

Contents

Chapter 1	The Molecular Biology of Cauliflower Mosaic Virus and Its Application as Plant Gene Vector B. Gronenborn, Köln, Federal Republic of Germany
I.	Introduction 1
II.	The Biology of Cauliflower Mosaic Virus 2
A.	The Virus Particle 3
B.	The DNA of CaMV 4
C.	The Genetic Organisation of the CaMV 4
1.	The Genes of CaMV 6
2.	Transcripts of CaMV 8
D.	The Replication of CaMV 9
E.	Structural Homologies Between CaMV and Retroid Elements 12
III.	The Development of CaMV into a Plant Gene Vector 13
A.	Mutants of CaMV 13
1.	Mutations in ORF II 13
2.	Mutations in Other Regions of the CaMV Genome 14
B.	Translational Polarity 14
C.	Transducing Cauliflower Mosaic Virus Variants 16
D.	Defective Complementing Mutants of CaMV 16
IV.	Elements of CaMV as Tools in Plant Genetic Engineering 18
V.	Vector Based on Other Plant Viruses 19
VI.	Conclusion and Outlook 20
VII.	References 21
Chapter 2	The Structure, Expression, Functions and Possible Exploitation of Geminivirus Genomes J. W. Davies, R. Townsend and J. Stanley, Norwich, U. K.
I.	Introduction 31
II.	Genome Organisation 32
A.	Coding Regions 32
B.	Non-coding Regions 36
III.	Gene Expression 37
IV.	Gene Functions 41
V.	The Potential of Geminiviruses as Gene Vectors 43
VI.	References 49

Chapter 3 **cDNA Cloning of Plant RNA Viruses and Viroids**

P. Vos, Wageningen, The Netherlands

- I. General Introduction 54
- II. Construction of Full-Length cDNA Clones 54
 - A. Introduction 54
 - B. Synthesis of Double-Stranded cDNA 54
 - C. Cloning Strategies 56
 - D. Cloning in Transcription Vectors 57
- III. DNA Copies as Tools to Study the Molecular Biology of Plant RNA Viruses 59
 - A. Introduction 59
 - B. Infectivity of cDNA Clones 60
 - i) Infectivity of DNA Copies 60
 - ii) Infectivity of *in vitro* Transcripts from DNA Copies 61
 - C. RNA Replication 62
 - D. RNA Recombination 65
 - E. Genetic Organisation and Gene Expression 66
- IV. Viroids and Satellites 71
 - A. Introduction 71
 - B. Molecular Cloning of Viroids 73
 - C. Application of cDNA Clones 74
 - i) Viroids 74
 - ii) Satellite Viruses 76
- V. Diagnosis of Plant Diseases Using DNA Copies of Plant Viruses and Viroids 77
 - A. Introduction 77
 - B. Spot Hybridisation 77
- VI. Conclusions and Future Aspects 78
- VII. References 79

Chapter 4 **Agroinfection**

N. Grimsley and D. Bisaro, Basel, Switzerland, and Auburn, Ala., U.S.A.

- I. Introduction 88
- II. Potential Applications of Agroinfection 89
 - A. *Agrobacterium* as an Organism for the Experimental Storage and Transmission of Plant Viruses 89
 - 1. Storage and Safety 89
 - 2. Efficiency and Flexibility 90
 - 3. Release of Viral Genomes from the T-DNA 93
 - 4. Analysis of T-DNA Transfer 94
 - B. Transformation of Plant Cells with Viral Genetic Information 96
 - 1. Transient Expression 96
 - 2. Expression of Viral Genes in Host and Non-Host Plants 97

3. Transgenic Plants Containing Oligomers of Viral Genomes or Genome Components 98
 - i) Complementation Between Different Components of a Multi-Component System 98
 - ii) Analysis of *in vitro* Produced Mutant Viral Strains 100
 - iii) Development of Proviral Vectors 100
 4. Super-Infection of Plants Transgenic for Viral Sequences 101
 - i) Cross-Protection 102
 - ii) Complementation of Defective Viral Genes with Integrated Wild-Type-Genes; Development of Complementation Vectors 103
- III. Perspectives 103
- IV. References 104

