

Cell Components

Edited by

H.F. Linskens and J.F. Jackson

Contributors

J.M. Anderson B. Andersson G.A. Berkowitz K. Cline
M. Gibbs R. Goldberg T. Hirokawa A.H.C. Huang
J.F. Jackson R.L. Jones B.A. Larkins Ch. Larsson
J.M. Miernyk G. Nagahashi C. Paech D.D. Sabnis
H. Takeda S.S. Thayer G. Wagner

With 96 Figures



Springer-Verlag
Berlin Heidelberg New York Tokyo

Contents

Cell-Wall-Isolation, General Growth Aspects

R. GOLDBERG (With 8 Figures)

1	Introduction	1
2	Isolation Procedures	1
2.1	Cell Breakage	2
2.2	Cell-Wall Recovery	2
2.3	Removal of Contaminants	2
3	Composition and Ultrastructure of Plant Cell Walls	6
3.1	Chemical Composition of Plant Cell Walls	6
3.1.1	Standard Extraction Procedures	6
3.1.2	Analysis of Polysaccharide Fractions	6
3.1.2.1	Chemical Methods	6
3.1.2.2	Physical Methods	8
3.2	Supramolecular Organization of Plant Cell Walls	8
3.2.1	Morphological Observations	8
3.2.2	Selective Staining of Polysaccharides	9
3.2.2.1	Visualization of Esterified Carboxyl Groups	9
3.2.2.2	Detection of Acidic Functions	9
3.2.2.3	Periodic Oxidation of Glycol Groups	9
3.2.3	Visualization of Lignin	12
3.2.4	Identification of Wall Components by Means of Affinity Methods	12
3.2.5	Detection and Estimation of Cations	12
3.2.6	Ultracryotomy	12
4	Properties of Plant Cell Walls	13
4.1	Exchange Properties of Plant Cell Walls	13
4.2	Enzymatic Properties	14
4.2.1	Cytochemical Investigations	14
4.2.1.1	Cell-Wall Phosphatase Activities	14
4.2.1.2	Cell-Wall Peroxidase Activities	14
4.2.2	Biochemical Investigations	16
4.2.2.1	Properties of Immobilized and Solubilized Cell-Wall Enzymes	16
4.2.2.2	Biological Functions	17
4.3	Mechanical Properties	18

5 Growth Aspects	18
5.1 Cell-Wall Loosening	19
5.1.1 Wall-Loosening-Inducing Agents	20
5.1.2 Nature of the Broken Bonds	20
5.2 Deposition of Wall Material	21
5.3 Growth Direction	22
References	22

Cell-Wall Chemistry, Structure and Components

H. TAKEDA and T. HIROKAWA (With 16 Figures)

1 Introduction	31
2 Histochemical Analysis of Cell Walls	32
2.1 Specific Stainings	33
2.2 Staining with Fluorescent Brightener	33
2.3 Anisotropy Test	33
2.4 Selective Dissolution	33
2.4.1 Alkali Treatment	33
2.4.2 Cuprammonium Solution (Schweitzer's Reagent) Treatment	34
2.4.3 Enzymatic Digestion	34
3 Quantitative Analysis of Cell Walls	36
3.1 Plant Materials	36
3.1.1 Pure Culture	37
3.1.2 Synchronous Culture	38
3.1.3 Harvesting of Cells	38
3.2 Measurement of Cell Growth	38
3.3 Preparation and Fractionation of Cell Walls	39
3.3.1 Disruption of Cells	39
3.3.2 Separation and Purification of Cell Walls	39
3.3.3 Fractionation of Cell Walls	40
3.4 Quantitative Analysis of Whole Cell Walls	41
3.4.1 Gravimetry	41
3.4.2 Turbidimetry	41
3.4.3 Colorimetry	41
4 Qualitative Analysis of Cell-Wall Materials	45
4.1 Acid Hydrolysis	45
4.2 Enzymatic Hydrolysis	46
5 Chromatographic Analysis of Cell-Wall Constituents	46
5.1 Thin-Layer Chromatography	47
5.1.1 Neutral Sugars and Uronic Acids	47
5.1.2 Amino Acids and Amino Sugars	47
5.1.3 Thin-Layer Chromatographic Analyses of the Constituents of Chlorella Cell Walls	48
5.2 Liquid Chromatography	48
5.2.1 Amino Acids and Amino Sugars	48
5.2.2 Neutral Sugars	49
References	51

