

# Biotechnology in Agriculture and Forestry

---

Edited by

T. Nagata (Managing Editor)

H. Lörz

J. M. Widholm

Springer-Verlag Berlin Heidelberg GmbH

---

*Volumes already published*

- Volume 1: Trees I (1986)  
Volume 2: Crops I (1986)  
Volume 3: Potato (1987)  
Volume 4: Medicinal and Aromatic Plants I (1988)  
Volume 5: Trees II (1989)  
Volume 6: Crops II (1988)  
Volume 7: Medicinal and Aromatic Plants II (1989)  
Volume 8: Plant Protoplasts and Genetic Engineering I (1989)  
Volume 9: Plant Protoplasts and Genetic Engineering II (1989)  
Volume 10: Legumes and Oilseed Crops I (1990)  
Volume 11: Somaclonal Variation in Crop Improvement I (1990)  
Volume 12: Haploids in Crop Improvement I (1990)  
Volume 13: Wheat (1990)  
Volume 14: Rice (1991)  
Volume 15: Medicinal and Aromatic Plants III (1991)  
Volume 16: Trees III (1991)  
Volume 17: High-Tech and Micropropagation I (1991)  
Volume 18: High-Tech and Micropropagation II (1992)  
Volume 19: High-Tech and Micropropagation III (1992)  
Volume 20: High-Tech and Micropropagation IV (1992)  
Volume 21: Medicinal and Aromatic Plants IV (1993)  
Volume 22: Plant Protoplasts and Genetic Engineering III (1993)  
Volume 23: Plant Protoplasts and Genetic Engineering IV (1993)  
Volume 24: Medicinal and Aromatic Plants V (1993)  
Volume 25: Maize (1994)  
Volume 26: Medicinal and Aromatic Plants VI (1994)  
Volume 27: Somatic Hybridization in Crop Improvement I (1994)  
Volume 28: Medicinal and Aromatic Plants VII (1994)  
Volume 29: Plant Protoplasts and Genetic Engineering V (1994)  
Volume 30: Somatic Embryogenesis and Synthetic Seed I (1995)  
Volume 31: Somatic Embryogenesis and Synthetic Seed II (1995)  
Volume 32: Cryopreservation of Plant Germplasm I (1995)  
Volume 33: Medicinal and Aromatic Plants VIII (1995)  
Volume 34: Plant Protoplasts and Genetic Engineering VI (1995)  
Volume 35: Trees IV (1996)  
Volume 36: Somaclonal Variation in Crop Improvement II (1996)  
Volume 37: Medicinal and Aromatic Plants IX (1996)  
Volume 38: Plant Protoplasts and Genetic Engineering VII (1996)  
Volume 39: High-Tech and Micropropagation V (1997)  
Volume 40: High-Tech and Micropropagation VI (1997)  
Volume 41: Medicinal and Aromatic Plants X (1998)  
Volume 42: Cotton (1998)  
Volume 43: Medicinal and Aromatic Plants XI (1999)  
Volume 44: Transgenic Trees (1999)  
Volume 45: Transgenic Medicinal Plants (1999)  
Volume 46: Transgenic Crops I (1999)  
Volume 47: Transgenic Crops II (2001)  
Volume 48: Transgenic Crops III (2001)  
Volume 49: Somatic Hybridization in Crop Improvement II (2001)  
Volume 50: Cryopreservation of Plant Germplasm II (2002)  
Volume 51: Medicinal and Aromatic Plants XII (2002)  
Volume 52: Brassicas and Legumes: From Genome Structure to Breeding (2003)  
Volume 53: Tobacco BY-2 Cells (2004)

*Volumes in preparation*

- Volume 54: *Brassica* (2004)  
Volume 55: Molecular Marker Systems in Plant Breeding and Crop Improvement  
Volume 56: Haploids in Crop Improvement II

---

# Biotechnology in Agriculture and Forestry 53

---

## *Tobacco BY-2 Cells*

Edited by

T. Nagata, S. Hasezawa, and D. Inzé

With 84 Figures, 12 in Color, and 8 Tables



Springer

Series Editors

Professor Dr. T OSHIYUKI NAGATA  
University of Tokyo  
Graduate School of Science  
Department of Biological Sciences  
7-3-1 Hongo, Bunkyo-ku  
Tokyo 113-0033, Japan

Professor Dr. H ORST LÖRZ  
Universität Hamburg  
Institut für Allgemeine Botanik  
Angewandte Molekularbiologie  
der Pflanzen II  
Ohnhorststraße 18  
22609 Hamburg, Germany

Professor Dr. JACK M. W IDHOLM  
University of Illinois  
285A E.R. Madigan Laboratory  
Department of Crop Sciences  
1201 W. Gregory  
Urbana, IL 61801, USA

Volume Editors

Professor Dr. T OSHIYUKI NAGATA (address see above)

Professor Dr. Seiichiro Hasezawa  
University of Tokyo  
Graduate School of Science  
Department of Integral Biosciences  
5-1-5 Kashiwanoha  
Kashiwa-shi 227-8562  
Chiba, Japan

Professor Dr. Dirk Inzé  
VIB/Ghent University  
Department of Plant Systems Biology  
Technologiepark 927  
9052 Ghent, Belgium

ISSN 0934-943X

ISBN 978-3-642-07305-2 ISBN 978-3-662-10572-6 (eBook)

DOI 10.1007/978-3-662-10572-6

Library of Congress Cataloging-in-Publication Data

Tobacco BY-2 cells / edited by T. Nagata and D. Inze.  
p. cm. – (Biotechnology in agriculture and forestry ; 53)  
Includes bibliographical references and index.

