Methods in Enzymology
Volume 139

Cellular Regulators

Part A
Calcium- and Calmodulin-Binding Proteins

EDITED BY
Anthony R. Means
DEPARTMENT OF CELL BIOLOGY
BAYLOR COLLEGE OF MEDICINE
HOUSTON, TEXAS

P. Michael Conn
DEPARTMENT OF PHARMACOLOGY
UNIVERSITY OF IOWA
COLLEGE OF MEDICINE
IOWA CITY, IOWA

1987

ACADEMIC PRESS, INC.
Harcourt Brace Jovanovich, Publishers
Orlando San Diego New York Austin
Boston London Sydney Tokyo Toronto
# Table of Contents

CONTRIBUTORS TO VOLUME 139 .................................................. xi
PREFACE ................................................................................. xvii
VOLUMES IN SERIES ........................................................... xix

## Section I. Isolation and Characterization of Calcium-Binding Proteins

1. The Use of Melittin-Sepharose Chromatography for Gram-Preparative Purification of Calmodulin
   **Randall L. Kincaid** 3

2. Calcinedins: Purification and Production of Antibodies
   **J. K. Mathew, R. R. Scully, V. L. Smith, E. Bernicker, and J. R. Dedman** 19

3. Purification of Calelectrins
   **Thomas C. Südhof and Dennis K. Stone** 30

   **Navin C. Khanna, Masaaki Tokuda, and David Morton Waismann** 36

5. Purification and Characterization of Acanthamoeba Calcium-Binding Proteins
   **David C. Wylie and Thomas C. Vanaman** 50

6. Purification of Novel Calcium-Binding Proteins from Bovine Brain
   **Masaaki Tokuda, Navin C. Khanna, and David Morton Waismann** 68

7. Calmodulin-Dependent Protein Phosphatase: Isolation of Subunits and Reconstitution to Holoenzyme
   **Dennis L. Merat and Wai Yiu Cheung** 79

8. Isolation and Characterization of a Novel 21-kDa Ca²⁺-Binding Protein from Bovine Brain
   **John R. McDonald, Michael P. Walsh, William D. McCubbin, and Cyril M. Kay** 88

9. Isolation and Characterization of Calmodulin-Dependent Myosin Heavy Chain Kinase from Intestinal Brush Border
   **James P. Rieker, Helena Swanljung-Collins, Judith Montibeller, and Jimmy H. Collins** 105
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Authors</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.</td>
<td>Immunoassay and Immuno purification of Macrophage Calmodulin-Binding Protein</td>
<td>Seth J. Orlow and Ora M. Rosen</td>
<td>148</td>
</tr>
<tr>
<td>15.</td>
<td>Isolation and Spectroscopic Analyses of S-100 Proteins and Their Interactions with Metal Ions</td>
<td>Rajam S. Mani and Cyril M. Kay</td>
<td>168</td>
</tr>
<tr>
<td>16.</td>
<td>Affinity Labeling of the Nucleotide-Binding Site of Myosin Light Chain Kinases</td>
<td>Joel C. Colburn, Peter J. Kenenelly, Edwin G. Krebs, and James T. Stull</td>
<td>188</td>
</tr>
<tr>
<td>17.</td>
<td>Study of Calmodulin-Peptide Interactions by NMR Spectroscopy</td>
<td>Rachel E. Klevit</td>
<td>197</td>
</tr>
<tr>
<td>18.</td>
<td>Novel Ca²⁺-Binding Proteins in Tumor Cells</td>
<td>Claus W. Heizmann</td>
<td>207</td>
</tr>
</tbody>
</table>

**Section II. Molecular Cloning of Calcium-Binding Proteins, cDNAs, and Genes**

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Authors</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>20.</td>
<td>The Calmodulin Gene of Drosophila melanogaster</td>
<td>Kathy Beckingham, Kevin E. Doyle, and John F. Maune</td>
<td>230</td>
</tr>
<tr>
<td>21.</td>
<td>Isolation of the Yeast Calmodulin Gene Using Synthetic Oligonucleotide Probes</td>
<td>Trisha N. Davis and Jeremy Thorner</td>
<td>248</td>
</tr>
<tr>
<td>22.</td>
<td>Calmodulin from Trypanosoma brucei: Immunological Analysis and Genomic Organization</td>
<td>Larry Ruben and Curtis L. Patton</td>
<td>262</td>
</tr>
</tbody>
</table>
TABLE OF CONTENTS