Protoplasts – for Compartmentation Studies

S. S. THAYER (With 2 Figures)

1	Introduction	54
2	Advantages of the Use of Protoplasts for Compartmentation Studies	54
3	Protoplast Isolation and Its Effect on Cellular Metabolism	55
3.1	Isolation Procedures	55
3.2	Effect of Isolation pH	56
3.3	Effect of Plasmolysis	56
3.4	Effect of Enzyme Contaminants	57
4	Protoplast Lysis	58
5	Protoplast Fractionation	58
5.1	Density Gradient Fractionation	58
5.2	Rapid Fractionation Procedures	60
6	Methods to Relate Protoplast Activity to That of Intact Tissue	62
7	Concluding Remarks	63
	References	63

The Marker Concept in Cell Fractionation

G. NAGAHASHI (With 3 Figures)

1	Introduction	66
2	The Marker Concept	66
2.1	Basic Concepts	66
2.2	Types of Marker	67
2.2.1	Morphological	67
2.2.2	Cytochemical	68
2.2.3	Biochemical	68
3	Preservation of Marker Enzyme Activity During Cell Disruption	69
3.1	Choice of Material	69
3.2	Homogenization Procedure	69
3.3	Use of Additives in the Homogenization Medium	70
3.4	Gel Filtration to Remove Soluble Hydrolytic Activity	72
4	Methods Used to Separate Markers	72
4.1	General Approaches to Cell Fractionation	72
4.2	Differential Centrifugation	73
4.2.1	Preparative vs. Analytical Cell Fractionation	73
4.2.2	Need for Quantitation	74
4.2.3	Problems with Complete Quantitation and Interpretation of Data	74
4.3	Linear Density Gradient Centrifugation	75
4.3.1	Density Gradient Material	75
4.3.2	Pelleted vs. Unpelleted Overlays	76
4.3.3	Soluble Enzyme Contamination in Gradients	76
4.3.4	Equilibrium Density Centrifugation (Isopycnic Conditions)	78
4.3.5	Other Factors Which Influence Marker Enzyme Profiles Across a Gradient	80
4.3.6	Need for Quantitation and Lack of Negative Marker Activity	81

5 Concluding Remarks	81
References	82

Plasma Membranes

CH. LARSSON (With 10 Figures)

1 Introduction	85
2 Theory of Phase Partition	86
2.1 The Phase System	86
2.2 Partition of Membrane Particles	87
2.3 Effects of Polymer Concentrations	87
2.4 Effects of Salts	88
2.5 Multistep Procedures	89
3 Experimentals	91
3.1 Chemicals	91
3.2 Preparation Procedure	92
4 Purity of the Preparations	94
4.1 Specific Staining	94
4.2 K^+ -Stimulated, Mg^{2+} -Dependent ATPase	94
4.3 Glucan Synthetase II	97
4.4 Light-Reducible b-Cytochrome	98
4.5 Markers for Contaminants	99
5 Protein and Lipid Composition	101
6 Surface Properties of the Isolated Vesicles	101
References	102

Vacuoles

G. WAGNER (With 1 Figure)

1 Introduction	105
2 Methods of Isolation	106
2.1 Isolation of Vacuoles from Meristematic Tissues	106
2.2 Isolation of Vacuoles from Mature Plant Tissue	108
2.2.1 Isolation of Mature Vacuoles from Protoplasts – Methods pre 1981	108
2.2.2 Isolation of Mature Vacuoles from Protoplasts – Methods post 1981	110
2.2.3 Isolation of Mature Vacuoles Directly from Tissue – Methods pre 1981	114
2.2.4 Isolation of Mature Vacuoles Directly from Tissue – Methods post 1981	115
2.2.5 Preparation of Lutoids from Hevea Latex	115
2.2.6 Comments on Methods for Isolating Vacuoles from Higher Plants	116
2.2.7 Isolation of Proton-Pumping Vesicles	118
2.2.8 Preparation of Vacuoles from Yeast Neurospora	120

3 Isolation of Tonoplast and Tonoplast Markers	121
4 Comments on Physiological Functions	123
5 Concluding Remarks	126
References	128

Protein Bodies

A. H. C. HUANG (With 5 Figures)

1 Introduction	134
2 Special Consideration in Isolation of Protein Bodies	135
3 Nonaqueous Preparation in Glycerol	136
4 Nonaqueous Preparation in Hexane and Carbon Tetrachloride	137
5 Aqueous Preparation in Sources Gradients	138
6 Subfractionation of Isolated Protein Bodies	139
7 Analyses	141
References	143