1. Plant cell cycle. 2. Plant cells and tissues. 3. Tobacco – Cytology. I. Nagata, T. (Toshiyuki)  
II. Inzé, D. (Dirk) III. Series.

QK725.T625 2004

This work is subject to copyright. All rights reserved, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilm or in any other way, and storage in data banks. Duplication of this publication or parts thereof is permitted only under the provisions of the German Copyright Law of September 9, 1965, in its current version, and permission for use must always be obtained from Springer-Verlag Berlin Heidelberg GmbH. Violations are liable for prosecution under the German Copyright Law.

springeronline.com

© Springer-Verlag Berlin Heidelberg 2004

Originally published by Springer-Verlag Berlin Heidelberg New York in 2004

The use of general descriptive names, registered names, trademarks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.

Cover design: Design & Production GmbH, Heidelberg

39/3150-WI-5 4 3 2 1 0-Printed on acid-free paper

*The editors would like to dedicate  
this volume to  
Professor Jeff Schell  
who inspired several generations of scientists  
to explore the fascinating world  
of plant sciences.*

*Please note that the decision to dedicate this volume  
to Prof. Schell was made before he passed away  
on April 17, 2003.*

## Preface

It is our pleasure to present the 53rd volume of the *Biotechnology in Agriculture and Forestry (BAF)* series. This is the second issue of the BAF series edited by the new editorial team consisting of Professors Horst Lörz, University of Hamburg, Hamburg, Germany, Jack Widholm, University of Illinois, Urbana, USA, and Toshiyuki Nagata, University of Tokyo, Tokyo, Japan. This series was originally founded by the late Professor Y.P.S. Bajaj, Delhi, India, in 1986. The current volume is somewhat unique, since in this volume only one plant cell line, the tobacco BY-2 cell line, is handled, while previous volumes mainly dealt with plants having certain economical importance. Nonetheless, the three editors of this volume, Professors Dirk Inzé, Seiichiro Hasezawa and Toshiyuki Nagata, believe that most scientists who are working in the field of plant sciences will enjoy seeing this volume as a kind of source book of the unique tobacco BY-2 cells. Indeed, tobacco BY-2 cells have, over the years, gained the status as a model plant system, comparable to HeLa cells for human research. The current book is very timely because a wealth of basic knowledge on plant cells related to, e.g., cell division, cytoskeleton, cytokinesis, plant hormone signaling, etc., has been gathered from experiments with this cell line. As reflected in the contents, the accumulated knowledge of the BY-2 cell line is enormous and there is no other cell line that has been so important for progress in the plant sciences. Such knowledge should be shared with scientists from the fields of both applied and basic plant science. Furthermore, systematic studies on BY-2 cells are a prerequisite for further progress. Thus, the cell line that has been correctly called the HeLa cells in plant cell cultures should have particular importance.

In this volume, the following themes are handled. In Chapter 1, the early cradle stage in the development of tobacco BY-2 cell line, which has not been previously reported, is described. Chapters 2–5 describe recent progress in cell cycle studies. Chapters 6–9 discuss cell biological aspects, focusing on the dynamic changes in the cytoskeleton. Contributions to physiological and developmental aspects are dealt with in Chapters 10–17. Finally, studies from the viewpoint of molecular biology are described in Chapters 18–22. Thus, many aspects that have not been reported in books or journals so far are presented.

DIRK INZÉ (Gent, Belgium), SEIICHO HASEZAWA (Kashiwa, Japan),  
and TOSHIYUKI NAGATA (Tokyo, Japan)  
November 2003

# Contents

## 1 When I Encountered Tobacco BY-2 Cells!

TOSHIYUKI NAGATA

1 Introduction .....	1
2 Encounter .....	1
3 Synchronization .....	3
4 Distribution .....	4
5 Concluding Remarks .....	5
References .....	5

## Section I: Cell Cycle

### 2 Improvements of the Molecular Toolbox for Cell Cycle Studies in Tobacco BY-2 Cells

JÉRÔME JOUBÈS, DIRK INZÉ, and DANNY GEELEN (With 5 Figures)

1 Introduction .....	7
2 Genome-Wide Expression Analysis of Cell Cycle Modulated Genes in Tobacco BY-2 Cells .....	8
3 A Set of Gateway™ Compatible Binary T-DNA Destination Vectors for Cell Cycle Gene Function Analysis in BY-2 Cells .....	11
4 Protein Localization in Tobacco BY-2 Cells by Means of GFP-Tagging .....	14
5 Localization of Cell Cycle Proteins .....	16
6 Conclusion .....	20
References .....	20

### 3 Transcriptional Regulation During the Plant Cell Cycle: Involvement of Myb Proteins in Cyclin B Transcription

MASAKI ITO (With 2 Figures)

1 Introduction .....	24
2 Mechanisms of G2/M Phase-Specific Transcription in Plants .....	25
2.1 G2/M Phase-Specific Expression of B-Type Cyclin Genes .....	25
2.2 The <i>cis</i> Element Controlling B-Type Cyclin Promoter Activity ..	26

