

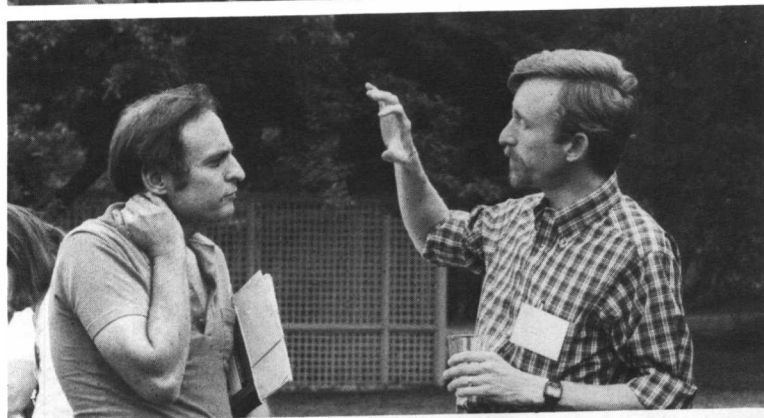
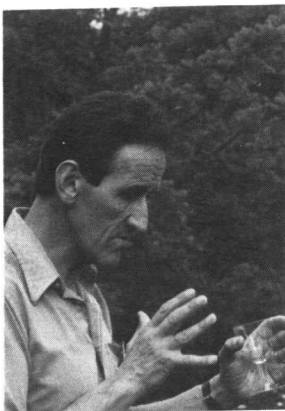
Symposium Participants

- ADELSTEIN, ROBERT, NHLBS, National Institutes of Health, Bethesda, Maryland
- ALBRECHT-BUEHLER, GUENTER, Cold Spring Harbor Laboratory, New York
- ALLEN, ROBERT D., Dept. of Biology, Dartmouth College, Hanover, New Hampshire
- AMATO, PHILIP, The Biological Laboratories, Harvard University, Cambridge, Massachusetts
- ASANO, AKIRA, Dept. of Protein Research, Osaka University, Japan
- AVILA DE GRADO, JESUS, Dept. of Molecular Biology, University of Madrid, Spain
- BADER, ARTRICE, NIGMS, National Institutes of Health, Bethesda, Maryland
- BADER, JOHN, NCI, National Institutes of Health, Bethesda, Maryland
- BAIER, ANDREW, Dept. of Biology, University of Oregon, Eugene
- BALCH, W. E., Dept. of Biochemistry, Stanford University, California
- BEN ZE'EV, AVRI, Dept. of Genetics, Weizmann Institute of Science, Rehovot, Israel
- BENNETT, VANN, Dept. of Anatomy, Johns Hopkins School of Medicine, Baltimore, Maryland
- BERNAL, SAM, Sidney Farber Cancer Institute, Harvard University, Boston, Massachusetts
- BIRCHMEIER, WALTER, Dept. of Biochemistry, Eidgenössische Technische Hochschule, Zürich, Switzerland
- BLACK, MARK M., Dept. of Anatomy, Temple University Medical School, Philadelphia, Pennsylvania
- BLOBEL, GUNTER, Laboratory of Cell Biology, Rockefeller University, New York, New York
- BLOODGOOD, ROBERT, Dept. of Anatomy, University of Virginia School of Medicine, Charlottesville
- BLOSE, STEPHEN, Cold Spring Harbor Laboratory, New York
- BORISY, GARY G., Dept. of Zoology, University of Wisconsin, Madison
- BRADY, SCOTT, Dept. of Anatomy, Case Western Reserve University, Cleveland, Ohio
- BRAELL, WILLIAM, Dept. of Biology, Massachusetts Institute of Technology, Cambridge
- BRANTON, DANIEL, The Biological Laboratories, Harvard University, Cambridge, Massachusetts
- BRENNER, SARI, Dept. of Cell Biology, Baylor College of Medicine, Houston, Texas
- BRETSCHER, ANTHONY, Dept. of Cell Biology, University of Texas Health Science Center, Dallas
- BRETSCHER, MARK, Laboratory for Molecular Biology, Medical Research Council, Cambridge, England
- BRIER, JONATHAN, The Biological Laboratories, Harvard University, Cambridge, Massachusetts
- BRINKLEY, BILL R., Dept. of Cell Biology, Baylor College of Medicine, Houston, Texas
- BROWN, MICHAEL, Dept. of Molecular Genetics, University of Texas Health Science Center, Dallas
- BURRIDGE, KEITH, Dept. of Cell Biology, University of North Carolina, Chapel Hill
- CAPUT, DANIEL, Dept. of Biophysics, University of California, San Francisco
- CARLEY, WILLIAM W., Dept. of Applied Engineering Physics, Cornell University, Ithaca, New York
- CELIS, JULIO E., Chemistry Institute, Aarhus University, Denmark
- CHAFIOULEAS, JAMES, Dept. of Cell Biology, Baylor College of Medicine, Houston, Texas
- CHALFIE, MARTIN, Laboratory of Molecular Biology, Medical Research Council, Cambridge, England
- CHEN, LAN BO, Sidney Farber Cancer Institute, Harvard University, Boston, Massachusetts
- CHEUNG, WAI YIU, Dept. of Biochemistry, St. Jude Children's Research Hospital, Memphis, Tennessee
- CHILD, FRANK, Dept. of Biology, Trinity College, Hartford, Connecticut
- CHOO, QUI-LIM, Dept. of Biochemistry, University of California, San Francisco
- CHOW, LOUISE, Cold Spring Harbor Laboratory, New York
- CLARKE, MARGARET, Dept. of Molecular Biology, Albert Einstein College of Medicine, Bronx, New York
- CLEGG, JAMES S., Dept. of Biology, University of Miami, Coral Gables, Florida
- CLEVELAND, DON, Dept. of Biochemistry, University of California, San Francisco
- COLMAN, DAVID, Dept. of Cell Biology, New York University Medical Center, New York
- CONDEELIS, JOHN, Dept. of Anatomy, Albert Einstein College of Medicine, Bronx, New York
- CRAIG, SUSAN, Dept. of Physiological Chemistry, Johns Hopkins University School of Medicine, Baltimore, Maryland
- CREUTZ, CARL, NIAMDD, National Institutes of Health, Bethesda, Maryland
- DALEY, ROGER, Dept. of Anatomical Science, State University of New York, Buffalo
- DAVIDSON, NORMAN, Dept. of Chemistry, California Institute of Technology, Pasadena
- DAVIS, JONATHAN, Dept. of Anatomy, Johns Hopkins School of Medicine, Baltimore, Maryland