23. Dissection of Tissues for Characterizing Nucleic Acids from Aplysia: Isolation of the Structural Gene Encoding Calmodulin  
   J. H. Schwartz and M. E. Swanson 277

24. The Use of Synthetic Oligodeoxyribonucleotides in the Examination of Calmodulin Gene and Protein Structure and Function  
   Daniel M. Roberts, Warren E. Zimmer, and D. Martin Watterson 290

25. Bacterial Expression Vectors for Calmodulin  
   John A. Putkey, Patricia V. Donnelly, and Anthony R. Means 303

   Martin W. Berchtold 317

27. The Cloning and the Codon and Amino Acid Sequence of the Quail Slow/Cardiac Troponin C cDNA  
   Peter C. Maisonpierre, Kenneth E. M. Hastings, Charles P. Emerson, Jr. 326

28. 9-kDa Vitamin D-Induced Intestinal Calcium-Binding Protein: Cloning and Characterization of a Specific cDNA  
   Monique Thomasset, Claude Desplan, and Christine Perret 337

29. Spec Proteins: Calcium-Binding Proteins in the Embryonic Ectoderm Cells of Sea Urchins  
   William H. Klein, Susan H. Hardin, and Bruce P. Brandhorst 354

30. Cloning and Evolution of Calcium-Dependent Protease, cDNA Cloning of a Novel Family of Calcium-Binding Proteins  
   Shigeo Ohno, Yasufumi Emori, Hindemitsu Sugihara, Shinobu Iimori, and Koich Suzuki 363

31. Cloning of the Gene for Protein S: A Development-Specific Ca2+-Binding Protein from Myxococcus xanthus  
   Sumiko Inouye and Masayori Inouye 380

Section III. Reagents and Methods for the Study of Calcium-Binding Proteins

32. Site-Directed Antibodies to Vertebrate and Plant Calmodulins  
   Linda J. Van Eldik and Thomas J. Lukas 393

33. Synthesis and Properties of CAPP1-Calmodulin  

34. Specific Chemical Modification as a Probe of Calmodulin Function  
   Dennis Mann and Thomas C. Vanaman 417

35. Use of the 125I-Labeled Protein Gel Overlay Technique to Study Calmodulin-Binding Proteins  
   Gayle R. Slaughter and Anthony R. Means 433
TABLE OF CONTENTS

36. Interaction of Calmodulin and Other Calcium-Modulated Proteins with Gap Junctions
   Elliot L. Hertzberg and Linda J. Van Eldik

37. Recognition and Characterization of Calmodulin-Binding Sequences in Peptides and Proteins
   Susan Erickson-Vitanen and William F. DeGrado

38. Immunolocalization of Calmodulin in Plants
   Chin-Taung Lin

39. Immunoperoxidase Staining of S-100 and Calmodulin in Tissue Sections and Cultured Cells
   Mamoru Sano, Akiko Seto-Ohshima, Satoko Kitajima, and Kanefusa Kato

40. Production and Characterization of a Monoclonal Antibody Cross-Reactive with Calmodulin, Calmodulin-Dependent Phosphodiesterase, and Protein Phosphatase
   Martin A. Winkler, John R. Zysk, and Wai Yiu Cheung

41. Monoclonal Antibodies as Specific Probes for the Study of CaM-Regulatory System
   Jerry H. Wang, Takashi Suzuki, Marilyn Mooibroek, Anne-Marie Adachi, Rajendra K. Sharma, and Y.-H. Peter Lam

42. Rat Calbindin D_{28k}: Purification, Quantitation, Immunocytochemical Localization, and Comparative Aspects
   Sylvia Christakos, William B. Rhoden, and Susan C. Feldman

43. Immunolocalization of Parvalbumin
   Claus W. Heizmann and Marco R. Celia

44. Transmembrane Ca^{2+} Signaling and a New Class of Inhibitors
   Hiroshi Hidaka and Toshio Tanaka

Section IV. Regulation of Calcium and Calcium-Binding Proteins

45. Determination of the Three-Dimensional Structure of Vitamin D-Dependent Calcium-Binding Protein from Bovine Intestine
   D. M. E. Szabonyi and K. Moffat