Lipid Bodies

A. H. C. HUANG (With 2 Figures)

1 Introduction	145
2 Ontogeny	146
3 Isolation	146
4 Markers of Lipid Bodies	147
5 Assays	149
5.1 Fluorometric Assay	149
5.2 Colorimetric Assay	149
References	151

Chloroplasts as a Whole

G. A. BERKOWITZ and M. GIBBS

1 Introduction	152
2 Considerations of Integrity and Purity	153
3 Chloroplasts from Protoplasts	154
4 The Use of Silica Sols in Density Gradient Purification of Chloroplasts	156
5 General Notes on Isolation Procedures	158
6 Specific Isolation Protocols	161
6.1 Higher Plants	161
6.1.1 C ₃ Plants	161
6.1.2 C ₄ Plants	164
6.1.3 CAM Plants	166
6.2 Algae	167
6.2.1 Volvocales	167
6.2.2 (Ceramiaceae, Rhodophyta) – <i>Griffithsia monilis</i>	169
6.2.3 Siphonales	170

6.2.4 (Xanthophyceae) <i>Bumilleriopsis filiformis</i>	173
6.2.5 (Euglenophyceae) <i>Euglena gracilis</i>	173
7 Additional Comments on Chloroplast Isolation	174
8 Abbreviations	176
References	176

Purification of Inner and Outer Chloroplast Envelope Membranes

K. CLINE (With 6 Figures)

1 Introduction	182
2 General Considerations	183
3 The Procedure	184
3.1 Reagents and Equipment	184
3.1.1 Solutions	184
3.1.2 Materials	185
3.2 Growth of Peas and Purification of Intact Chloroplasts	185
3.3 Purification of Inner and Outer Envelope Membranes	187
4 Properties of the Isolated Membranes	190
4.1 Purity	190
4.1.1 Cross-Contamination by Envelope Membranes	190
4.1.2 Contamination by Thylakoids	193
4.1.3 Contamination by Stroma	194
4.2 Other Properties	194
5 Modifications of the Procedure	194
5.1 Alternate Methods of Chloroplast Rupture	194
5.2 Purification Subsequent to Rupture	195
5.3 Application to Other Tissues	195
6 Other Procedures	196
References	196

The Major Protein of Chloroplast Stroma, Ribulosebisphosphate Carboxylase

C. PAECH (With 2 Figures)

1 Introduction	199
2 Characteristics of RuBP Carboxylase	200
2.1 Molecular Arrangement and Physical Structure of Subunits	200
2.2 Molecular Structure	202
2.3 Biosynthesis and Assembly of Subunits	203
2.3.1 Large Subunit	203
2.3.2 Small Subunit	204
2.3.3 Subunit Heterogeneity	205
2.3.4 Coordinate Control of Subunit Synthesis	205
2.4 Catalytic Mechanism	206
2.4.1 Activation and Role of Mg^{2+}	206
2.4.2 Carboxylation of RuBP	207
2.4.3 Oxygenation of RuBP	210
2.4.4 Localization of Catalytic and Activator Site	210

3	Practical Aspects	211
3.1	Purification	211
3.1.1	Summary of Techniques	211
3.1.2	Interfering Compounds	211
3.1.3	Choice of Extraction Buffer and Grinding Procedures	212
3.1.4	Protein Determination	213
3.1.5	Example: RuBP Carboxylase from Soybean Leaves	214
3.2	Assay	214
3.2.1	Substrates	214
3.2.2	Activation of RuBP Carboxylase	215
3.2.3	Continuous Spectrophotometric Assay for RuBP Carboxylase Activity	216
3.2.4	Discontinuous Assays for RuBP Carboxylase Activity	216
3.2.4.1	Radiochemical Assay with $[^{14}\text{C}]\text{NaHCO}_3$	216
3.2.4.2	Radiochemical Assay Using $[^{14}\text{C}]\text{NaHCO}_3$ and $[1\text{-}^3\text{H}]\text{RuBP}$	218
3.2.4.3	Discontinuous Assay Using Nonlabeled Substrates	218
3.2.5	Assays for RuBP Oxygenase	218
3.2.6	Kinetic Parameters of RuBP Carboxylase and RuBP Oxygenase	219
4	Conclusion	219
	References	221

The Chloroplast Thylakoid Membrane – Isolation, Subfractionation and Purification of Its Supramolecular Complexes