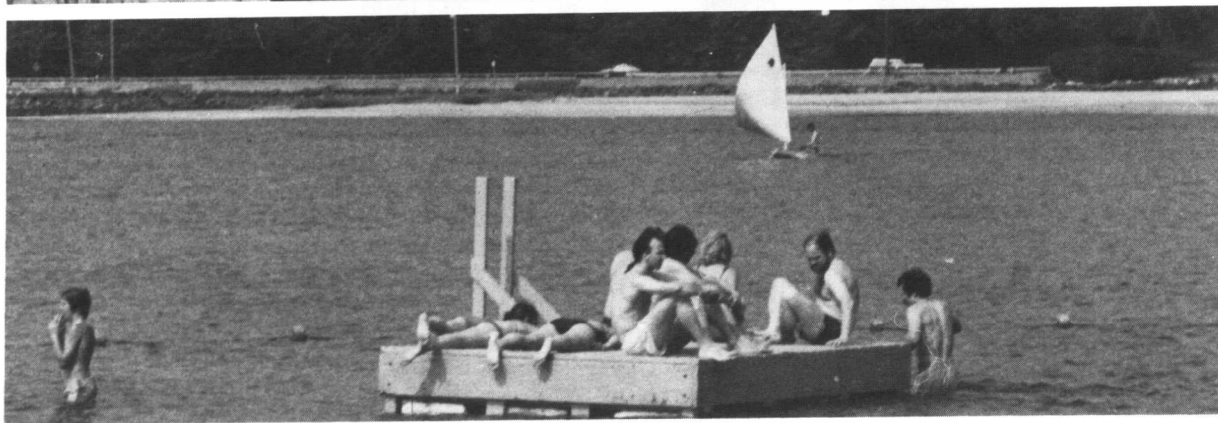
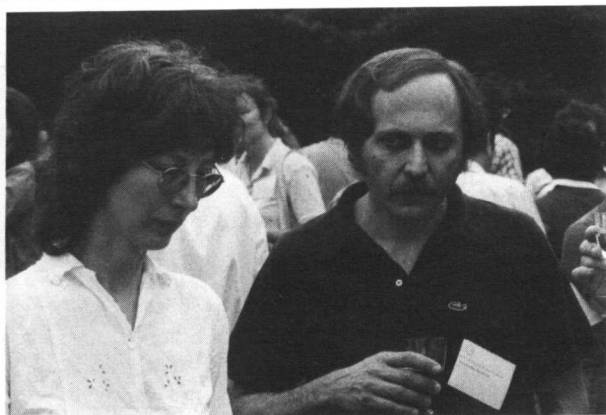
- DE BRABANDER, MARC, Dept. of Oncology, Janssen Pharmaceutical Research Laboratory, Beerse, Belgium
- DENNIS, MICHAEL, Dept. of Anatomy, University of North Carolina, Chapel Hill
- DRAKE, PETER, Dept. of Anatomy, Case Western Reserve University, Cleveland, Ohio
- DUERR, ANN, Dept. of Biology, Massachusetts Institute of Technology, Cambridge
- ECKERT, BARRY, Dept. of Anatomical Science, State University of New York, Buffalo
- ERIKSON, RAYMOND, Dept. of Pathology, University of Colorado Health Science Center, Denver
- FARMER, STEPHEN, Dept. of Biochemistry, Boston University, Massachusetts
- FECHHEIMER, MARCUS, The Biological Laboratories, Harvard University, Cambridge, Massachusetts
- FERAMISCO, JAMES, Cold Spring Harbor Laboratory, New York
- FOWLER, VELIA, NIADDK, National Institutes of Health, Bethesda, Maryland
- FRANKE, WERNER, Dept. of Membrane Biology, German Cancer Center, Heidelberg, Federal Republic of Germany
- FULTON, ALICE, Dept. of Biology, Massachusetts Institute of Technology, Cambridge
- GARRELS, JAMES, Cold Spring Harbor Laboratory, New York
- GEIGER, BENJAMIN, Dept. of Chemical Immunology, Weizmann Institute of Science, Rehovot, Israel
- GERACE, LARRY, Dept. of Cell Biology, Johns Hopkins School of Medicine, Baltimore, Maryland
- GLENNEY, JOHN R., JR., Max-Planck-Institut für Biophysische Chemie, Göttingen, Federal Republic of Germany
- GOLDBERG, DANIEL J., Dept. of Pharmacology, Columbia University College of Physicians & Surgeons, New York, New York
- GOLDMAN, ROBERT D., Mellon Institute, Carnegie-Mellon University, Pittsburg, Pennsylvania
- GOLDSTEIN, JOSEPH, Dept. of Molecular Genetics, University of Texas Health Science Center, Dallas
- GRANGER, BRUCE, Dept. of Biology, California Institute of Technology, Pasadena
- GREEN, HOWARD, Dept. of Biology, Massachusetts Institute of Technology, Cambridge
- GRIFFITH, LINDA M., Dept. of Biology, University of Colorado, Boulder
- HANSBURG, DANIEL, NIAID, National Institutes of Health, Bethesda, Maryland
- HARTWIG, JOHN, Massachusetts General Hospital, Boston
- HAYASHI, TERU, Papanicolaou Cancer Research Institute, Miami, Florida
- HEIPLE, JEANNE, The Biological Laboratories, Harvard University, Cambridge, Massachusetts
- HELENIUS, ARI, Dept. of Biochemistry, European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany
- HELFMAN, DAVID, Dept. of Pharmacology, Emory University, Atlanta, Georgia
- HERTZBERG, ELLIOT L., Dept. of Biology, Rockefeller University, New York, New York
- HIGASHI-FUJIME, SUGIE, Dept. of Molecular Biology, Nagoya University, Japan
- HOLTZER, HOWARD, Dept. of Anatomy, University of Pennsylvania, Philadelphia
- HOROWITZ, SAMUEL, Depts. of Molecular Pharmacology and Cell Biology, Michigan Cancer Foundation, Detroit
- HORWITZ, SUSAN, Albert Einstein College of Medicine, Bronx, New York
- HUNTER, TONY, Tumor Virology Laboratory, Salk Institute, San Diego, California
- HUXLEY, HUGH, Laboratory of Molecular Biology, Medical Research Council, Cambridge, England
- HYNES, RICHARD, Dept. of Biology, Massachusetts Institute of Technology, Cambridge
- ISENBERG, GERHARD, Max-Planck-Institute for Psychiatry, Munich, Federal Republic of Germany
- JOCKUSCH, BRIGITTE, European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany
- KAMIYA, NOBURO, Dept. of Cell Biology, National Institute for Basic Biology, Okazaki, Japan
- KAULFUS, PHYLLIS, Max-Planck-Institut für Biophysische Chemie, Göttingen, Federal Republic of Germany
- KEITH, CHARLES, Dept. of Pharmacology, New York University, New York
- KELLER, THOMAS, Dept. of Biology, Yale University, New Haven, Connecticut
- KENDRICK-JONES, JOHN, Laboratory of Molecular Biology, Medical Research Council, Cambridge, England
- KENNETT, SHERMIN, Dept. of Pathology, New York University, New York
- KIRSCHNER, MARC, Dept. of Biophysics, University of California Medical School, San Francisco
- KOBAYASHI, YOSHITERU, University of Tokyo, Japan
- LANE, E. BIRGITTE, Imperial Cancer Research Fund Laboratories, London, England
- LASEK, RAYMOND, Dept. of Anatomy, Case Western Reserve University, Cleveland, Ohio
- LAZARIDES, ELIAS, Dept. of Biology, California Institute of Technology, Pasadena
- LEVINE, MARK, NIADDK, National Institutes of Health, Bethesda, Maryland
- LIEM, RONALD, Dept. of Pharmacology, New York University, New York
- LIN, JAMES, Cold Spring Harbor Laboratory, New York
- LIN, SHIN, Dept. of Biophysics, Johns Hopkins University, Baltimore, Maryland
- LOCKWOOD, ARTHUR, Dept. of Cancer Research, University of North Carolina Medical School, Chapel Hill
- LOUVARD, DANIEL, European Molecular Biological Laboratory, Heidelberg, Federal Republic of Germany