2.3 MSA Elements Provide a Common Mechanism for G2/M Phase Transcription .....	26
2.4 Factors that Bind to the MSA Motif .....	27
2.5 Putative Factors that Bind to <i>cis</i> Elements Involved in Cell Cycle Phase-Independent Activation .....	28
3 Mechanisms of G2/M Phase-Specific Transcription in Animal Cells .....	29
3.1 Genes for B-Type Cyclins and Co-Expressed Genes .....	29
3.2 CCAAT Box-Mediated Activation .....	29
3.3 Cell Cycle-Dependent Repression .....	30
3.3.1 The CDE/CHR Tandem Element .....	30
3.3.2 Mechanisms of Repression Mediated by CDE/CHR .....	31
4 Conclusion .....	32
5 Perspective .....	33
References .....	34
4 Control of the G1/S Phase Transition in Tobacco BY-2 Cells MASAMI SEKINE and ATSUHIKO SHINMYO (With 2 Figures)	
1 Introduction .....	37
2 Control of the G1/S Transition in Animals .....	38
3 The Rb/E2F Pathway Regulates the G1/S Transition in Plants .....	39
3.1 Retinoblastoma-Related Protein .....	39
3.2 E2F Transcription Factor Family .....	40
3.3 Cyclin D .....	43
3.4 CDK Inhibitors .....	44
4 Conclusions .....	46
References .....	48
5 Expression, Localisation and Stability of Mitotic Cyclins in Tobacco BY-2 Cells YVES PARMENTIER, MARIE CLAIRE CRIQUI, THOMAS POTUSCHAK, and PASCAL GENSHCHIK (With 4 Figures)	
1 Introduction .....	52
2 Tight Control of Mitotic Cyclin Gene Expression .....	53
3 APC/C-Dependent Ubiquitylation: After 20 Years of Investigation .....	53
4 Mitotic Cyclin Degradation in BY-2 Cells .....	55
5 Cyclin Subcellular Localisation: a First Step to Identify Functions .....	57
6 The Spindle Checkpoint and Cyclin Stability .....	60
7 Mitotic Cyclin Destruction: the Essential Step to Exit Mitosis .....	60



Contents	XI
8 Conclusion	61
References	62

## Section II: Cell Biology and Cytoskeleton

### 6 Molecular Mechanisms of Microtubule Nucleation in Tobacco BY-2 Cells

ANNE-CATHERINE SCHMIT, JEAN CANADAY, VIRGINIE SELTZER,  
SARAH CAMPAGNE, ETIENNE HERZOG, JEAN-LUC EVRARD,  
and ANNE-MARIE LAMBERT (With 6 Figures)

1 Introduction	66
2 The Microtubular Cycle in Tobacco BY-2 Cells	67
3 The Molecular Mechanism of Microtubule Nucleation in Metazoans and Fungi: $\gamma$ -Tubulin Complexes	68
4 Identification of Plant Homologues of $\gamma$ -TuSC Components in Tobacco BY-2 Cells	69
4.1 Plant $\gamma$ -Tubulin	69
4.2 Plant $\gamma$ -Tubulin Level During the Cell Cycle of Tobacco BY-2 Synchronized Cells, and $\gamma$ -Tubulin RNA Expression in Tobacco Plants	71
4.3 Identification of <i>Arabidopsis</i> Spc97p and Spc98p Homologues in Tobacco BY-2 Cells	71
5 $\gamma$ -Tubulin Complexes in Plant Cell Extracts	72
6 Functional Assays: Inhibition of Microtubule Nucleation on BY-2 Nuclei Using Anti- $\gamma$ -Tubulin and Anti-Spc98p Antibodies	72
7 The Plant Spc98p as a Microtubule Nucleation Marker in Tobacco BY-2 Cells	74
8 Expression of the Spc98p-GFP Fusion Protein in Living Tobacco BY-2 Cells	74
9 Conclusion and Perspectives: a Model for Plant Microtubule Nucleation	75
References	78

### 7 Dynamic Behavior of Microtubules and Vacuoles at M/G<sub>1</sub> Interface Observed in Living Tobacco BY-2 Cells

FUMI KUMAGAI, ARATA YONEDA, NATSUMARO KUTSUNA,  
and SEIICHIRO HASEZAWA (With 6 Figures)

1 Introduction	81
2 Visualization of Microtubules with GFP- $\alpha$ -Tubulin Fusion Protein	83
3 Observation of Microtubule Dynamics During M/G <sub>1</sub> Transition	84

