11
Fluorescent Actin Probes	11
Antibodies	12
Myosin	13
Introduction	13
Structural Studies	13
Filament Formation	16
ATPase Activity	18
General Considerations	18
Actin-Activated ATPase Activity	18
Ca ²⁺ Control of Actin – Activation of Myosin	20

Contents	VI
Antibodies to Myosin	22
Tubulin	23
General Considerations	23
Tubulin Characteristics	23
Tubulin Polymerization	24
Effects of Accessory Proteins	24
Effects of Small Molecules	26
Covalent Modification	28
Seeding of Polymerization	28
Structure of Microtubules	28
Dynein	28
Cytoplasmic Dynein	29
Cytoplasmic Motility	29
Antibodies to Tubulin	30
Conclusion	30
Summary	31
Acknowledgments	31
References	31

The Cytoskeleton and Plasma Membrane

<i>Robert T. Weihsing</i> , Shrewsbury, Mass.	42
Introductory Remarks	43
Cytoskeleton and Plasma Membrane — General Comments and Examples	43
Cytoskeletal Role of Microfilaments near Plasma Membrane	44
Cytoskeletal Role of Microtubules near Plasma Membrane	46
Cytoskeletal Role of 10-nm Filaments near Plasma Membrane	46
Extent of Cytoskeleton in Tissues	48
Influence of Cytoskeleton on Shape of Erythrocytes	48
Attachments	52
Attachment without Local Differentiation	52
Attachment by Electron-Dense Material	52
Attachment to Cell-Cell and Cell-Substrate Attachment Sites	54
Attachment to Cell-Cell Junctions	54
Attachment to Cell-Substrate Attachments	58
Attachment by Side Arms	58
Structure and Chemistry of Attachment Sites	60
No Local Differentiation	60
Attachment Devices	60
Polarity	62
Polarity of Actin Relative to Plasma Membrane	62
Polarity of Microtubules Relative to Plasma Membrane	63
Polarity of 10-nm Filaments Relative to Plasma Membrane	64
Biochemistry	64
Isolation of 'Total' Plasma Membrane with Attached Cytoskeleton	64
Isolation of Specialized Regions of Plasma Membrane with Attached Cytoskeleton	65
Isolation of Attachment Devices	69

Contents	VII
----------	-----

Isolation of Cytoskeleton	71
Addition of Cytoskeletal Proteins to Plasma Membrane	71
Organization and Reorganization of Plasma Membrane-Associated Cytoskeleton	72
Actin-Based Cytoskeleton – Networks and Cables	72
Cables	72
Networks	76
Reorganization of Actin-Based Cytoskeleton – Relation of Cables to Networks	79
Organization and Reorganization of Microtubule-Based Cytoskeleton	80
Organization and Reorganization of 10-nm Filament-Based Cytoskeleton	83
Effects of Chemical and Physical Agents on Cytoskeleton	83
Cytochalasin B	83
Phalloidin	84
Colchicine and Other Microtubule Drugs	84
Cold and Pressure	85
Capping	85
Concluding Remarks	88
Summary	89
Acknowledgments	89
References	90

The Cytoskeleton and Cell Division

<i>Joseph W. Sanger and Jean M. Sanger, Philadelphia, Pa.</i>	110
---	-----

I. Introduction	110
A. Microtubules	112
B. Actin and Myosin	114
C. Intermediate (100-A) Filaments	116
II. Mitosis	117
A. Fate of Microtubules during Mitosis	118
B. Fate of Actin and Myosin during Mitosis	122
C. Fate of Intermediate Filaments during Mitosis	129
III. Cytokinesis	129
IV. Future Directions for Exploring the Role of the Cytoskeleton in Cell Division	131
A. Calcium Regulation of the Cytoskeleton	131
B. <i>In vitro</i> Models of Chromosome Movement	132
C. Interactions of Microtubules, Actin and Myosin, and Intermediate Filaments	133
Summary	133
Acknowledgements	134
References	134

The Unpolymerised Form of Actin in Non-Muscle Cells

<i>U. Lindberg, L. Carlsson, F. Markey and L.E. Nyström, Uppsala</i>	143
--	-----

Introduction	143
Organisational States of Actin	144
The Dynamic Nature of Microfilament Organisation	148

The Biochemistry of Non-Muscle Actin	150
Purification of Actin from Non-Muscle Sources	150
Characteristics of Proteins Associated with Actin	152
Speculations on the Role of Unpolymerised Actin <i>in vivo</i>	156
Summary	159
Acknowledgements	160
References	160

The Cytoskeleton and Cytoarchitecture in Embryogenesis — An Overview

<i>D.R. Burgess and T.E. Schroeder, Hanover, N.H.</i>	171
---	-----

Introduction	171
Embryogenesis and Morphogenesis	171
A Conceptual Framework for Morphogenesis	172
Early Events	173
The Egg Cortex	173
Spermiogenesis and the Acrosome	174
Sperm-Egg Contact	174
Post-Fertilization Cortical Contractions	175
Sperm Aster	176
Cell Cleavage	176
Epithelial Morphogenesis	178
Gastrulation	178
Neurulation	180
Other Invaginations and Evaginations	181
Metamorphosis	182
Cellular Morphogenesis and Supramolecular Function	182
Postscript on Pathology	184
Summary	185
References	185
Subject Index	190

The Cytoskeleton in Normal and Pathologic Processes

Cell Physiopathology

Papers, with emphasis on '*Cell Physiopathology*' are published under the title '*Cytoskeleton in Normal and Pathologic Processes – Cell Physiopathology*', forming vol. 9 of the series '*Methods and Achievements in Experimental Pathology*'.