- LUNA, ELIZABETH, The Biological Laboratories, Harvard University, Cambridge, Massachusetts
- MAIHLE, NITA, Dept. of Anatomy, Albert Einstein College of Medicine, Bronx, New York
- MANDELKOW, ECKHARD, Dept. of Biophysics, Max-Planck-Institut für Medizin, Heidelberg, Federal Republic of Germany
- MANDELKOW, EVA-MARIA, Dept. of Biophysics, Max-Planck-Institut für Medizin, Heidelberg, Federal Republic of Germany
- MANFREDI, JAMES, Depts. of Molecular Pharmacology and Cell Biology, Albert Einstein College of Medicine, Bronx, New York
- MATSUDAIRA, PAUL, Max-Planck-Institut für Biophysische Chemie, Göttingen, Federal Republic of Germany
- MATSUMURA, FUMIO, Cold Spring Harbor Laboratory, New York
- MATSUMURA-YAMASHIRO, SHIGEKO, Cold Spring Harbor Laboratory, New York
- McKEOWN, MICHAEL, Dept. of Biology, University of California, San Diego
- MEANS, ANTHONY, Dept. of Cell Biology, Baylor College of Medicine, Houston, Texas
- MIAKE, RYN, Dept. of Biochemistry, University of California, San Francisco
- MINDEN, JONATHAN, Dept. of Molecular Biology, Albert Einstein College of Medicine, Bronx, New York
- MITCHISON, TIMOTHY, Dept. of Biochemistry and Biophysics, University of California, San Francisco
- MOOSEKER, MARK, Dept. of Biology, Yale University, New Haven, Connecticut
- MORRIS, JAMES, Dept. of Anatomy, Case Western Reserve University, Cleveland, Ohio
- MORRIS, RONALD N., Dept. of Pharmacology, Rutgers Medical School, Piscataway, New Jersey
- MUELLER, SUSETTE, The Biological Laboratories, Harvard University, Cambridge, Massachusetts
- MULLET, JOHN, Dept. of Cell Biology, Rockefeller University, New York, New York
- NADAKAVUKAREN, KAREN, Sidney Farber Cancer Institute, Harvard University, Boston, Massachusetts
- NOVIKOFF, ALEX B., Dept. of Pathology, Albert Einstein College of Medicine, Bronx, New York
- NOVIKOFF, PHYLLIS, Albert Einstein College of Medicine, Bronx, New York
- OSBORN, MARY, Max-Planck-Institut für Biophysische Chemie, Göttingen, Federal Republic of Germany
- PALLAS, DAVID, Dept. of Biology, Massachusetts Institute of Technology, Cambridge
- PARNESS, JEROME, Dept. of Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, New York
- PAULIN, DENISE, Dept. of Molecular Biology, Institut Pasteur, Paris, France
- PEARL, MIRILEE L., Dept. of Biology, Yale University, New Haven, Connecticut
- PEARSE, B. M. F., Laboratory of Molecular Biology, Medical Research Council, Cambridge, England
- PENMAN, SHELDON, Dept. of Biology, Massachusetts Institute of Technology, Cambridge
- PEPPER, DANIEL, High Voltage Electron Microscopy Laboratory, University of Wisconsin, Madison
- PHILLIPS, M. JAMES, Dept. of Pathology, Hospital for Sick Children, Toronto, Canada
- POLLARD, HARVEY, NIAMDD, National Institutes of Health, Bethesda, Maryland
- POLLARD, THOMAS, Dept. of Cell Biology, Johns Hopkins University School of Medicine, Baltimore, Maryland
- PONSTINGL, HERWIG, Dept. of Cell and Tumor Biology, German Cancer Research Center, Heidelberg, Federal Republic of Germany
- PORTER, KEITH R., Dept. of Biology, University of Colorado, Boulder
- PRIVES, JOAV, Dept. of Anatomical Sciences, State University of New York, Stony Brook
- PUDNEY, JEFFREY A., Dept. of Anatomy, University of Massachusetts, Worcester
- RACKER, EFRAIM, Dept. of Molecular Biology, Cornell University, Ithaca, New York
- RAMAEKER, FRANS C. S., Dept. of Pathology, University of Nijmegen, The Netherlands
- REVEL, JEAN-PAUL, Dept. of Biology, California Institute of Technology, Pasadena
- ROBERTS, THOMAS, Dept. of Biological Sciences, Florida State University, Tallahassee
- ROHRSCHEIDER, LARRY, Fred Hutchinson Cancer Research Center, Seattle, Washington
- ROSENBAUM, JOEL L., Dept. of Biology, Yale University, New Haven, Connecticut
- ROSENBERG, SHARON, Dept. of Biochemistry, Downstate Medical Center, Brooklyn, New York
- ROTHMAN, JAMES E., Dept. of Biochemistry, Stanford University, California
- RUBENSTEIN, JOHN L. R., Dept. of Biochemistry, Stanford University, California
- SABATINI, DAVID, Dept. of Cell Biology, New York University School of Medicine, New York
- SALISBURY, JEFFREY, Dept. of Anatomy, Albert Einstein College of Medicine, Bronx, New York
- SANDOVAL, IGNACIA, California Institute of Technology, Pasadena
- SATIR, PETER, Dept. of Anatomy, Albert Einstein College of Medicine, Bronx, New York
- SCHLIWA, MANFRED, Dept. of Biology, University of Colorado, Boulder
- SCHLOSS, JEFFREY, Dept. of Biology, Yale University, New Haven, Connecticut
- SCHMID, ERIKA, Dept. of Tumor Biology, German Cancer Research Center, Heidelberg, Federal Republic of Germany
- SCOTT, JANET, NIADDK, National Institutes of Health, Bethesda, Maryland
- SHELANSKI, MICHAEL, Dept. of Pharmacology, New York University Medical Center, New York

- SHERMAN, JANE, Dept. of Cell Biology, New York University Medical Center, New York
- SHRIVER, KATHLEEN, Fred Hutchinson Cancer Research Center, Seattle, Washington
- SIEGEL, DON, The Biological Laboratories, Harvard University, Cambridge, Massachusetts
- SILVERSTEIN, SAMUEL, Dept. of Immunology, Rockefeller University, New York, New York
- SINGER, ROBERT, Dept. of Anatomy, University of Massachusetts, Worcester
- SINGER, JOHN S., Dept. of Biology, University of California, La Jolla
- SMALL, J. V., Austrian Academy of Sciences, Institute of Molecular Biology, Salzburg
- SNYDER, JUDITH, Dept. of Biological Sciences, University of Denver, Colorado
- SOLOMON, FRANK, Dept. of Biology, Massachusetts Institute of Technology, Cambridge
- SORBARA, LYNN, Albert Einstein College of Medicine, Bronx, New York
- SPUDICH, JAMES, Dept. of Structural Biology, Stanford University, California
- SPUDICH, ANNAMMA, Dept. of Structural Biology, Stanford University, California
- STEINERT, PETER, NCI, National Institutes of Health, Bethesda, Maryland
- STOLLMAN, YACOV, Liver Research Center, Albert Einstein College of Medicine, Bronx, New York
- STOSSEL, THOMAS, Dept. of Oncology, Massachusetts General Hospital, Boston
- STRACHER, ALFRED, Dept. of Biochemistry, Downstate Medical Center, Brooklyn, New York
- STROUS, GER J., Dept. of Cell Biology, University of Utrecht, The Netherlands
- SUMMERHAYES, IAN, Sidney Farber Cancer Institute, Harvard University, Boston, Massachusetts
- SUNG, SUN-SANG, Dept. of Cellular Physiology and Immunology, Rockefeller University, New York, New York
- SWANSON, JOEL A., The Biological Laboratories, Harvard University, Cambridge, Massachusetts
- TANASUGARN, LERSON, The Biological Laboratories, Harvard University, Cambridge, Massachusetts
- TAYLOR, D. LANSING, The Biological Laboratories, Harvard University, Cambridge, Massachusetts
- TELZER, BRUCE R., Dept. of Biology, Pomona College, Claremont, California
- THOENEN, HANS, Dept. of Neurochemistry, Max-Planck-Institute for Psychiatry, Munich, Federal Republic of Germany
- TILNEY, LEWIS, Dept. of Physiology, Washington University, St. Louis, Missouri
- TRAKTMAN, PAULA, Center for Cancer Research, Massachusetts Institute of Technology, Cambridge
- TSEN, SU DER, Sidney Farber Cancer Institute, Harvard University, Boston, Massachusetts
- TUCKER, ROBERT, Dept. of Cell Proliferation, Johns Hopkins Oncology Center, Baltimore, Maryland
- UNGEWICKELL, ERNST, The Biological Laboratories, Harvard University, Cambridge, Massachusetts
- VANDERKERCKHOVE, JOEL, Laboratory for Histology and Genetics, Ghent, Belgium
- WALLACE, ROBERT, Dept. of Biochemistry, St. Jude Children's Research Hospital, Memphis, Tennessee
- WANG, EUGENIA, Virology Laboratory, Rockefeller University, New York, New York
- WANG, KUAN, Dept. of Chemistry, University of Texas, Austin
- WANG, YU-LI, Sidney Farber Cancer Institute, Harvard University, Boston, Massachusetts
- WEATHERBEE, JAMES, Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts
- WEBB, WATT, Cornell University, Ithaca, New York
- WEBER, KLAUS, Max-Planck-Institute für Biophysische Chemie, Göttingen, Federal Republic of Germany
- WEBSTER, ROBERT, Dept. of Biochemistry, Duke University Medical Center, Durham, North Carolina
- WEEDS, ALAN G., Dept. of Structural Biology, Stanford University, California
- WEHLAND, JURGEN, NCI, National Institutes of Health, Bethesda, Maryland
- WEIHING, ROBERT, Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts
- WEINBERG, UZI, Dept. of Neurology, Albert Einstein College of Medicine, Bronx, New York
- WELCH, WILLIAM, Cold Spring Harbor Laboratory, New York
- WELLER, NANCY, Dept. of Biology, Massachusetts Institute of Technology, Cambridge
- WENSINK, PIETER, Rosenstiel Basic Medical Science Research Center, Brandeis University, Waltham, Massachusetts
- WICHE, GERHARD, Dept. of Biochemistry, University of Vienna, Austria
- WILLIAMS, ROBLEY C., JR., Dept. of Molecular Biology, Vanderbilt University, Nashville, Tennessee
- WILSON, LESLIE, Dept. of Biological Sciences, University of California, Santa Barbara
- WOJCIESZYN, JOHN, Dept. of Anatomy, University of North Carolina, Chapel Hill
- WOLFARTH-BOTTERMANN, K. E., Institute for Cytology, University of Bonn, Federal Republic of Germany
- WOLOSEWICK, JOHN, Dept. of Anatomy, University of Illinois Medical Center, Chicago
- YIN, HELEN LU, Dept. of Oncology, Massachusetts General Hospital, Boston
- ZANER, KENNETH, Dept. of Oncology, Massachusetts General Hospital, Boston
- ZIEVE, GARY, Dept. of Biology, Massachusetts Institute of Technology, Cambridge
- ZILBERSTEIN, ASHER, Dept. of Biology, Massachusetts Institute of Technology, Cambridge



*First row: B. R. Brinkley; S. Higashi-Fujime; H. Thoenen, K. E. Wolfarth-Bottermann
 Second row: K. R. Porter, W. Franke, G. Albrecht-Buehler; R. O. Hynes, J. Wolosewick
 Third row: L. G. Tilney, T. D. Pollard; E. Ungewickell
 Fourth row: Viewing 3-D slides in Vannevar Bush Lecture Hall*