4 Visualization of Vacuoles with FM4-64 .....	89
5 Vacuolar Reorganization and Microtubule Dynamics During Mitosis .....	89
6 Summary and Prospects .....	92
7 Protocols .....	94
7.1 Cell Culture and Synchronization .....	94
7.2 GFP-Tubulin Construct, Transformation and Selection of Transformants .....	94
7.3 Staining of Vacuolar Membranes .....	95
7.4 Microscopy .....	95
References .....	95
8 Tobacco BY-2 Cells as an Ideal Material for Biochemical Studies of Plant Cytoskeletal Proteins SEIJI SONOBE, ETSUO YOKOTA, and TERUO SHIMMEN (With 4 Figures)	
1 Introduction .....	98
2 Microtubule and Related Proteins .....	98
2.1 Tubulin .....	101
2.2 65-kDa Microtubule-Associated Proteins .....	101
2.3 190-kDa Protein .....	103
2.4 MBP200 .....	103
3 Actin and Related Proteins .....	105
3.1 Actin .....	105
3.2 Myosin .....	106
3.3 Actin Binding Proteins .....	109
4 Future Perspectives .....	109
5 Protocol .....	110
References .....	111
9 Cell Plate Formation: Knowledge from Studies Using Tobacco BY-2 Cells TETSUHIRO ASADA and HIROKI YASUHARA (With 3 Figures)	
1 Introduction .....	116
2 Organization and Redistribution of the Phragmoplast Microtubule Array .....	117
3 Production, Accumulation, and Fusion of Cell Plate Vesicles .....	122
4 Chains Between Vesicle Accumulation and Microtubule Redistribution Which Bring About Cell Plate Expansion .....	123
4.1 Caffeine-Sensitive Process .....	124
4.2 Kinesin – MAPKKK Complex-Mediated Process .....	125
5 Prediction and Future Verification of a Cycle that Coordinates Cell Plate Formation .....	127
References .....	128

**Section III: Physiological and Developmental Aspects****10 Hormonal Control of the Plant Cell Cycle**

LUC ROEF and HARRY VAN ONCKELEN (With 1 Figure)

1 Introduction .....	132
2 Cytokinins .....	132
3 Auxins .....	136
4 Abscisic Acid .....	138
5 Jasmonates .....	139
6 Other Hormones .....	140
7 Conclusion .....	140
8 Protocol for the Analysis of Cytokinins, IAA and ABA from BY-2 Cells .....	141
8.1 Cytokinin, IAA and Abscisic Acid Extraction from BY-2 for Mass Spectrometric Analysis .....	141
8.2 Mass Spectrometric Analysis of Cytokinins .....	143
8.3 Mass Spectrometric Analysis of IAA and Abscisic Acid .....	143
References .....	144

**11 Block Points in the Cell Cycle Progression of Plant Cells:**

Deduced Lessons from Tobacco BY-2 Cells

TOSHIO SANO, TAKASHI SHIMIZU, KENICHI SAKAMOTO,

and TOSHIYUKI NAGATA (With 3 Figures)

1 Introduction .....	149
2 Auxin as a Sole Growth Factor for the Proliferation of Plant Cells .....	150
3 2B-13 Cell Line as an Auxin-Autotrophic Cells .....	151
4 Cell Cycle Block by Phosphate Starvation .....	153
5 Conclusion and Perspectives .....	157
6 Protocols .....	157
6.1 Auxin Starvation of Tobacco BY-2 Cells and Their Re-Entry into the Cell Cycle with the Addition of Auxin .....	157
6.2 Phosphate Starvation of Tobacco BY-2 Cells and Their Re-Entry into the Cell Cycle with Phosphate Addition .....	158
References .....	158

**12 Growth and Physiology of Suspension-Cultured Plant Cells:**

the Contribution of Tobacco BY-2 Cells to the Study of Auxin Action

JEAN-PIERRE RENAUDIN

1 Introduction .....	160
2 The Main Features of Plant Cell Cultures .....	161
2.1 General Parameters .....	161
2.2 Growth of Plant Cell Cultures .....	162
2.3 The Extracellular Medium .....	163

3	Auxin Effects on Cultured Plant Cells . . . . .	164
3.1	General Hormone Requirement of Plant Cell Cultures . . . . .	164
3.2	Auxin Requirement of Plant Cell Cultures . . . . .	164
3.2.1	Nature of the Auxin Dose-Response Relationship; Auxin Agonists and Antagonists . . . . .	164
3.2.2	Metabolism and Transport of Auxin; Cross Talk with Other Hormones . . . . .	165
3.2.3	Lethality of Auxin Absence . . . . .	167
3.2.4	Toxicity of High Auxin Levels . . . . .	167
3.3	The Contribution of Cultured Plant Cells to Study the Effect of Auxin on Cell Expansion . . . . .	168
3.3.1	Cell Expansion Occurs at Low Auxin Levels in Cell Cultures . . . . .	168
3.3.2	Cell Expansion Is Coupled to the Onset of Differentiation in Cell Cultures . . . . .	169
3.3.3	Functional Genetics of Cell Expansion in Cell Cultures . . . . .	170
3.4	The Contribution of Cultured Plant Cells to Study the Effect of Auxin on the Cell Cycle . . . . .	171
3.5	The Issue of the Cell Cycle Step(s) Affected by Auxin . . . . .	172
3.5.1	The Control of Ploidy . . . . .	173
4	Auxin Signalling and Effect on Gene Expression in Cultured Plant Cells . . . . .	174
4.1	Receptors and Sensitivity . . . . .	174
4.2	Early Transduction Events . . . . .	175
4.3	The Control of Gene Expression . . . . .	176
5	Conclusion . . . . .	177
	References . . . . .	177

### 13 Dual Pathways for Auxin Regulation of Cell Division and Expansion

ALAN M. JONES, HEMAYET ULLAH, and JIN-GUI CHEN (With 5 Figures)