Contents

Interaction of Microtubules and Microfilaments in Platelet Contractile Physiology

James G. White and Jonathan M. Gerrard, Minneapolis, Minn. 1

I. Introduction	1
II. Platelet Structure and Function	4
A. Physiology	4
B. Morphology	6
1. Peripheral Zone	8
2. Sol-Gel Zone	12
3. Organelle Zone	12
4. Membrane Systems	14
III. Structural Physiology	16
IV. Functions of Platelet Microtubules	20
A. The Origin of the Circumferential Band of Microtubules	20
B. Microtubules and Platelet Discoid Shape	20
C. Microtubules and Platelet Contraction	24
V. Structure and Biochemistry of Platelet Microtubules	28
VI. Recent Studies	30
VII. Conclusion	32
References	36

The Contractile System of Blood Platelets and Its Function

Isaac Cohen, Evanston, Ill. 40

I. Introduction	41
II. Major Building Units of the Platelet Contractile System – Isolated Components	41
A. Natural Actomyosin (Thrombosthenin)	41
1. Preparation	41
2. Physico-Chemical Properties	42
3. Localization	43
4. Biosynthesis	43

Contents	X
B. Myosin	43
1. Preparation	43
2. Physico-Chemical Properties	44
3. Assembly	48
4. Localization	48
C. Actin	49
1. Preparation	50
2. Physico-Chemical Properties	50
3. Assembly	52
4. Localization	53
III. Proteins and Other Factors Involved in Contractile Regulation	54
A. Calcium Sensitivity of Contractile Processes	55
1. Tropomyosin	57
2. Troponin	58
B. Actinins and Actin-Binding Protein	58
C. Allosteric Properties of Platelet Myosin	59
D. Role of ADP	59
E. Phosphorylation	60
F. Cofactors	60
IV. Is Platelet Activation a Corollary of Contractile Activity?	61
A. Excitation-Contraction Coupling in Platelets	61
B. Model for Platelet Contraction	63
1. First Stage of the Shape Change	64
2. Second Stage of Shape Change	66
3. Final Stage of Release	69
4. Mechanism of Clot Retraction	69
C. Energy Metabolism of the Contracting Platelet	70
V. Physiopathology and Concluding Remarks	73
Summary	75
Note Added in Proof	76
References	76

The Role of Cytoskeleton in Neuron Activity

Saul Puszkin and William Schook, New York, N.Y. 87

Introduction	87
Theoretical Background	88
Major Neuronal Cytoskeletal Components	89
A. Microfilaments	89
B. Neurofilaments	91
C. Microtubules	95
The Cytoskeleton in Specialized Neuronal Functions	97
A. Axonal Transport	97
B. Neurotransmitter Release	99
Pathology of the Neuronal Cytoskeleton	102
Summary	104
Acknowledgments	104
References	104

The Role of the Cytoskeleton in Pancreatic B-Cell Function

Willy J. Malaisse and Lelio Orci, Brussels 112

Introduction	112
Ultrastructural Organization of the B-Cell Cytoskeleton	113
Experimental Alteration of the B-Cell Microtubular System	116
Experimental Alteration of the B-Cell Microfilamentous System	118
B-Cell Cytoskeleton and the Dynamics of Insulin Release	120
Biochemical Aspects	122
Microtubules and the Biosynthetic Machinery of Insulin-Producing Cells	124
Motile Events and the Microtubular-Microfilamentous System	126
Cell Surface Organization and the Microtubular-Microfilamentous System	130
Physiopathological Implications	130
Glucagon Release and the Microtubular-Microfilamentous System	132
Conclusion	133
Summary	133
References	133

The Role of Cytoskeleton in Adreno-Medullary Secretion

P.H. Cooke and Alan M. Poisner, Farmington, Conn. 137

I. Introduction	137
II. Structural Organization of the Chromaffin Cell	139
III. Alterations in Cytoplasmic Organization	140
VI. Alterations in Catecholamine Secretion	142
V. Discussion	142
Summary	145
References	145

Distribution and Function of Cytoskeletal Proteins in Lung Cells
with Particular Reference to 'Contractile Interstitial Cells'*Y. Kapanci, P. Mo Costabella, P. Cerutti and A. Assimacopoulos, Geneva* 147