First row: J. Lin, N. Davidson, A. Klar; W. Webb, A. Bretscher
Second row: J. S. Singer, G. G. Borisy; M. Hogg, R. D. Goldman
Third row: J. D. Watson, B. M. F. Pearse, J. V. Small, K. R. Porter; R. S. Adelstein, R. Erikson

Contents

Principles of Organization: Functional and Spatial Order in the Cytoplasm

Membrane Cytoskeletal Interactions in the Human Erythrocyte	<i>D. Branton</i>	1
Regulation of Intracellular Protein Traffic	<i>G. Blobel</i>	7
Organizing the Cytoplasm for Motility	<i>F. Solomon</i>	17

Principles of Organization: Water and the Cytoplasmic Architecture

Interrelationships between Water and Cell Metabolism in <i>Artemia</i> Cysts. IX. Evidence for Organization of Soluble Cytoplasmic Enzymes	<i>J. S. Clegg</i>	23
Measurements of the Diffusion of Macromolecules Injected into the Cytoplasm of Living Cells	<i>J. W. Wojcieszyn, R. A. Schlegel, and K. A. Jacobson</i>	39
Does Blebbing Reveal the Convulsive Flow of Liquid and Solutes through the Cytoplasmic Meshwork?	<i>G. Albrecht-Buehler</i>	45
Structural Organization of the Cytoplasm	<i>M. Schliwa, J. van Blerkom, and K. B. Pryzwansky</i>	51

Cytoplasmic and Axonal Flow

Active Movement of Bundles of Actin and Myosin Filaments from Muscle: A Simple Model for Cell Motility	<i>S. Higashi-Fujime</i>	69
Contraction-Relaxation Cycle of <i>Physarum</i> Cytoplasm: Concomitant Changes in Intraplasmoidal ATP and Ca^{++} Concentrations	<i>N. Kamiya, Y. Yoshimoto, and F. Matsumura</i>	77
Cytoplasmic Transport: Moving Ultrastructural Elements Common to Many Cell Types Revealed by Video-enhanced Microscopy	<i>R. D. Allen, J. L. Travis, J. H. Hayden, N. S. Allen, A. C. Breuer, and L. J. Lewis</i>	85
Fine Structure and Organization of Microfilaments in <i>Physarum</i> as Revealed by Chemofixation and Freeze-etching	<i>K. E. Wohlfarth-Bottermann and W. Stockem</i>	89
Cellular and Molecular Aspects of Amoeboid Movement	<i>D. L. Taylor, J. Heiple, Y.-L. Wang, E. J. Luna, L. Tanasugarn, J. Brier, J. Swanson, M. Fechheimer, P. Amato, M. Rockwell, and G. Daley</i>	101
The Axon: A Prototype for Studying Expressional Cytoplasm	<i>R. J. Lasek and S. T. Brady</i>	113
Communication between Target Organs and Nerve Cells: Retrograde Axonal Transport and Site of Action of Nerve Growth Factor	<i>M. E. Schwab, R. Heumann, and H. Thoenen</i>	125
Studies on the Mechanism of Fast Axoplasmic Transport Using Microinjection into Single Giant Neurons	<i>D. J. Goldberg, D. A. Harris, and J. H. Schwartz</i>	135
Probing Mitochondria in Living Cells with Rhodamine 123	<i>L. B. Chen, I. C. Summerhayes, L. V. Johnson, M. L. Walsh, S. D. Bernal, and T. J. Lampidis</i>	141

Elements of Organization: Microtubules

Expression of Flagellar Protein Genes during Flagellar Regeneration in <i>Chlamydomonas</i> C. D. Silflow, P. A. Lefebvre, T. W. McKeithan, J. A. Schloss, L. R. Keller, and J. L. Rosenbaum	157
Autoregulatory Control of the Expression of α - and β -Tubulins: Implications for Microtubule Assembly D. Cleveland and M. Kirschner	171
<i>Drosophila</i> α -Tubulin Genes and Their Transcription Patterns L. Kalfayan, J. Loewenberg, and P. C. Wensink	185
Amino Acid Sequence of α - and β -Tubulins from Pig Brain: Heterogeneity and Regional Similarity to Muscle Proteins H. Ponstingl, E. Krauhs, M. Little, T. Kempf, R. Hofer-Warbinek, and W. Ade	191
Microtubule Treadmills and Their Possible Cellular Functions L. Wilson and R. L. Margolis	199
Dynein-Microtubule Interactions: ATP-sensitive Dynein Binding and the Structural Polarity of Mitotic Microtubules L. T. Haimo and B. R. Telzer	207
Taxol: A New Probe for Studying the Structure and Function of Microtubules S. B. Horwitz, J. Parness, P. B. Schiff, and J. J. Manfredi	219
Microtubule Stability and Assembly in Living Cells: The Influence of Metabolic Inhibitors, Taxol and pH M. De Brabander, G. Geuens, R. Nuydens, R. Willebrords, and J. De Mey	227
Tubulin Assembly Sites and the Organization of Microtubule Arrays in Mammalian Cells S. L. Brenner and B. R. Brinkley	241
Microtubule Structure in <i>Caenorhabditis elegans</i> Neurons M. Chalfie	255
Asters, Poles, and Transport Properties within Spindlelike Microtubule Arrays A. S. Bajer and J. Molè-Bajer	263
Approaches to Potential Sliding Mechanisms of Cytoplasmic Microtubules P. Satir	285

Elements of Organization: Intermediate Filaments

Differentiated Structural Components of the Keratinocyte H. Green, E. Fuchs, and F. Watt	293
Immunolabeling Studies of Cytoskeletal Associations in Cultured Cells S. J. Singer, E. H. Ball, B. Geiger, and W.-T. Chen	303
Intermediate-size Filaments: Changes in Synthesis and Distribution in Cells of the Myogenic and Neurogenic Lineages H. Holtzer, G. S. Bennett, S. J. Tapscott, J. M. Croop, and Y. Toyama	317
Use of Antibodies to Intermediate Filaments in the Characterization of Human Tumors F. C. S. Ramaekers, J. J. G. Puts, A. Kant, O. Moesker, P. H. K. Jap, and G. P. Vooijs	331
Chemistry and Biology of Neuronal and Glial Intermediate Filaments R. K. H. Liem, C. H. Keith, J. F. Leterrier, E. Trenkner, and M. L. Shelanski	341
Desmin- and Vimentin-containing Filaments and Their Role in the Assembly of the Z Disk in Muscle Cells E. Lazarides, B. L. Granger, D. L. Gard, C. M. O'Connor, J. Breckler, M. Price, and S. I. Danto	351
Modification of Vimentin Polypeptides during Mitosis R. Bravo, S. J. Fey, P. Mose Larsen, and J. E. Celis	379
Epithelial Tonofilaments: Investigating Their Form and Function Using Monoclonal Antibodies E. B. Lane and M. W. Klymkowsky	387
In Vivo Disruption of the Cytokeratin Cytoskeleton in Cultured Epithelial Cells by Microinjection of Antikeratin: Evidence for the Presence of an Intermediate-filament-organizing Center B. S. Eckert, R. A. Daley, and L. M. Parysek	403
Intermediate Filaments M. Osborn, N. Geisler, G. Shaw, G. Sharp, and K. Weber	413
Differentiation-related Patterns of Expression of Proteins of Intermediate-size Filaments in Tissues and Cultured Cells W. W. Franke, E. Schmid, D. L. Schiller, S. Winter, E. D. Jarasch, R. Moll, H. Denk, B. W. Jackson, and K. Illmensee	431
Structure of Vimentin 10-nm Filaments Probed with a Monoclonal Antibody That Recognizes a Common Antigenic Determinant on Vimentin and Tropomyosin S. H. Blose, F. Matsumura, and J. J.-C. Lin	455

Heterogeneity of Intermediate Filaments Assembled In Vitro <i>P. Steinert, W. Idler, M. Aynardi-Whitman, R. Zackroff, and R. D. Goldman</i>	465
Plectin: A High-molecular-weight Cytoskeletal Polypeptide Component That Copurifies with Intermediate Filaments of the Vimentin Type <i>C. Wiche, H. Herrmann, F. Leichtfried, and R. Pytela</i>	475
Formation of an ATP-dependent Microtubule-Neurofilament Complex In Vitro <i>M. S. Runge and R. C. Williams, Jr.</i>	483