B. ANDERSSON and J. M. ANDERSON (With 8 Figures)

1	Introduction	231
2	Function and Organization of the Thylakoid Membrane	231
3	Isolation of Thylakoid Membranes	234
4	Thylakoid Membrane Subfractionation	237
4.1	Photosystem I Stroma Lamellae Thylakoids	238
4.2	Photosystem II Oxygen Evolving Thylakoid Preparations	241
4.2.1	Isolation by Press Treatment and Phase Partition	241
4.2.2	Isolation by Detergent Fractionation	243
4.2.3	Choice of Preparation	244
4.3	Separation of Inside-Out and Right-Side-Out Thylakoid Vesicles with the Same Composition	245
5	Isolation of Thylakoid Supramolecular Complexes	247
5.1	The Photosystem I Complex and the Light-Harvesting Complex of Photosystem II (LHC II)	247
5.2	The Light-Harvesting Complex of Photosystem I (LHC I)	249
5.3	The Inner Core Complex of Photosystem II (CC II)	249
5.4	The Cytochrome b/f Complex	250
5.5	The ATP Synthase ($\text{CF}_0\text{-CF}_1$)	253
	References	254

The Isolation and Characterization of Nongreen Plastids

J. A. MIERNYK (With 9 Figures)

1	Introduction	259
2	The Terminology of Nongreen Plastids	260
2.1	Proplastids	260
2.2	Etioplasts	260
2.3	Chromoplasts	261
2.4	Amyloplasts	261
2.5	Leucoplasts	261
2.6	Other Nongreen Plastids	262
3	Basics of Plastid Isolation and Separation	262
3.1	Experimental Design	262
3.2	Isolation Medium	263
3.3	Tissue Disruption	264
3.4	General Methods of Chloroplast Isolation	265
4	Isolation of Nongreen Plastids from Developing <i>Ricinus</i> Endosperm	265
4.1	Rate-Zonal Sedimentation	265
4.1.1	Protocol	266
4.1.2	Analysis	267
4.1.3	Comments	268
4.2	Isopycnic Banding on Linear Sucrose Gradients	268
4.2.1	Protocol	269
4.2.2	Comments	269
4.3	Rate-Zonal Sedimentation on Linear Sucrose-Magnesium Co-Gradients	270
4.3.1	Protocol	270
4.3.2	Comments	270
4.4	Rate-Zonal Sedimentation on Discontinuous Sucrose Gradients	271
4.4.1	Protocol	271
4.4.2	Comments	272
4.5	Rate-Zonal Sedimentation on Discontinuous Percoll Gradients	274
4.5.1	Protocol	274
4.5.2	Comments	274
4.6	Nonaqueous Methods	276
4.6.1	Isopycnic Banding on Linear Hexane-CCl Gradients	276
4.6.2	Silicon Oil Centrifuged Filtration	277
4.7	Noncentrifugal Methods	277
4.7.1	Gel Permeation	278
4.7.1.1	Materials	278
4.7.1.2	Protocol	278
4.7.1.3	Comments	278
4.7.2	Phase Partition	279
4.7.3	Unit-Gravity Sedimentation	279
5	Metabolic Capabilities of <i>Ricinus</i> Endosperm Plastids	280
5.1	Glycolysis, the Pentose-Phosphate Pathway and Fatty Acid Synthesis	280
5.2	The Calvin Cycle	282

5.3 Nitrogen Metabolism	282
5.4 Terpenoid Metabolism	283
6 Composition and Biochemical Properties	283
6.1 Structure	283
6.2 Protein Composition	283
6.3 Membranes	284
6.4 Nucleic Acids	287
7 Future Prospects	289
References	290

Mitochondria

J. F. JACKSON (With 2 Figures)

1 Introduction	296
2 Preparation for DNA Analysis	297
2.1 Cytoplasmic Male Sterility and Structure of Mitochondrial DNA	297
2.2 Isolation of Mitochondria for DNA Preparation	298
2.3 Preparation of Mitochondrial DNA	298
2.4 Electrophoresis of Mitochondrial DNA	298
2.5 Restriction Analysis of Mitochondrial DNA	299
2.6 Notes on Mitochondrial DNA Studies	300
3 Preparation of Intact Mitochondria for Oxidative Studies	300
3.1 Introduction	300
3.2 Mitochondrial Preparation and Purification	301
3.3 Tests for Integrity of Mitochondria	302
3.3.1 Succinate: Cytochrome c Reductase	302
3.4 Tests for Integrity	302
3.4.1 Measurement for Oxygen Consumption for Respiratory Control and P/O Ratios	302
3.5 Notes on the Methods	302
References	303