46. Molecular Structure of Troponin C and its Implications for the Ca^{2+} Triggering of Muscle Contraction
   Osnat Herzberg, John Moul, and Michael N. G. James

47. X-Ray Diffraction Studies of Calmodulin
   Y. Sudhakar Babu, Charles E. Bugg, and William J. Cook

48. Methods for Analyzing Bovine Papilloma Virus-Based Calmodulin Expression Vectors
   Colin D. Rasmussen, Rosalia C. M. Simmen, Elizabeth A. MacDougall, and Anthony R. Means
<table>
<thead>
<tr>
<th>Page</th>
<th>Title</th>
<th>Authors</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>49</td>
<td>Ca(^{2+})/Calmodulin Regulation of Prolactin Gene Expression</td>
<td>BRUCE A. WHITE and CARTER BANCROFT</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>Calmodulin as an Activator and a Substrate of Methyltransferase Enzymes</td>
<td>FRANK L. SIEGEL, LYNSA S. WRIGHT, and PAUL M. ROWE</td>
<td></td>
</tr>
<tr>
<td>51</td>
<td>Fluorometric and Flow Cytometric Analysis of the Cyclosporine–Calmodulin Interaction in T Lymphocytes</td>
<td>PAUL M. COLOMBANI and ALLAN D. HESS</td>
<td></td>
</tr>
<tr>
<td>52</td>
<td>Role of Autophosphorylation in Regulating Calmodulin-Dependent Protein Kinases</td>
<td>PAUL T. KELLY and SHIRISH SHENOLIKAR</td>
<td></td>
</tr>
<tr>
<td>53</td>
<td>DNA Repair in Human Cells: Methods for the Determination of Calmodulin Involvement</td>
<td>PAUL A. CHARP</td>
<td></td>
</tr>
<tr>
<td>54</td>
<td>Calmodulin-Stimulated Estradiol Receptor–Tyrosine Kinase</td>
<td>FERDINANDO AURICCHIO, ANTIMO MIGLIACCIO, GABRIELLA CASTORIA, ANDREA ROTONDI, and MARINA DI DOMENICO</td>
<td></td>
</tr>
<tr>
<td>55</td>
<td>Interaction of Calmodulin with Phosphofructokinase: Binding Studies and Evaluation of Enzymatic and Physicochemical Changes</td>
<td>GEORGE W. MAYR</td>
<td></td>
</tr>
<tr>
<td>56</td>
<td>Regulation of Parvalbumin Concentration in Mammalian Muscle</td>
<td>EKKEHARD LEBERER, GARY A. KLUG, UDO SEEDORF, and DIRK PETTE</td>
<td></td>
</tr>
<tr>
<td>57</td>
<td>Reconstitution of Calmodulin-Sensitive Adenylate Cyclase from Bovine Brain with Phospholipids, Calmodulin, and (\beta)-Adrenergic Receptors</td>
<td>GARY B. ROSENBerg, ARDA-E-VIRAF M., MINOCHERHOMJEE, and DANIEL R. STORM</td>
<td></td>
</tr>
<tr>
<td>58</td>
<td>Purification, Reconstitution, and Molecular Characterization of the Ca(^{2+}) Pump of Plasma Membranes</td>
<td>V. NIGGIL, M. ZURINI, and E. CARAFOLI</td>
<td></td>
</tr>
<tr>
<td>59</td>
<td>Ca(^{2+}) Regulation of Sperm Axonemal Motility</td>
<td>JOSEPH S. TASH and ANTHONY R. MEANS</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>The Use of Microinjection and Video Microscopy for the Study of Calmodulin and Calcium in Living Cells</td>
<td>MICHAEL SHELANSKI and RAJIV RATAN</td>
<td></td>
</tr>
<tr>
<td>61</td>
<td>Calmodulin and Cold-Label Microtubules</td>
<td>YOUNG C. LEE and J. WOLFF</td>
<td></td>
</tr>
<tr>
<td>62</td>
<td>Calmodulin and Actin Polymerization</td>
<td>ROBERT W. WAlLACE and GARY A. PIAZZA</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>AUTHOR INDEX</strong></td>
<td><strong>SUBJECT INDEX</strong></td>
</tr>
</tbody>
</table>