Elements of Organization: Microfilaments

Actin Multigene Family of <i>Dictyostelium</i> <i>M. McKeown and R. A. Firtel</i>	495
Approaches for Isolating Genes Encoding the Cytoskeletal Proteins and for Reintroducing the Genes into Cultured Cells <i>S. H. Hughes, J. Sorge, G. P. Thomas, J. Feramisco, L. Chow, and J. Garrels</i>	507
Actin Structure, Polymerization, and Gelation <i>T. D. Pollard, U. Aebi, J. A. Cooper, W. E. Fowler, and P. Tseng</i>	513
How Actin Filaments Pack into Bundles <i>D. J. DeRosier and L. G. Tilney</i>	525
Calcium-modulated Multifunctional Proteins Regulating F-Actin Organization <i>K. Weber and J. R. Glenney, Jr.</i>	541
Control of Assembly of <i>Dictyostelium</i> Myosin and Actin Filaments <i>J. A. Spudich, E. R. Kuczmarski, J. D. Pardee, P. A. Simpson, K. Yamamoto, and L. Stryer</i>	553
Function of Cytoplasmic Flow in Photosensory Transduction and Phase Regulation of Contractile Activities in <i>Physarum</i> <i>K. E. Wohlfarth-Bottermann and I. Block</i>	563
Actin Gelation and the Structure of Cortical Cytoplasm <i>T. P. Stossel, J. H. Hartwig, H. L. Yin, K. S. Zaner, and O. I. Stendahl</i>	569
Actinogelin: A Ca^{++} -sensitive Regulatory Protein of Microfilament Organization <i>N. Mimura and A. Asano</i>	579
α -Actinin and Vinculin from Nonmuscle Cells: Calcium-sensitive Interactions with Actin <i>K. Burridge and J. R. Feramisco</i>	587
Organization of Actin Meshworks in Cultured Cells: The Leading Edge <i>J. V. Small, G. Rinnerthaler, and H. Hinssen</i>	599
Vinculin and α -Actinin: Interaction with Actin and Effect on Microfilament Network Formation <i>B. M. Jockusch and G. Isenberg</i>	613
Proteins and Complexes That Affect Actin-filament Assembly and Interactions <i>S. Lin, J. A. Wilkins, D. H. Cribbs, M. Grumet, and D. C. Lin</i>	625

Cell Surface: Surface Organization

Partial Sequence and Turnover of Rat Liver Gap Junction Protein <i>J.-P. Revel, B. J. Nicholson, and S. B. Yancey</i>	633
Liver Gap Junctions and Lens Fiber Junctions: Comparative Analysis and Calmodulin Interaction <i>E. L. Hertzberg and N. B. Gilula</i>	639
Immunoreactive Forms of Human Erythrocyte Ankyrin Are Localized in Mitotic Structures in Cultured Cells and Are Associated with Microtubules in Brain <i>V. Bennett and J. Davis</i>	647
Relationships between Microfilaments, Cell-substratum Adhesion, and Fibronectin <i>R. O. Hynes, A. T. Destree, and D. D. Wagner</i>	659
Involvement of Vinculin in Contact-induced Cytoskeletal Interactions <i>B. Geiger</i>	671
Flagellum as a Model System for Studying Dynamic Cell-surface Events <i>R. A. Bloodgood</i>	683
Directed Membrane Flow on the Pseudopods of <i>Caenorhabditis elegans</i> Spermatozoa <i>T. M. Roberts and S. Ward</i>	695

Cell Surface: Clathrin

Packing of Clathrin into Coats <i>B. M. F. Pearse and R. A. Crowther</i>	703
Surface Uptake by Fibroblasts and Its Consequences <i>M. S. Bretscher</i>	707
Recycling of Cell-surface Receptors: Observations from the LDL Receptor System <i>M. S. Brown, R. G. W. Anderson, S. K. Basu, and J. L. Goldstein</i>	713
Functional and Structural Studies on Clathrin Triskelions and Baskets <i>E. Unge- wickell, E. R. Unanue, and D. Branton</i>	723
Receptor-mediated Endocytosis by Clathrin-coated Vesicles: Evidence for a Dynamic Pathway <i>J. L. Salisbury, J. S. Condeelis, N. J. Maihle, and P. Satir</i>	733
Microinjection of Anticlatrin Antibodies into Cultured Fibroblasts: Clathrin-coated Structures in Receptor-mediated Endocytosis and in Exocytosis <i>J. Wehland, M. C. Willingham, M. G. Gallo, A. V. Rutherford, J. Rudick, R. B. Dickson, and I. Pastan</i>	743
Intracellular and Extracellular Components Involved in the Formation of Ventral Surfaces of Fibroblasts <i>W. Birchmeier, T. A. Libermann, B. A. Imhof, and T. E. Kreis</i>	755

Cell Surface: Synthesis, Exocytosis, Endocytosis

Mapping Structural Proteins of Cultured Cells by Monoclonal Antibodies <i>J. J.-C. Lin</i>	769
Synthesis and Assembly of the Vesicular Stomatitis Virus Glycoprotein <i>A. Zilber- stein, M. D. Snider, and H. F. Lodish</i>	785
The Golgi Apparatus, Coated Vesicles, and the Sorting Problem <i>J. E. Rothman, E. Fries, W. G. Dunphy, and L. J. Urbani</i>	797
Mechanisms for the Incorporation of Proteins into the Plasma Membrane <i>D. Sabatini, D. Colman, E. Sabban, J. Sherman, T. Morimoto, G. Kreibich, and M. Adesnik</i>	807
Calcium-dependent Regulation of Chromaffin Granule Movement, Membrane Contact, and Fusion during Exocytosis <i>H. B. Pollard, C. E. Creutz, V. Fowler, J. Scott, and C. J. Pazoles</i>	819
Are Lysosomes a Site of Enveloped-virus Penetration? <i>M. Marsh, K. Matlin, K. Simons, H. Reggio, J. White, J. Kartenbeck, and A. Helenius</i>	835

Cell Surface: Microvilli

Structure and Function of the Brush-border Cytoskeleton <i>P. T. Matsudaira and D. R. Burgess</i>	845
Regulation of Contractility, Cytoskeletal Structure, and Filament Assembly in the Brush Border of Intestinal Epithelial Cells <i>M. S. Mooseker, E. M. Bonder, B. G. Grimwade, C. L. Howe, T. C. S. Keller III, R. H. Wasserman, and K. A. Wharton</i>	855
Characterization and Ultrastructural Role of the Major Components of the Intestinal Microvillus Cytoskeleton <i>A. Bretscher</i>	871
The Cytoskeleton of Intestinal Microvilli Contains Two Polypeptides Immunologically Related to Proteins of Striated Muscle <i>E. Coudrier, H. Reggio, and D. Louvard</i>	881

Mechanisms of Organization: Calcium Regulation and Phosphorylation of Cytoplasmic Components

Multifunctional Role of Calmodulin in Biologic Processes <i>R. W. Wallace, E. A. Tallant, and W. Y. Cheung</i>	893
Regulation by and of Calmodulin in Mammalian Cells <i>A. R. Means and J. G. Chaf- ouleas</i>	903
Molecular Events in cAMP-mediated Reverse Transformation <i>A. H. Lockwood, D. D. Trivette, and M. Pendergast</i>	909
Regulation of Actin-Myosin Interaction by Reversible Phosphorylation of Myosin and Myosin Kinase <i>R. S. Adelstein, M. D. Pato, J. R. Sellers, P. de Lanerolle, and M. A. Conti</i>	921

Regulation of Myosin-filament Assembly by Light-chain Phosphorylation <i>J. Kendrick-Jones, P. Tooth, K. A. Taylor, and J. M. Scholey</i>	929
Cytoskeletal Targets for Viral Transforming Proteins with Tyrosine Protein Kinase Activity <i>B. M. Sefton, T. Hunter, E. A. Nigg, S. J. Singer, and G. Walter</i>	939
Mechanism of Transformation by Rous Sarcoma Virus: Events within Adhesion Plaques <i>L. Rohrschneider, M. Rosok, and K. Shriver</i>	953
 Nucleus and Cytoplasm	
Nuclear Lamina and the Structural Organization of the Nuclear Envelope <i>L. Gerace and G. Blobel</i>	967
Synthesis and Assembly of the Polypeptide Subunits of Photosystem I <i>J. E. Mullet, A. R. Grossman, and H.-H. Chua</i>	979
Molecular and Cellular Effects of Heat-shock and Related Treatments of Mammalian Tissue-culture Cells <i>G. P. Thomas, W. J. Welch, M. B. Mathews, and J. R. Feramisco</i>	985
Function of Cytoplasmic Fibers in Syncytia <i>E. Wang, D. S. Roos, M. H. Heggeness, and P. W. Choppin</i>	997
Cytoplasmic and Nuclear Architecture in Cells and Tissue: Form, Functions, and Mode of Assembly <i>S. Penman, A. Fulton, D. Capco, A. Ben Ze'ev, S. Witeltsburger, and C. F. Tse</i>	1013
 Summary Organization of the Cytoplasm <i>B. R. Brinkley</i>	1029
 Author Index	1041
 Subject Index	1043

