

Contents

Section I Cell Cycle and Cellular Dynamics

I.1	Novel Approaches for Cell Cycle Analysis in BY-2	3
	O.A. KOROLEVA, G.R. ROBERTS, M.L. TOMLINSON, and J.H. DOONAN	
1	Introduction	3
2	A Modified alc-Inducible System for Transgene Expression in BY-2	3
3	Use of the alc System to Define the Time of Action of Induced Protein	5
4	An Example of Functional Analysis Using the AlcR-GR Gene Switch	6
5	Transient Protein Expression	9
6	Emerging Techniques	13
7	Protocols	16
8	Composition of Media	19
	References	19
I.2	Dynamics and Structure of the Preprophase Band and the Phragmoplast	23
	D. GEELEN and D. INZÉ	
1	Introduction	23
2	The Preprophase Band (PPB)	24
3	The Phragmoplast	31
4	Future Perspectives	35
	References	36
I.3	Formation of Cortical Microtubules in a Cell-Free System Prepared from Plasma Membrane Ghosts and a Cytosolic Extract of BY-2 Cells	41
	T. MURATA and M. HASEBE	
1	Introduction	41
2	Microtubules Nucleate as Branches on Existing Microtubules in the Cortical Arrays of Plant Cells	41
3	Analysis of the Molecular Mechanisms of Microtubule Nucleation in a BY-2 Cell-Free System	44
4	Future Perspectives	47
	References	47

I.4	Chromosome Dynamics in Tobacco BY-2 Cultured Cells	51
	S. MATSUNAGA, N. OHMIDO, and K. FUKUI	
1	Introduction	51
2	Dynamic Analysis of Condensin Complexes	52
3	Dynamic Analysis of Heterochromatic Protein 1	53
4	Dynamic Analysis of Aurora Kinases	57
5	Conclusion and Perspectives	60
	References	61
I.5	Ion Channels Meet Cell Cycle Control	65
	R. HEDRICH and D. BECKER	
1	Introduction	65
2	Cell Elongation	66
3	Cell Cycle	66
4	Discussion	70
	References	75
I.6	The NACK-PQR MAP Kinase Cascade Controls Plant Cytokinesis .	79
	M. SASABE, Y. TAKAHASHI, T. SOYANO, H. TANAKA, K. KOUSETSU, T. SUZUKI, and Y. MACHIDA	
1	Introduction	79
2	Plant Cytokinesis	81
3	The MAPK Cascade Involved in Cytokinesis	82
4	Regulation of the NACK-PQR Pathway in the Cell Cycle Machinery	86
5	Downstream Factors of the NACK-PQR Pathway	88
6	Future Perspectives	91
	References	92

Section II Physiological and Developmental Aspects

II.1	Characterization of a Cell Division Factor from Auxin-Autotrophic 2B-13 Cells Derived from the Tobacco BY-2 Cell Line	97
	T. SHIMIZU, K. EGUCHI, I. NISHIDA, K. LAUKENS, E. WITTERS, and T. NAGATA	
1	Introduction	97
2	Characterization of Cell Division-Inducing Activity in the Culture Filtrates of 2B-13 Cells	98
3	Purification of the CDF	100
4	Searches for CDFs in the Culture Filtrates of Tobacco BY-2 Cells .	102
5	Concluding Remarks and Future Perspectives	103
6	Protocols	104
	References	106

II.2	The BY-2 Cell Line as a Tool to Study Auxin Transport	107
	J. PETRÁŠEK and E. ZAŽÍMALOVÁ	
1	Introduction	107
2	Present State of the Art of Cell-to-Cell Transport of Auxins	107
3	Auxin Transport Studies in <i>Planta</i>	109
4	Auxin Transport Studies in Simplified Models	110
5	Concluding Remarks and Future Prospects	114
	References	115
II.3	Tobacco BY-2 Cells as a Model for Differentiation in Heterotrophic Plant Cells	119
	Y. MIYAZAWA and A. SAKAI	
1	Introduction	119
2	Hormonal Factors Affecting Starch-Storing Cell Differentiation in BY-2 Cells	120
3	Regulations of Gene Expressions Required for Differentiation and Dedifferentiation of Starch-Storing Cells	124
4	Conclusions and Perspectives	127
	References	130

Section III Intracellular Traffic

III.1	Imaging the Early Secretory Pathway in BY-2 Cells	135
	D.G. ROBINSON and C. RITZENTHALER	
1	The Early Secretory Pathway in Plants: A Brief Introduction	135
2	General Description of the BY-2 Endomembrane System	136
3	The Golgi Apparatus: Structure, Motility and Behaviour During Mitosis	137
4	The Endoplasmic Reticulum: Distribution and ER-Export Sites (ERES)	140
5	BY-2 Cells: A Model System for Studying the Action of BFA	143
6	Appendix: Standard Fixation Protocols for Electron Microscopy and Immunostaining	147
	References	148
III.2	Molecular Study of Prevacuolar Compartments in Transgenic Tobacco BY-2 Cells	153
	S.W. LO and L. JIANG	
1	Introduction	153
2	Results and Discussion	155
3	Conclusions	163
4	Protocols	164
	References	165

III.3	Autophagy and Non-Classical Vacuolar Targeting in Tobacco BY-2 Cells	167
	K. TOYOOKA and K. MATSUOKA	
1	Introduction	167
2	Autophagy	168
3	Protein Aggregation and Degradation	174
4	Direct Transport from the ER to the Vacuole	175
5	Concluding Remarks	177
	References	178