1	Background . . . . .	181
1.1	Dual Auxin Pathways in Plant Cell Expansion and Division . . . . .	181
1.2	Auxin-Binding Protein 1 . . . . .	183
1.3	Heterotrimeric G proteins in Plants . . . . .	183
2	Evidence for Auxin Binding Protein 1-Mediated Cell Expansion . . . . .	184
2.1	Loss of Function Analysis in Tobacco BY-2 Cells . . . . .	184
2.2	Gain of Function Analysis in Tobacco Leaves . . . . .	185
3	Evidence for G Protein Involvement in Cell Division . . . . .	186
3.1	Pharmacological Evidence . . . . .	186
3.2	Genetic Evidence . . . . .	187
4	Conclusions . . . . .	190
	References . . . . .	190

## 14 Studies on Dynamic Changes of Organelles Using Tobacco BY-2 as the Model Plant Cell Line

ATSUSHI SAKAI, YUTAKA MIYAZAWA, and TSUNEYOSHI KUROIWA (With 5 Figures)

1	Introduction	192
2	Characteristics of Tobacco BY-2 Cells as Material for Analysis of Plant Organelles	193
3	Tobacco BY-2 Cells as a Model of Undifferentiated Plant Cells	193
3.1	Comparative Analyses of Plastid Gene Expression in Proplastids of Tobacco BY-2 Cells and in Chloroplasts of Leaf Mesophyll Cells	193
3.2	Isolation of Proplastid- and Chloroplast-Nuclei, and Comparison of Their Molecular Architectures	194
3.3	Comparison of Transcriptional Activities in Proplastid-Nuclei from Tobacco BY-2 Cells and Chloroplast-Nuclei from Mature Leaves	195
3.4	Roles of Distinct RNA Polymerases in Transcriptional Regulation	197
3.5	Role of PEP in Nongreen Plastids	198
4	Tobacco BY-2 Cells as a Model of Proliferating Plant Cells	199
4.1	Organelle Dynamics During Proliferation of Tobacco BY-2 Cells	199
4.2	Morphological Changes in Organelles and Organelle Nuclei During Culture	200
4.3	Changes in Organelle DNA Synthesis During Culture	202
4.4	Organelle DNA Polymerases	203
5	Tobacco BY-2 Cells as a Model of Differentiating Plant Cells	204
5.1	Hormone-Induced Amyloplast Formation in Tobacco BY-2 Cells	204
5.2	Amyloplast Formation in Tobacco BY-2 Cells	205
5.3	Effects of Auxin and Cytokinin on Amyloplast Formation and Accompanying Changes	205
5.4	Requirement for Transcription and Translation in Nucleo-Cytoplasmic and Organelle Compartments	207
5.5	Other Changes Associated with Amyloplast Formation in Tobacco BY-2 Cells	208
6	Summary	209
7	Protocol	209
7.1	Plastid Genes	209
7.2	Preparation and Disruption of Protoplasts	211
7.3	Isolation of Proplastid-Nuclei	211
7.4	Isolation of Mitochondrial-Nuclei	212
7.5	In Vitro Transcription/DNA Synthesis Using Isolated Organelle-Nuclei	213

References .....	213
15 Cell Wall Dynamics in Tobacco BY-2 Cells RYUSUKE YOKOYAMA, DAISUKE TANAKA, TAKESHI FUJINO, TAKAO ITOH, and KAZUHIKO NISHITANI (With 3 Figures)	
1 Introduction .....	217
2 Suspension Culture of Tobacco BY-2 Cells as a Model System for Cell Wall Studies .....	218
2.1 Preparation of Cell Wall Enzymes .....	218
2.2 Direct Delivery of Molecular Probes to Cell Wall Space .....	219
2.3 Tobacco BY-2 Cell Lines with Altered Cell Wall Components by Acclimatization .....	219
2.4 Cell Wall Regeneration from Protoplasts .....	221
2.5 Cell Wall Analyses Using Transformant Cell Lines .....	222
2.6 Cell Wall Dynamics During Cell Division .....	224
3 Concluding Remarks .....	227
References .....	228
16 Regulation of Secondary Metabolism in Tobacco Cell Cultures SUVI T. HÄKKINEN and KIRSI-MARJA OKSMAN-CALDENTÉY (With 5 Figures)	
1 Introduction .....	231
2 Alkaloids .....	232
2.1 Pharmacological Effects of Tobacco Alkaloids .....	232
2.2 Nicotine Biosynthesis .....	234
2.3 Nicotine Degradation and Other Nicotine-Related Alkaloids ...	236
2.4 Precursor Feeding .....	238
2.5 Tobacco BY-2 Cell Culture .....	239
3 Other Secondary Compounds .....	240
3.1 Putrescine Derivatives and Polyamines .....	240
3.2 Phenylpropanoids .....	240
3.3 Sesquiterpenes .....	242
4 Discovering Secondary Metabolite Pathways – Combining Transcriptomics and Metabolomics .....	243
5 Conclusions .....	244
References .....	245
17 Boron Nutrition of Cultured Tobacco BY-2 Cells MASARU KOBAYASHI and TORU MATOH (With 7 Figures)	
1 Introduction .....	250
2 Intracellular Localization of Boron in Tobacco BY-2 Cells .....	251
3 Boron-Polysaccharide Complex .....	252

4 Selection and Characterization of Cells that Tolerate Low Levels of Boron .....	254
5 Responses to Boron Deprivation in Tobacco BY-2 Cells .....	258
6 Future Perspective .....	261
References .....	262