Membrane Cytoskeletal Interactions in the Human Erythrocyte

D. BRANTON

Cell and Developmental Biology, The Biological Laboratories, Harvard University, Cambridge, Massachusetts 02138

When an erythrocyte membrane is extracted with a nonionic detergent such as Triton X-100, an interconnected reticulum or shell remains (Yu et al. 1973). This reticulum constitutes the membrane skeleton or erythrocyte cytoskeleton. Interest in the erythrocyte membrane and its associated proteins has been heightened by the realization that membrane skeletons exist in other cell types (Ben-Ze'ev et al. 1979; Luna et al. 1981; Mescher et al. 1981) and that the transcellular cytoskeletal structures found in all eukaryotic cells contain some of the same elements (actin, actin-binding proteins, and cytoskeletal anchorage sites) present in the erythrocyte cytoskeleton. The results of biochemical and electron microscopic investigations have now indicated the major molecular features of the erythrocyte cytoskeleton (Fig. 1). Although it is doubtful that these features per se can serve as a model for all membrane skeletons or cytoskeletons, many of the approaches and lessons that have emerged from studying the erythrocyte cytoskeleton will continue to serve as guidelines during the analysis of more complex cytoskeletal structures.

In this laboratory, strategies for analyzing the erythrocyte cytoskeleton have involved selective elution and purification of membrane components, assays of binding between eluted components and membrane vesicles, association assays in solution, and visualization of site-specific binding by electron microscopy. Here, I summarize how these strategies have served to develop our current understanding of the erythrocyte cytoskeleton (for details, see Branton et al. 1981), and I discuss why these strategies are limited to providing insight into the first-order interactions of individual molecules but few predictions about the next hierarchical levels of structure involving the overall organization of a cytoskeleton.

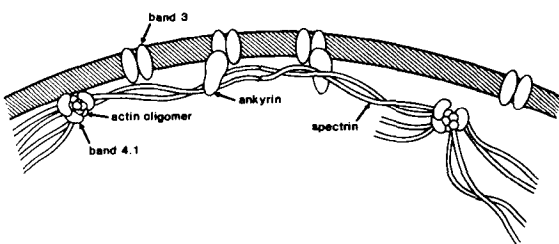


Figure 1. Drawing showing the general structure and linkages of the major polypeptides of the erythrocyte cytoskeleton (Branton et al. 1981).

Membrane Binding Studies

The first evidence for a specific association between the membrane and a cytoskeletal protein came from studies (Bennett and Branton 1977) in which purified spectrin, one of the major cytoskeletal components, was reassociated with spectrin-depleted membranes (Fig. 2). Once this assay, which quantified the reassociation of cytoskeletal and membrane components, was available, it became possible to identify the component that was binding the spectrin. The crucial step that led to this identification was the isolation from spectrin-depleted inside-out membranes of a chymotryptic fragment capable of competing with inside-out vesicles for the rebinding of spectrin (Bennett 1978). Using antibodies to this fragment, Bennett and Stenbuck (1979a) were able to show that it was derived from band 2.1, a polypeptide they named ankyrin.

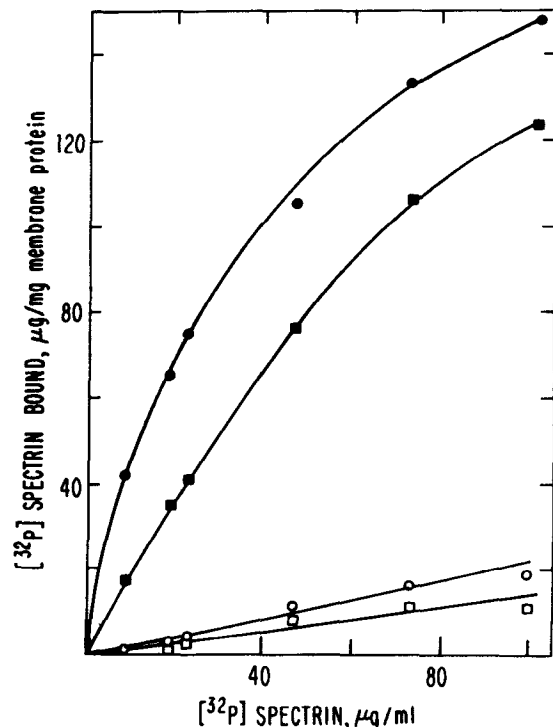


Figure 2. The binding of spectrin to erythrocyte membranes. The amount of [³²P]spectrin which binds to spectrin-depleted inside-out vesicles (●, ■) or right-side-out vesicles (○, □) is plotted as a function of spectrin concentration at pH 6.6 (●, ○) and pH 7.6 (■, □) (Bennett and Branton 1977).

Using a different approach, Luna et al. (1979) and Yu and Goodman (1979) showed by two-dimensional peptide mapping that the fragment was derived from band 2.1 and the sequence-related group of polypeptides designated as bands 2.2–2.6. Bands 2.2–2.6 are not simply breakdown products formed from band 2.1 during ghost production (Siegel et al. 1980), but the similarities between these polypeptides, band 2.1, and the fragment suggest that all are spectrin-binding proteins. They have been referred to as syndeins (Yu and Goodman 1979), but the name ankyrin has found widespread acceptance.

Having identified ankyrin as a crucial spectrin-binding site, it was important to determine how ankyrin itself, which could be eluted from the membrane with high-salt treatments, was anchored. The first clue that band 3, a major transmembrane protein and the erythrocyte anion transporter, was the binding site for ankyrin came from detergent solubilization studies that showed band 3 together with ankyrin and band 4.2 in detergent complexes (Bennett and Stenbuck 1979b). Subsequent steps to elucidate the role of band 3 as the membrane binding site for ankyrin paralleled the approaches used in studying the association of spectrin with membranes: The reassociation of purified ankyrin with ankyrin-depleted membranes was quantified, and competition assays using proteolytic fragments of putative binding sites were performed (Bennett and Stenbuck 1980; Hargreaves et al. 1980). Although these studies made it clear that band 3 is the integral protein which is the receptor that binds ankyrin to the membrane, the number of ankyrin molecules in an erythrocyte is far less than the number of band-3 polypeptides. The significance of this discrepancy remains to be clarified.

Although the association of spectrin with the membrane is well understood, the membrane association of actin is just beginning to be characterized. It has been shown that binding of preformed F-actin to spectrin- and actin-depleted inside-out vesicles is promoted by prior reconstitution of the vesicles with purified spectrin (Cohen and Foley 1980; Fowler et al. 1981). Binding of F-actin to spectrin-reconstituted vesicles is further enhanced by addition of purified band 4.1, another cytoskeletal polypeptide. Furthermore, spectrin exists as a heterodimer and as a tetramer, and spectrin tetramers are twice as effective as spectrin dimers (per mole) at stimulating actin binding (Cohen and Foley 1980). In vivo, spectrin tetramers far outnumber the heterodimers (Goodman and Weidner 1980; Liu and Palek 1980).

The binding of F-actin to membranes has also been measured by a low-shear viscometric technique (Fowler et al. 1981). The rationale for these measurements is that if membranes have binding sites for F-actin, they will be able to cross-link and increase the viscosity of an actin preparation. Indeed, spectrin- and actin-depleted inside-out vesicles reconstituted with purified spectrin induce large increases in the viscosity of actin, whereas spectrin alone or inside-out vesicles not re-

constituted with spectrin have no effect on the viscosity of actin (Fig. 3). These and other results using low-shear viscometry confirm that spectrin provides a crucial binding site that attaches actin filaments to the erythrocyte membrane.

Association Assays in Solution

Reassociation assays using selectively depleted membranes provided the first clues that identified the binding interactions of cytoskeletal proteins. Many of these interactions have now been confirmed and extended by solution studies using purified polypeptides. By using purified components in the absence of membranes, potentially complicating effects of multiple protein-protein or protein-lipid interactions can be avoided.