Chapter 5 **The Mechanism of T-DNA Transfer from *Agrobacterium tumefaciens* to the Plant Cell**
 Z. Koukolíková-Nicola, L. Albright and B. Hohn, Boston, Mass., U.S.A., and Basel, Switzerland

- I. General Introduction 110
 - A. Scope of the Review 110
 - B. Crown Gall Disease 110
 - C. Molecular Basis of Neoplastic Transformation 111
 - a) The Ti Plasmid and Its Organisation 111
 - b) Functional Organisation of the T-DNA 113
- II. Early Events of Transformation 114
 - A. Virulence Functions 114
 - a) Chromosomal Virulence Region 114
 - b) Organisation of the Virulence Region 115
 - c) Regulation of *vir* Region Expression 116
 - d) Nature of the Inducer 118
 - B. T-DNA-Transfer 119
 - a) The 25-bp Terminal Sequence 119
 - b) Overdrive 122
 - c) Analysis of the T-DNA/Plant DNA Junctions 123
 - d) T-DNA Localisation and Structure Within the Plant Genome 124
- III. T-DNA Processing 124
 - A. The Search of Processing Intermediates 124
 - a) Genetic Assays 125
 - b) Physical Assays 127
 - c) Summary and Discussion 129
 - B. Proteins Involved in T-DNA Processing 133
 - a) *Vir* D Locus 133
 - b) *Vir* C Locus 134
 - c) *Vir* E Locus 135

	d) <i>Vir</i> F Locus	137
IV.	Conclusions	137
V.	References	138
Chapter 6	Molecular Analysis of Root Induction by <i>Agrobacterium rhizogenes</i>	
	F. F. White and V. P. Sinkar, Manhattan, Kans., U.S.A., and Seattle, Wash., U.S.A.	
	I. Introduction	149
	II. Taxonomy	150
	III. Ri Plasmid Structure	153
	IV. Ri T-DNA Organization	157
	V. T-DNA of Ri Transformed Plants	163
	VI. Endogenous T-DNA of Plants	168
	VII. Conclusions and Further Speculations	170
	VIII. References	172
Chapter 7	Pathways to Plant Genetic Manipulation Employing <i>Agrobacterium</i>	
	St. G. Rogers and H. Klee, St. Louis, Mont., U.S.A.	
	I. Introduction	179
	II. Biology of <i>Agrobacterium tumefaciens</i> Ti Plasmid	180
III.	Strategies for Inserting Genes into T-DNA	181
	A. Homogenotization	181
	B. Cointegrating Intermediate Vectors	182
	C. Binary Vectors	183
	D. Disarming the T-DNA	184
	E. Specific Examples	187
	1. Use of pMON200: A Cointegrating Vector	187
	2. Use of pMON505: A Binary Vector	190
	F. Border Sequences and Binary Vector T-DNA Structure	191
IV.	Practical Catalogue	193
	A. Survey of Binary Vectors	193
	B. Selectable Markers	195
	C. Expression Cassette Vectors	196
	V. Getting Genes into Plants	197
VI.	Novel Applications of Ti Transformation	197
	A. Gene Isolation by Complementation or Direct Selection	197
	B. T-DNA as a Transposon for Mutation and Promoter Probe	198
VII.	Prospects	199
VIII.	References	199