Section IV As a Host for Infectious Diseases

IV.1	In Vitro Translation and Replication of Tobamovirus RNA in a Cell-Free Extract of Evacuolated Tobacco BY-2 Protoplasts	183
	K. ISHIBASHI, K. KOMODA, and M. ISHIKAWA	
1	Introduction	183
2	Commercial Wheat Germ Extract and Rabbit Reticulocyte Lysate Can Support the Translation but not the Replication of Tobamovirus RNA	186
3	Establishment of an In Vitro Translation–Replication System for Plant Viral RNA with an Extract of Evacuolated BY-2 Protoplasts	186
4	Conclusion and Perspective	189
5	Protocols	190
	References	192
IV.2	Using BY-2 Cells to Investigate <i>Agrobacterium</i> –Plant Interactions .	195
	S.B. GELVIN	
1	Introduction	195
2	In What Form is T-DNA Transferred from <i>Agrobacterium</i> to Plant Cells?	196
3	How Soon after <i>Agrobacterium</i> Infection Can we Detect Transgene Expression?	196
4	Using Tobacco BY-2 Cells to Follow the Journey of T-DNA Through the Plant Cell	198
5	Using Tobacco BY-2 Cells to Investigate the Response of Plant Cells to <i>Agrobacterium</i> Infection	200
6	Conclusion	205
	References	205

IV.3	Regulation of Elicitor-Induced Defense Responses by Ca ²⁺ Channels and the Cell Cycle in Tobacco BY-2 Cells	207
	Y. KADOTA and K. KUCHITSU	
1	Introduction	207
2	Elicitor-Induced Cytosolic Free Ca ²⁺ Concentration ([Ca ²⁺] _{cyt}) Change and its Regulatory Mechanisms	208
3	Involvement of Putative Voltage-Dependent Ca ²⁺ Permeable Channels, NtTPC1A/B, in Elicitor Signaling	210
4	Elicitor-Induced Cell Cycle Arrest	213
5	Cell Cycle Dependence of Elicitor-Induced Defense Signaling	214
6	Conclusion	217
7	Protocols	218
	References	218

Section V Other Cellular Functions

V.1	Dynamic Mitochondria, their Fission and Fusion in Higher Plants .	225
	S. ARIMURA and N. TSUTSUMI	
1	Introduction	225
2	Mitochondrial Fission	227
3	Mitochondrial Fusion	232
4	Conclusions and Perspectives	235
	References	236
V.2	The Use of Tobacco BY-2 Cells to Elucidate the Biosynthesis and Essential Functions of Isoprenoids	241
	A. HEMMERLIN, E. GERBER, M.-A. HARTMANN, D. TRITSCH, D.N. CROWELL, M. ROHMER, and T.J. BACH	
1	Introduction	241
2	The Use of Tobacco BY-2 Cells for Biosynthetic Studies	242
3	Incorporation of Stably Labelled Glucose into Selected Isoprenoid End-Products	243
4	Incorporation of Additional Pathway-Specific, Stably Labelled Precursors	248
5	Incorporation of Pathway-Specific Radiolabelled Precursors	253
6	In Vivo Effects of Isoprenoid Precursors and Pathway Intermediates	255
7	Isoprenylation of Proteins in BY-2 Cells	262
8	Conclusion and Outlook	263
	References	265

Section VI Omics

VI.1	Tobacco BY-2 Proteome Display, Protein Profiling and Annotation Using Two-Dimensional Electrophoresis and Mass Spectrometry-Based Cross-Species Identification	275
K.	LAUKENS and E. WITTERS	
1	Introduction	275
2	Gel-Based Analysis of the BY-2 Proteome	276
3	MS-Based Protein Structure Analysis	281
4	Searching Sequence Databases with BY-2 Peptide Mass Spectra	285
5	Proteomic Data Integration	287
6	Concluding Remarks	288
7	Protocols	289
	References	291
VI.2	EST and Microarray Analysis of Tobacco BY-2 Cells	293
K.	MATSUOKA and I. GALIS	
1	Introduction	293
2	The RIKEN Tobacco BY-2 EST and BY-2 Microarray	293
3	An Example of Microarray-Based Analysis: Jasmonate-Dependent Gene Expression and Metabolic Studies	298
4	Future Perspectives	308
	References	309
VI.3	Proteomics of Tobacco Bright Yellow-2 (BY-2) Cell Culture Plastids	313
M.A.	SIDDIQUE, W. GRUISEM, and S. BAGINSKY	
1	Introduction	313
2	The Proteome of BY-2 Cell Culture Plastids	316
3	Concluding Remarks	322
4	Protocols for BY-2 Plastid Isolation and Protein Fractionation	322
	References	325

Section VII Technical Advances

VII.1 Cryopreservation of Tobacco BY-2 Suspension Cell Cultures Using an Encapsulation – Simple Prefreezing Method	329
T. KOBAYASHI, T. NIINO, and M. KOBAYASHI	
1 Introduction	329
2 Methods for Cryopreservation of Cultured Plant Cells	330
3 Conditions for Cryopreservation of Cell Suspension Cultures	331
4 The Encapsulation-Simple Prefreezing Method for Cryopreservation of BY-2	332
5 Conclusion	334
6 Protocol	334
References	336
VII.2 High Throughput Microinjection Technology for the Single-Cell Analysis of BY-2 in Vivo	339
H. MATSUOKA, Y. YAMADA, K. MATSUOKA, and M. SAITO	
1 Microinjection in the Post-Genome Era	339
2 Single-Cell Manipulation Supporting Robot (SMSR)	339
3 Microinjection of a Dominant-Negative Protein into BY-2 Cells . .	343
4 Concluding Remarks	345
References	346
Subject Index	347