## Section IV: Molecular Biological Aspects

### 18 In Vitro Transcription Systems from BY-2 Cells

YASUSHI YUKAWA and MASAHIRO SUGIURA (With 9 Figures)

1 Introduction .....	265
2 Advantages of the BY-2 Cell as Starting Material for In Vitro Transcription Systems .....	266
3 In Vitro Transcription from Pol I-Dependent Genes .....	266
4 In Vitro Transcription from Pol II-Dependent Genes .....	267
5 In Vitro Transcription from Pol III-Dependent Genes .....	269
5.1 Transcription from <i>Arabidopsis</i> U6 snRNA Genes .....	269
5.2 Transcription from Nuclear tRNA Genes .....	272
5.3 Transcriptional Regulation by DNA Methylation of Pol III-Dependent Genes .....	274
6 In Vitro Splicing of pre-tRNA .....	275
7 Conclusion .....	277
8 Protocol .....	278
References .....	279

### 19 Protein Sorting and Protein Modification Along the Secretory Pathway in BY-2 Cells

KEN MATSUOKA (With 2 Figures)

1 Introduction .....	283
2 Secretion and Vacuolar Targeting .....	284
2.1 Endomembrane Organelles, Their Structure and Function .....	284
2.1.1 The Endoplasmic Reticulum .....	284
2.1.2 The Golgi Apparatus and the Trans-Golgi Network .....	285
2.1.3 Prevacuolar Compartment, Endosome and Autolysosome .....	286
2.1.4 Vacuole .....	287
2.2 Protein Transport in the Secretory Pathway .....	288
2.2.1 Translocation Through the Endoplasmic Reticulum Membrane .....	288
2.2.2 Export from the Endoplasmic Reticulum .....	289
2.2.3 Golgi-to-Endoplasmic Reticulum and Intra-Golgi Transport .....	290

2.2.4	Vacuolar Targeting and Secretion .....	290
2.2.5	Secretion to the Extracellular Space and Cell Plate Formation .....	292
3	Advantages of Using BY-2 Cells for the Characterization of the Endomembrane System in Plant Cells .....	292
3.1	An Ideal Tool to Study Protein Transport and Protein Modification with Stably Transformed Cells .....	292
3.2	Easy Transient Expression Analysis .....	293
3.3	Easy Detection of Fluorescence in Living Cells .....	294
3.4	Efficient In Vivo Labeling .....	295
3.5	Easy Pharmacological Analysis .....	295
4	Concluding Remarks .....	298
	References .....	298

## 20 Characterisation of an Inducible/Repressible Gene Expression System in Tobacco BY-2 Cells

SÉVERINE PLANCHAIS, GACHAO KIUNA, GRAHAM ARMSTRONG,  
and JAMES A. H. MURRAY (With 5 Figures)

1	Introduction .....	302
2	Development of the TGV System .....	304
2.1	Precursors to the TGV System .....	304
2.2	Detailed Description of the TGV System .....	306
3	The TGV System in Tobacco BY-2 Cells .....	308
3.1	Tobacco BY-2 Transformation Strategy .....	308
3.2	Reporter Genes Mark Successful Dexamethasone-Induced Expression .....	308
3.2.1	$\beta$ -Glucuronidase Reporter Gene .....	308
3.2.2	Green Fluorescent Protein Reporter Gene .....	309
3.3	The TGV Protein Contributes to Leakiness in Calli, but not in Liquid-Grown Cells .....	311
4	Toxicity Studies .....	312
5	Discussion .....	312
	References .....	313

## 21 The Tobacco BY-2 Cell Line as a Model System to Understand in Planta Nuclear Coactivator Interactions

RIYAZ A. BHAT and RICHARD D. THOMPSON (With 7 Figures)

1	Introduction .....	316
1.1	Rationale for the Use of Tobacco BY-2 Cells for Analysis of Coactivator Function .....	316
1.2	GCN5 and ADA: Coactivator Proteins Involved in Histone Acetylation .....	317



Contents	XIX
2 Results and Discussion	318
2.1 ZmGCN5 and ZmADA2 Are Nuclear Proteins	318
2.2 The Extended N-Terminal Region of ZmGCN5 Contains a Functional Nuclear Localisation Sequence	319
2.3 The <i>ZmGCN5</i> Promoter Drives the Expression of GFP in Transiently and Stably Transformed Tobacco BY-2 Cells	321
2.4 ZmGCN5 Interacts with ZmADA2 in a Modified Split-Ubiquitin System	322
2.5 Establishment of Split-Ubiquitin System to Study the in Vivo Interaction in Tobacco BY-2 Protoplasts	323
2.6 The Split-Ubiquitin System Detects a Strong in Vivo Interaction Between ZmGCN5 HAT and the Adaptor ZmADA2	325
2.7 Split Ubiquitin as a Sensor for In Vivo Protein – Protein Interaction Studies in Living Plant Cells	326
3 Concluding Remarks	326
4 Protocols	327
4.1 Plasmid Constructs	327
4.1.1 SubCellular Localisation of ZmGCN5 and ZmADA2	327
4.1.2 Functionality of N-Terminally Located NLS in ZmGCN5	327
4.1.3 Functionality Test of ZmGCN5 Promoter	327
4.1.4 In Planta Interaction Between ZmGCN5 and ZmADA2	327
4.2 Plant Material	328
4.3 Preparation and Transfection of Protoplasts	328
4.4 Tobacco BY-2 Cell Line Transformation	328
4.5 Fluorescence Microscopy	328
References	329