I. Introduction	147
II. 'Contractile Interstitial Cells'	148
Ultrastructural Studies	148
Immunofluorescence Studies	150
Quantitative Estimations of 'Contractile Interstitial Cells'	152
Location and Distribution of 'Contractile Interstitial Cells'	152
Reactions of 'Contractile Interstitial Cells'	156
Function of 'Contractile Interstitial Cells'	158
III. Distribution and Function of Cytoskeletal Proteins in Other Lung Cells	158
Bronchial Epithelium	158
Alveolar Epithelium	158

Capillary Endothelium	161
Pericytes	162
IV. Discussion	162
Summary	166
References	166

Leucocytic Movement and Contractile Protein

N. Senda, N. Shibata, H. Tamura and J. Yoshitake, Osaka 169

I. Introduction	169
II. The Organization of Leucocytes in Movement	170
A. Dynamic Patterns in the Movement of Neutrophils	170
1. Classification of the Motile Type	170
2. The Periodicity of the Movement	172
3. The Distance and Mean Time of One Period in each Motile Type	172
B. Tropic Reaction	173
C. The Tail	173
D. Protoplasmic Streaming of Neutrophils	175
III. The Coordinate Control of the Motile Form and Function by ATP	176
IV. The Contraction Wave	177
V. Contractile Protein from Leucocytes	179
A. Biochemical Properties	179
1. Actomyosin or Myosin B	179
2. Myosin	179
3. Actin	181
4. Interaction of Myosin and Actin	181
B. Intracellular Location of Actin in Neutrophils during the Movement	181
VI. Considerations on the Mechanism of Leucocytic Movement	183
Summary	184
References	184

The Role of Contractile Proteins in Wound Healing and Fibrocontractive Diseases

G. Gabbiani, Geneva 187

I. Introduction	187
II. The Normal Fibroblast	188
III. The Cultivated Fibroblasts	190
IV. The Fibroblast of Granulation Tissue (Myofibroblast)	191
A. Morphology	191
B. Pharmacology	192
C. Chemistry	192
D. Immunology	194
V. Epithelialization of a Wound	198
VI. Conclusions	202
Summary	203
References	203

The Cytoskeleton in Cultured Cells: Coordinate *in vitro* Regulation of Cell Growth and Shape

R.E. Pollack and L. Kopelovich, New York, N.Y. 207

Introduction	208
Viral Transformation	208
Serum Sensitivity	209
Density Sensitivity	210
Anchorage Sensitivity	210
Transition Probability and the Cell Cycle	211
Viral Antigens in SV40-Transformed Cells	211
Other Tumor Viruses	212
Anchorage and Tumorigenicity	213
Proteolysis and Anchorage Transformation	215
Cytoskeleton and Anchorage Transformation	215
A Syndrome of Changes in Oncogenic Transformation of Cultured Murine Cells	217
Cultured Cells from Individuals at Increased Risk of Cancer	217
ACR: Clinical Studies	217
ACR: Cell Culture Studies	218
ACR: Cytoskeleton of Skin Fibroblasts	219
Cultured Cells from Human Tumors	221
Cultures Cells from Vascular Endothelium	222
Summary	224
References	224

The Cytoskeleton in Cancer Cells in Animals and Humans

G. Gabbiani, Geneva 231

I. Introduction	231
II. Electron Microscopic Studies	233
III. Immunofluorescent Studies	237
IV. Conclusions	237
Summary	240
References	240

Contractile Proteins as Autoantigens

E.J. Holborow, London 244

Introduction	244
Smooth Muscle Autoantibodies	246
SMA in Human Disease	247
The Specificity of Human SMA	248
Relationship of SMA and Skeletal Muscle Autoantibodies	251
Rabbit Antisera against Contractile Proteins	252
What Makes Contractile Proteins Autoimmunogenic?	253
Summary	256
References	256

Subject Index	261
-------------------------	-----

The Cytoskeleton and Cell Movement: General Considerations

R.S. Adelstein, S.P. Scordilis¹ and J.A. Trotter²

Section on Molecular Cardiology, Cardiology Branch, National Heart, Lung, and Blood Institute, Bethesda, Md.

Contents

Introduction	2
Actin and Myosin: Historical Perspective	3
Actin	4
General Considerations	4
Sites of Interaction	4
Polymerization	4
Critical Concentration	6
Interactions with Non-Contractile Proteins	6
Sequence	7
Isoelectric Points	7
Actin Filaments	8
Decoration	10
Polarity	11
Fluorescent Actin Probes	11
Antibodies	12
Myosin	13
Introduction	13
Structural Studies	13
Filament Formation	16
ATPase Activity	18
General Considerations	18
Actin-Activated ATPase Activity	18
Ca ²⁺ Control of Actin-Activation of Myosin	20
Antibodies to Myosin	22

¹ Department of Biological Sciences, Clark Science Center, Smith College, Northampton Mass.

² Department of Anatomy, University of New Mexico School of Medicine, Albuquerque, N. Mex.