For example, solution studies with ankyrin and spectrin or with spectrin and band 4.1 (Tyler et al. 1980) allowed separate determinations of the dissociation constants and stoichiometric values for each of these pairs. Because band 4.1 remained associated with the spectrin-depleted inside-out vesicles used in the spectrin-membrane binding studies (Bennett and Branton 1977), its contribution to spectrin binding was hard to evaluate using membrane binding studies alone. By measuring the associations of the purified components, it became clear that ankyrin and spectrin and spectrin and band 4.1 interactions are distinct associations, each of which can occur in the absence of the other. Solution studies have also been useful in confirming

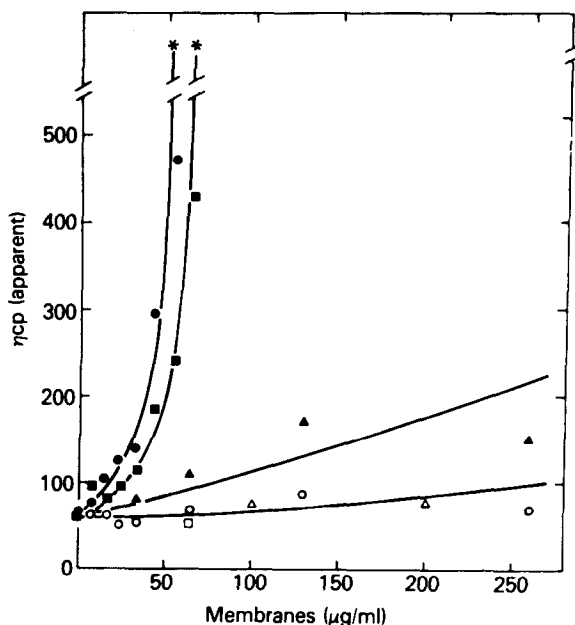


Figure 3. Viscosity changes as a measure of actin-membrane interaction. Changes of viscosity in a preparation of actin mixed with varying concentrations of spectrin-depleted inside-out vesicles reconstituted with no spectrin (○), 2 $\mu\text{g/ml}$ spectrin (▲), 5 $\mu\text{g/ml}$ spectrin (■), 30 $\mu\text{g/ml}$ spectrin (●), or 5 $\mu\text{g/ml}$ heat-treated spectrin (□). Controls using intact, right-side-out ghosts (△) are also shown (Fowler et al. 1981).

the association between spectrin and ankyrin (Tyler et al. 1979), between ankyrin and the cytoplasmic domain of band 3 (Bennett and Stenbuck 1980), between spectrin and actin (Brenner and Korn 1979; Ungewickell et al. 1979; Cohen and Korsgren 1981), and between spectrin, band 4.1, and actin in ternary complexes (Ungewickell et al. 1979; Cohen and Korsgren 1981).

Structural Analysis

Because a membrane skeleton or cytoskeleton is defined in terms of its structural role, it is important that the biochemical data showing protein-protein connections be supplemented by studies that provide information about the structure of these interconnecting elements. Direct electron microscopic examination of the interacting molecules was facilitated by examining the purified polypeptides that had been allowed to associate in solution and then mixed with glycerol. When these solutions were sprayed onto mica, dried in vacuo, and rotary-shadowed with heavy metals, the protein-protein interactions were not disturbed and the individual molecular shapes were displayed in a readily interpretable manner (Tyler and Branton 1980).

Electron micrographs of rotary-shadowed spectrin showed the heterodimer as two loosely intertwined, long, flexible strands joined at their ends (Shotton et al. 1979). The heterodimer was approximately 100 nm long, and the tetramer was 200 nm long. These simple electron microscopic observations indicated that the tetramer must be formed by the end-to-end association of two heterodimers. Similar electron micrographs of dried solutions in which two or more of the erythrocyte cytoskeletal proteins were reassociated made it possible to map the location of specific binding sites along the length of the spectrin molecule. Thus, ankyrin binding sites are located approximately 200 Å from the head end of the spectrin heterodimer that participates in tetramer formation (Fig. 4a), and band 4.1 and actin-binding sites are located at the tail end of the heterodimer farthest from the end that participates in tetramer formation (Fig. 4b,c). Long actin filaments, such as those shown in Figure 4c, do not exist in the erythrocyte where short actin oligomers probably predominate (Branton et al. 1981). Electron micrographs show that such oligomers also bind at a site located at the tail ends of the spectrin molecule (Cohen et al. 1980).

Membrane-Protein Interactions and the Organization of the Erythrocyte Cytoskeleton

The view of the erythrocyte cytoskeleton that emerges from these biochemical and structural studies is that of a reticulum composed of spectrin tetramers together with band 4.1 cross-linked by their association with short actin oligomers (Fig. 1). This reticulum is bound to the membrane by spectrin-ankyrin and ankyrin-band-3 interactions.

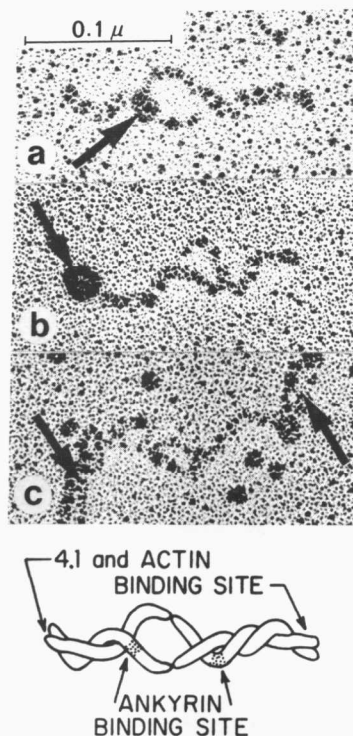


Figure 4. Electron micrographs that localize binding sites of spectrin for ankyrin (a), band 4.1 (b), and actin (c). The binding sites (←) are demonstrated by observing the position of the bound ankyrin molecule (a) or actin filament (c), or by observing the bound band-4.1 molecule that has been labeled with ferritin to make it clearly visible (b). (a,b,c) Adapted from Tyler et al. (1979), Tyler et al. (1980), and Cohen et al. (1980), respectively.

These studies of the protein interactions on the erythrocyte membrane have mapped out some of the major connections that link the proteins to each other. The role of other minor polypeptides, such as band 4.9, and the manner in which the known interactions are regulated remain to be established. But even before we answer these questions, it is useful to ask how the overall reticulum is organized and what the relationship of the individual molecular interactions is to the contours of the membrane and the shape of the cell. The substance of this question is made clear (Fig. 5) if one attempts to extrapolate from the known associations of an individual spectrin tetramer to the unknown arrangements that the many tetramers in one erythrocyte cytoskeleton can assume.

There are several reasons why it is impossible to make many predictions about the overall organization of the erythrocyte cytoskeleton from what we have learned about the first-order interactions of individual molecules: (1) The cytoskeletal reticulum is dominated by an elongated actin-binding protein, spectrin, that is extremely flexible and can assume a wide variety of configurations (Shotton et al. 1979). We do not know the extent to which the spectrin molecule is folded upon itself *in vivo*. (2) The cytoskeleton is anchored to the membrane at sites whose position is not fixed

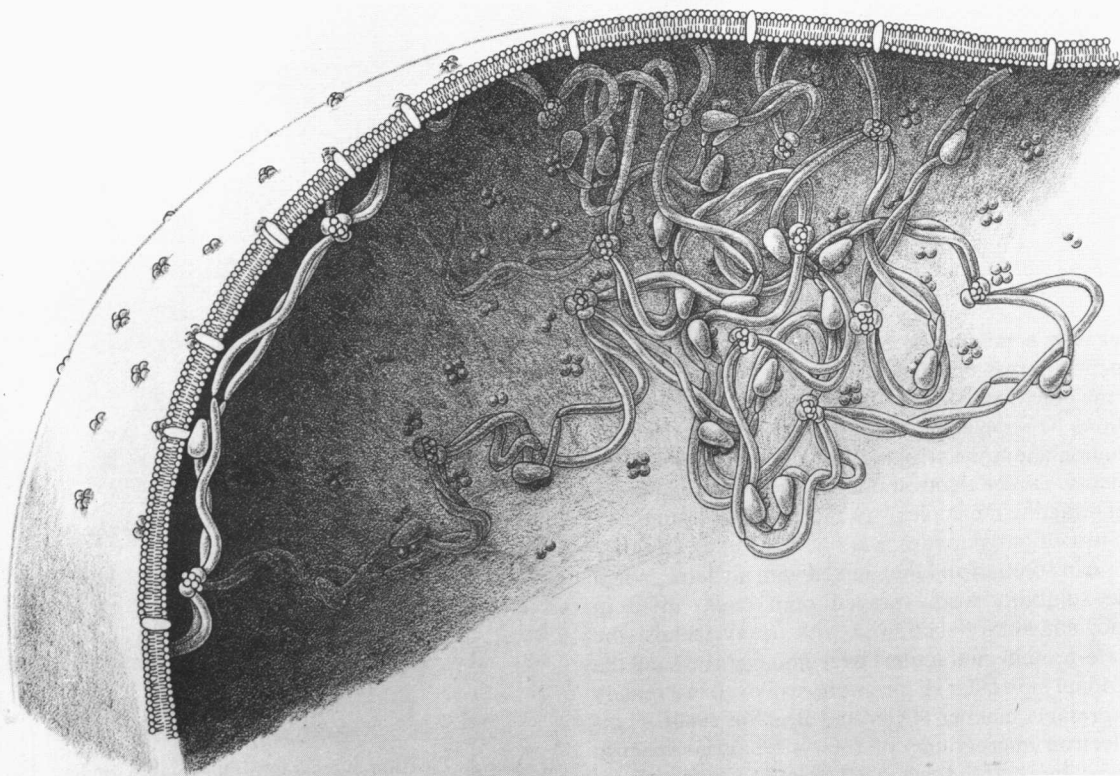


Figure 5. Associations of proteins in the erythrocyte cytoskeleton. An individual spectrin tetramer is shown at the left to illustrate the known first-order interactions discussed in the text. The overall structure of the cytoskeleton is not known. The meshwork at the right is drawn to indicate one of many ways in which the proteins could assemble to approximate the known density of molecules on the membrane (Cohen and Branton 1981).