## 22 Tobacco BY-2 Proteomics

KRIS LAUKENS and ERWIN WITTERS

1 Introduction	332
2 How Proteomics Can Resolve Biological Questions	333
3 Tobacco BY-2 Proteomics	334
3.1 Why Use Tobacco BY-2 for Proteome Studies?	334
3.2 Present Situation	334
4 Prospects	336
5 Protocols	336
5.1 Protein Separation	336
5.1.1 Protocol for Extraction and Separation of the Tobacco Proteome BY-2 by Two-Dimensional Electrophoresis (pH 3–10, 12% T)	337
5.2 Protein Detection	339
5.3 Protein Identification	339

5.3.1 Protocol for Tryptic Digestion of BY-2 Proteins (Adapted from the Protocol of the Protein and Peptide Group, EMBL-Heidelberg) .....	340
References .....	342
Subject Index .....	344

## List of Contributors

G. ARMSTRONG, Institute of Biotechnology, University of Cambridge, Tennis Court Road, Cambridge, CB2 1QT, UK

T. ASADA, Department of Biology, Graduate School of Science, Osaka University, Machikaneyama 1-1, Toyonaka, Osaka 560-0043, Japan

R.A. BHAT, Max-Planck-Institut für Züchtungsforschung, Carl-von-Linné Weg 10, 50829, Köln, Germany

S. CAMPAGNE, Institut de Biologie Moléculaire des Plantes, Centre National de la Recherche Scientifique CNRS, UPR 2357, Université Louis Pasteur, Strasbourg, France

J. CANADAY, Institut de Biologie Moléculaire des Plantes, Centre National de la Recherche Scientifique CNRS, UPR 2357, Université Louis Pasteur, Strasbourg, France

J.-G. CHEN, Department of Biology, University of North Carolina, Chapel Hill, NC 27599, USA

M.C. CRIQUI, Institut de Biologie Moléculaire des Plantes du CNRS, 12, rue du Général Zimmer, 67084 Strasbourg Cedex, France

J.-L. EVRARD, Institut de Biologie Moléculaire des Plantes, Centre National de la Recherche Scientifique CNRS, UPR 2357, Université Louis Pasteur, Strasbourg, France

T. FUJINO, Wood Research Institute, Kyoto University, Gokasho, Uji, Kyoto 611-0011, Japan

D. GEELLEN, VIB/Ghent University, Department of Plant Systems Biology, Technologiepark 927, 9052 Ghent, Belgium

P. GENSCHIK, Institut de Biologie Moléculaire des Plantes du CNRS, 12, rue du Général Zimmer, 67084 Strasbourg Cedex, France

S.T. HÄKKINEN, VTT Biotechnology, (Tietotie 2), P.O. Box 1500, 02044 VTT, Finland

S. HASEZAWA, Department of Integrated Biosciences, Graduate School of Frontier Sciences, The University of Tokyo, 5-1-5 Kashiwanoha, Kashiwa, 277-8562, Chiba, Japan

E. HERZOG, Institut de Biologie Moléculaire des Plantes, Centre National de la Recherche Scientifique CNRS, UPR 2357, Université Louis Pasteur, Strasbourg, France

D. INZÉ, VIB/Ghent University, Department of Plant Systems Biology, Technologiepark 927, 9052 Ghent, Belgium

M. ITO, Department of Regulation of Biological Signals, Graduate School of Bioagricultural Sciences, Nagoya University, Furocho, Chikusa-ku, 464-8601, Nagoya, Japan

T. ITOH, Wood Research Institute, Kyoto University, Gokasho, Uji, Kyoto 611-0011, Japan

A.M. JONES, Department of Biology, University of North Carolina, Chapel Hill, NC 27599, USA

J. JOUBÈS, present address: Laboratoire de Biogenèse Membranaire – FRE 2694 CNRS, Université Victor Ségalen Bordeaux 2, 146, rue Léo Saignat, F-33076, Bordeaux Cedex, France

J. JOUBÈS, VIB/Ghent University, Department of Plant Systems Biology, Technologiepark 927, 9052 Ghent, Belgium

G. KIUNA, Institute of Biotechnology, University of Cambridge, Tennis Court Road, Cambridge, CB2 1QT, UK

M. KOBAYASHI, Plant Nutrition Laboratory, Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, 606-8502, Kyoto, Japan

F. KUMAGAI, Department of Integrated Biosciences, Graduate School of Frontier Sciences, The University of Tokyo, 5-1-5 Kashiwanoha, Kashiwa, 277-8562, Chiba, Japan

T. KUROIWA, Department of Life Science, College of Science, Rikkyo (St. Paul's) University, 3-34-1 Nishiikebukuro, Toshima-ku, 171-8501, Tokyo, Japan

N. KUTSUNA, Department of Integrated Biosciences, Graduate School of Frontier Sciences, The University of Tokyo, 5-1-5 Kashiwanoha, Kashiwa, 277-8562, Chiba, Japan