Endoplasmic Reticulum

R. L. JONES (With 6 Figures)

1 Introduction	304
2 Structure and Organization of the ER	304
3 Interactions Between Tubular and Cisternal ER	306
3.1 Role in Protein Transport	306
3.2 Role in Cell Division	306
4 Synthesis and Degradation of ER	307
4.1 Membrane Proteins	307
4.2 Membrane Lipids	308
5 Isolation and Characterization of ER	309
5.1 Isolation Media	309
5.2 Tissue Homogenization	310

5.3	Organelle Isolation	310
5.3.1	Molecular Sieve Chromatography	311
5.3.2	Differential Centrifugation	311
5.3.3	Density Gradient Centrifugation	313
5.4	Identification of ER Membranes	315
5.4.1	Magnesium Shift	315
5.4.2	Marker Enzymes	317
5.4.3	Auxin Binding	322
5.4.4	Calcium Transport	323
5.4.5	Structural Proteins	323
5.5	Concluding Remarks	323
	References	324

Polyribosomes

B. A. LARKINS (With 10 Figures)

1	Introduction	331
2	Isolation of Polysomes from Plant Cells	332
2.1	Factors that Affect the Stability and Recovery of Polyribosomes	332
2.2	Tissue Preparation	333
2.3	Subcellular Fractionation and Polysome Isolation	334
3	Purification and Analysis of Polyribosomes	336
3.1	Sucrose Gradient Centrifugation	336
3.2	Purification of Polysomes with Discontinuous Sucrose Gradients	338
3.3	Analysis of Polyribosome Profiles	340
4	Polyribosome Extraction Buffers	340
4.1	pH	342
4.2	Potassium Chloride	342
4.3	Magnesium Chloride	343
4.4	Reducing Agents	345
4.5	Chelation of Divalent Metals	345
4.6	Proteinase K	348
4.7	Other Ribonuclease Inhibitors	348
5	Uses of Purified Polyribosomes	349
5.1	Changes in Protein Synthetic Activity	349
5.2	In Vitro Protein Synthesis	349
5.3	Purification of mRNA's	350
5.4	Subcellular Distribution of mRNA's	350
	References	350

The Nucleus – Cytological Methods and Isolation for Biochemical Studies

J. F. JACKSON (With 3 Figures)

1	Introduction	353
2	Structure of the Plant Nucleus and Implications for Nuclear Isolation and Staining	354

3	Cytology	357
3.1	Nuclei of Whole Cells	357
3.1.1	Feulgen Microspectrophotometric Methods	358
3.1.2	Microfluorometric DNA Determination	359
3.2	Staining Nuclei During Isolation	360
3.3	Nucleolus Staining	360
3.4	Chromosome Staining	361
3.5	Other Nuclear Stains	361
4	Isolation of Plant Nuclei – General	362
4.1	Isolation of Plant Nuclei – Methods	366
4.1.1	Nuclei from Tobacco Callus Cultures for RNA Synthesis Studies	366
4.1.2	Nuclei from Tobacco Cells in Culture – for General Purpose Studies	367
4.1.3	Nuclei from Soybean Cells – for DNA Studies	368
4.1.4	Plant-Root Nuclei – for DNA Analysis	369
5	Summary	370
	References	370

Microtubules

D. D. SABNIS (With 3 Figures)

1	Introduction	375
2	Extraction of Microtubule Proteins	376
3	Purification of Tubulin and MAP's	377
3.1	DEAE-Sephadex Ion Exchange Chromatography	377
3.2	Phosphocellulose Chromatography	378
3.3	Affinity Chromatography	378
3.4	Cycles of Polymerisation and Depolymerisation	379
3.4.1	Pre-Conditions for Microtubule Assembly	379
3.4.2	Microtubule Assembly in the Presence or Absence of Glycerol	380
3.4.3	The Dynamics of Polymerisation and the Use of Taxol	381
3.4.4	Co-Polymerisation	382
4	Fractionation and Identification of Tubulin by SDS-PAGE	382
5	Colchicine-Binding Assay for Tubulin	384
6	Immunochemical Methods of Analysis	386
6.1	Radioimmunoassay	387
6.2	Enzyme-Linked Immunosorbent Assay (ELISA)	387
6.3	Antibody Purification of Antigen-Affinity Column	388
6.4	Western Blots	388
7	Concluding Remarks	389
	References	389

Subject Index	395
--------------------------------	-----