(Elgsaeter and Branton 1974). It is therefore not known whether neighboring spectrin molecules cross over each other or lie curled next to each other. (3) Both our biochemical and our structural analyses measure relatively high-affinity interactions; but low-affinity interactions may be very important at the very high protein concentrations in the immediate neighborhood of the membrane. Low-affinity interactions could, for example, promote further oligomerization of cytoskeletal components (Morrow and Marchesi 1981). (4) Although it is believed widely that the shape of the red cell is primarily controlled by its cytoskeleton, there is also evidence that the cytoskeleton conforms to shape-determining factors inherent in the membrane bilayer (Steck and Lange 1981). Furthermore, many cellular components, some of which may be important in determining the overall assembly of the cytoskeleton, are lost during erythroid development.

Taken together, these facts make it unlikely that even a detailed description of the interactions which can be probed by binding studies and electron microscopy will make it possible to predict the next hierarchical level of structure involving the organization of the entire cytoskeleton and its role in determining cell shape.

CONCLUSION

The problems we face in the erythrocyte in extending from one level of organization to the next are encountered many times in probing the organization of a eukaryotic cell cytoplasm. It is essential that we understand the molecular interactions to discover the constraints which these interactions place on the next hierarchical level of structure. But it is also clear that all of the rules that dictate cytoplasmic organization will not be found in those relatively high-affinity interactions that are most easily measured and interpreted using current approaches. The challenge is to discover the approaches that will make it possible to understand the logic of more elaborate systems in terms of the rules of molecular interactions.

REFERENCES

- BENNETT, V. 1978. Purification of an active proteolytic fragment of the membrane attachment site for human erythrocyte spectrin. *J. Biol. Chem.* **253**: 2292.
- BENNETT, V. and D. BRANTON. 1977. Selective association of spectrin with the cytoplasmic surface of human erythrocyte plasma membranes. *J. Biol. Chem.* **252**: 2753.

- BENNETT, V. and P. J. STENBUCK. 1979a. Identification and partial purification of ankyrin, the high-affinity membrane attachment site for human erythrocyte spectrin. *J. Biol. Chem.* **254**: 2533.
- . 1979b. The membrane attachment protein for spectrin is associated with band 3 in human erythrocyte membranes. *Nature* **280**: 468.
- . 1980. Association between ankyrin and the cytoplasmic domain of band 3 isolated from the human erythrocyte membrane. *J. Biol. Chem.* **255**: 6424.
- BEN-ZE'EV, A., A. DUERR, F. SOLOMON, and S. PENMAN. 1979. The outer boundary of the cytoskeleton: A lamina derived from plasma membrane proteins. *Cell* **17**: 859.
- BRANTON, D., C. M. COHEN, and J. M. TYLER. 1981. Interaction of cytoskeletal proteins on the human erythrocyte membrane. *Cell* **24**: 24.
- BRENNER, S. L. and E. D. KORN. 1979. Spectrin-actin interaction. Phosphorylated and dephosphorylated spectrin tetramer cross-link F-actin. *J. Biol. Chem.* **254**: 8620.
- COHEN, C. M. and D. BRANTON. 1981. The normal and abnormal red cell cytoskeleton: A renewed search for molecular defects. *Trends Biochem. Sci.* **6**: 266.
- COHEN, C. M. and S. F. FOLEY. 1980. Spectrin dependent and independent association of F-actin with the erythrocyte membrane. *J. Cell Biol.* **86**: 694.
- COHEN, C. M. and C. KORSGREN. 1981. Band 4.1 causes spectrin-actin gels to become thixotropic. *Biochem. Biophys. Res. Commun.* **97**: 1429.
- COHEN, C. M., J. M. TYLER, and D. BRANTON. 1980. Spectrin-actin associations studied by electron microscopy of shadowed preparations. *Cell* **21**: 875.
- ELGSAETER, E. and D. BRANTON. 1974. Intramembrane particle aggregation in erythrocyte ghosts. I. The effects of protein removal. *J. Cell Biol.* **63**: 1018.
- FOWLER, V. M., E. J. LUNA, W. R. HARGREAVES, D. L. TAYLOR, and D. BRANTON. 1981. Spectrin promotes the association of F-actin with the cytoplasmic surface of the human erythrocyte membrane. *J. Cell Biol.* **88**: 388.
- GOODMAN, S. R. and S. A. WEIDNER. 1980. Binding of spectrin α - β tetramers to human erythrocyte membranes. *J. Biol. Chem.* **255**: 8082.
- HARGREAVES, W. R., K. N. GIEDD, A. VERKLEIJ, and D. BRANTON. 1980. Reassociation of ankyrin with band 3 in erythrocyte membranes and in lipid vesicles. *J. Biol. Chem.* **255**: 11965.
- LIU, S. C. and J. PALEK. 1980. Spectrin tetramer-dimer equilibrium and the stability of erythrocyte membrane cytoskeletons. *Nature* **285**: 586.
- LUNA, E. J., G. H. KIDD, and D. BRANTON. 1979. Identification by peptide analysis of the spectrin-binding protein in human erythrocytes. *J. Biol. Chem.* **254**: 2526.
- LUNA, E. J., V. M. FOWLER, J. SWANSON, D. BRANTON, and D. L. TAYLOR. 1981. A membrane cytoskeleton from *Dicystostelium discoideum*. I. Identification and partial characterization of an actin-binding activity. *J. Cell Biol.* **88**: 396.
- MESCHER, M. F., M. J. L. JOSE, and S. P. BALK. 1981. Actin-containing matrix associated with the plasma membrane of murine tumour and lymphoid cells. *Nature* **289**: 139.
- MORROW, J. S. and V. T. MARCHESI. 1981. Self-assembly of spectrin oligomers in vitro: A basis for a dynamic cytoskeleton. *J. Cell Biol.* **88**: 463.
- SHOTTON, D. M., B. BURKE, and D. BRANTON. 1979. Molecular structure of human erythrocyte spectrin: Biophysical and electron microscopic studies. *J. Mol. Biol.* **131**: 303.
- SIEGEL, D. L., S. R. GOODMAN, and D. BRANTON. 1980. The effect of endogenous proteases on the spectrin-binding proteins of human erythrocytes. *Biochim. Biophys. Acta* **598**: 517.
- STECK, T. L. and Y. LANGE. 1981. Determinants of the contour of the isolated red cell membrane. In *Perspectives in differentiation and hypertrophy* (ed. W. A. Anderson). Elsevier, New York. (In press.)
- TYLER, J. and D. BRANTON. 1980. Rotary shadowing of extended molecules dried from glycerol. *J. Ultrastruct. Res.* **71**: 95.
- TYLER, J., W. HARGREAVES, and D. BRANTON. 1979. Purification of two spectrin-binding proteins: Biochemical and electron microscopic evidence for site-specific reassociation between spectrin and bands 2.1 and 4.1. *Proc. Natl. Acad. Sci.* **76**: 5192.
- TYLER, J., B. N. REINHARDT, and D. BRANTON. 1980. Associations of erythrocyte membrane proteins: Binding of purified bands 2.1 and 4.1 to spectrin. *J. Biol. Chem.* **255**: 7034.
- UNGEWICKELL, E., P. M. BENNETT, R. CALVERT, V. OHANIAN, and W. B. GRATZER. 1979. In vitro formation of a complex between cytoskeletal proteins of the human erythrocyte. *Nature* **280**: 811.
- YU, J. and S. R. GOODMAN. 1979. Syndeins: The spectrin-binding protein(s) of the human erythrocyte membrane. *Proc. Natl. Acad. Sci.* **76**: 2340.
- YU, J., D. A. FISCHMAN, and T. L. STECK. 1973. Selective solubilization of proteins and phospholipids from red blood cell membrane by nonionic detergents. *J. Supramol. Struct.* **1**: 233.