A.-M. LAMBERT, IBMP, 12, rue du Général Zimmer, 67084, Strasbourg Cedex, France

K. LAUKENS, Laboratory of Plant Biochemistry and Physiology, Department of Biology, University of Antwerp (UIA), Universiteitsplein 1, 2610, Antwerp, Belgium

T. MATOH, Plant Nutrition Laboratory, Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, 606-8502, Kyoto, Japan

K. MATSUOKA, Plant Science Center Riken, 1-7-2 Suehirocho, Tsurumi-ku, 230-0045, Yokohama, Japan

J.A.H. MURRAY, Institute of Biotechnology, University of Cambridge, Tennis Court Road, Cambridge, CB2 1QT, UK

Y. MIYAZAWA, RIKEN (The Institute of Physical and Chemical Research), 2-1 Hirosawa, Wako-shi, 351-0198, Saitama, Japan

T. NAGATA, Department of Biological Sciences, Graduate School of Science, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, 113-0033, Tokyo, Japan

K. NISHITANI, Biological Institute, Graduate School of Sciences, Tohoku University, Aoba, Aramaki, Aobaku, Sendai 981-0945, Japan

K.-M. OKSMAN-CALDENTNEY, VTT Biotechnology, (Tietotie 2), P.O. Box 1500, 02044 VTT, Finland

Y. PARMENTIER, Institut de Biologie Moléculaire des Plantes du CNRS, 12, rue du Général Zimmer, 67084 Strasbourg Cedex, France

S. PLANCHAIS, Institute of Biotechnology, University of Cambridge, Tennis Court Road, Cambridge, CB2 1QT, UK

T. POTUSCHAK, Institut de Biologie Moléculaire des Plantes du CNRS, 12, rue du Général Zimmer, 67084 Strasbourg Cedex, France

J.-P. RENAUDIN, UMR PBV (Physiologie et Biotechnologies Végétale), INRA-IBVM, BP 8133883, Villenave d'Ornon Cedex, France

L. ROEF, Laboratory of Plant Biochemistry and Physiology, Department Biology, University of Antwerp (UIA), Universiteitsplein 1, 2610, Antwerp, Belgium

A. SAKAI, Department of Biological Science, Faculty of Science, Nara Women's University, Kitauoya, Nara-shi, Nara 630-8506, Japan

K. SAKAMOTO, Department of Biological Sciences, Graduate School of Science, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, 113-0033, Tokyo, Japan

T. SANO, Department of Biological Sciences, Graduate School of Science, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, 113-0033, Tokyo, Japan

A.-C. SCHMIT, Institut de Biologie Moléculaire des Plantes, Centre National de la Recherche Scientifique CNRS, UPR 2357, Université Louis Pasteur, Strasbourg, France

M. SEKINE, Graduate School of Biological Sciences, Nara Institute of Science and Technology (NAIST), Takayama 8916-5, Ikoma, 630-0101, Nara, Japan

V. SELTZER, Institut de Biologie Moléculaire des Plantes, Centre National de la Recherche Scientifique CNRS, UPR 2357, Université Louis Pasteur, Strasbourg, France

T. SHIMIZU, Department of Biological Sciences, Graduate School of Science, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, 113-0033, Tokyo, Japan

T. SHIMMEN, Department of Life Science, Graduate School of Science, Himeji Institute of Technology, Harima Science Park City, 678-1297, Hyogo, Japan

A. SHINMYO, Graduate School of Biological Sciences, Nara Institute of Science and Technology (NAIST), Takayama 8916-5, Ikoma, 630-0101, Nara, Japan

S. SONOBE, Department of Life Science, Graduate School of Science, Himeji Institute of Technology, Harima Science Park City, 678-1297, Hyogo, Japan

M. SUGIURA, Graduate School of Natural Sciences, Nagoya City University, Yamanohata, Mizuho, 467-8501, Nagoya, Japan

D. TANAKA, Biological Institute, Graduate School of Sciences, Tohoku University, Aoba, Aramaki, Aobaku, Sendai 981-0945, Japan

R.D. THOMPSON, INRA-URGELP Legume Unit, BP 86510, 21065 Dijon Cédex, France

H. ULLAH, Department of Biology, University of North Carolina, Chapel Hill, NC 27599, USA

H.VAN ONCKELEN, Laboratory of Plant Biochemistry and Physiology, Department Biology, University of Antwerp (UIA), Universiteitsplein 1, 2610, Antwerp, Belgium

E. WITTERS, Laboratory of Plant Biochemistry and Physiology, Department of Biology, University of Antwerp (UIA), Universiteitsplein 1, 2610, Antwerp, Belgium

H. YASUHARA, Department of Biotechnology, Faculty of Engineering, Kansai University, Yamate-cho Suita, 564-8680, Osaka, Japan

E. YOKOTA, Department of Life Science, Graduate School of Science, Himeji Institute of Technology, Harima Science Park City, 678-1297, Hyogo, Japan

R. YOKOYAMA, Biological Institute, Graduate School of Sciences, Tohoku University, Aoba, Aramaki, Aobaku, Sendai 981-0945, Japan

A. YONEDA, Department of Integrated Biosciences, Graduate School of Frontier Sciences, The University of Tokyo, 5-1-5 Kashiwanoha, Kashiwa, 277-8562, Chiba, Japan

Y. YUKAWA, Graduate School of Natural Sciences, Nagoya City University, Yamanohata, Mizuho, 467-8501, Nagoya, Japan