- Chapter 8 Plant Transposable Elements: Unique Structures for Gene Tagging and Gene Cloning**
 U. Wienand and H. Saedler, Köln, Federal Republic of Germany
- I. Introduction 205
 - II. Isolation and Characterization of a Transposable Element 207
 - A. Recognition of a Transposable Element 207
 - B. Genes Suitable for the Isolation of Transposable Elements 207
 - C. Transposon Tagging of a Gene in *Zea mays* 211
 - D. Genetical Analysis of the Tagged Mutants 213
 - E. Frequency of Mutation 215
 - III. Transposable Elements as Molecular Probes for Gene Isolation 216
 - A. General Aspects 216
 - B. Isolation of Mutants Induced by Autonomous Elements (*Ac* and *En [Spm]*) 216
 - C. Isolation of Clones Carrying Receptor Elements 218
 - D. Identification of Gene-Specific Sequences 219
 - IV. Conclusions 220
 - V. References 221
- Chapter 9 Direct Gene Transfer to Plants**
 I. Potrykus, J. Paszkowski, R. D. Shillito, and M. W. Saul, Basel, Switzerland
- I. Direct Gene Transfer 230
 - A. Introduction 230
 - B. A Representative Experiment 231
 - C. Protocol and Transformation Frequency 232
 - D. Electroporation 233
 - E. No Hostrange Limitations 233
 - F. Foreign Gene Mendelian Inheritance 233
 - G. Stability of the Foreign Gene 234
 - H. Instability of the Foreign Gene 234
 - I. Molecular Proof for Transformation 235
 - J. Gene Localization by *in situ* Hybridization 236
 - K. Arrangement of Foreign DNA in the Host Genome 236
 - L. Co-Transformation with Non-Selectable Genes 237
 - M. Gene Transfer from Total Genomic DNA 239
 - N. Limitations for Direct Gene Transfer 240
 - II. Other Vectorless Gene Transfer Systems 240
 - A. Liposome Fusion 240
 - B. Spheroplast Fusion 240
 - C. Microinjection 240
 - III. Direct Gene Transfer in Theoretical and Applied Genetics 240
 - A. Gene Isolation 241

- B. Gene Identification 241
- C. Replication 241
- D. Gene Replacement 241
- E. Gene Regulation 242
- F. Stability and Instability 242
- G. Gene Transfer to Chloroplasts and Mitochondria 242
- H. Gene Transfer into Cereals 242
- I. Gene Transfer into Potentially Totipotent Cells 243
- J. Gene Transfer Without Pre Cloning in Bacteria 243
- K. Gene Transfer into Organelles 244
- L. Tagging of Chromosomes 244
- M. Modulation of Expression 244
- N. Conclusions 244
- IV. References 245

Chapter 10 **Microinjection: An Experimental Tool for Studying and Modifying Plant Cells**

L. A. Miki, T. J. Reich, and V. N. Iyer, Ottawa, Ont., Canada

- I. Introduction 249
- II. Recipient Cell Systems 250
 - A. Cell Types 250
 - B. Protoplasts 251
 - C. Cell Culture Conditions 251
- III. Resolution of Intracellular Compartments 253
 - A. Microscopy 253
 - B. Fluorescent Stains 255
- IV. Microinjection Methodology 256
 - A. Micromanipulation Techniques 256
 - B. Equipment 259
- V. Genetic Transformation 260
- VI. Other Applications 260
- VII. Concluding Remarks 261
- VIII. References 262

Chapter 11 **Transformation of *Chlamydomonas Reinhardtii***

J.-D. Rochaix, Geneva, Switzerland

- I. Introduction 267
- II. Nuclear Transformation 270
 - A. Selection 271
 - i) ARG7 Locus 271
 - ii) Resistance to Kanamycin 271
 - iii) Other Selective Markers 272
 - B. ARS Sequences of *C. reinhardtii*
 - C. ARC Sequences 275
 - D. Natural Plasmids 276

- E. Is *Agrobacterium tumefaciens* a Possible Transformation Vector for *C. reinhardtii*? 278
- III. Prospects of Chloroplast Transformation in *C. reinhardtii* 278
- IV. Conclusions 279
- V. References 280
- Chapter 12 **Induction of Expression in and Stable Transformation of an Algal Cell by Nuclear Microinjection with Naked DNA**
H.-G. Schweiger and G. Neuhaus, Heidelberg, Federal Republic of Germany
- I. Introduction 285
- II. Acetabularia 286
- III. Techniques 287
- IV. Expression of Genomic RNA 289
- V. Expression of Genomic DNA 291
- VI. Expression of Genes and Gene Constructions 292
- VII. Regulation of Expression 294
- VIII. Transformation 295
- IX. Genetics 298
- X. Discussion 299
- XI. References 300
- Chapter 13 **Transient Expression of DNA in Plant Cells**
M. Fromm and V. Walbot, Stanford, Calif., U.S.A.
- I. Overview of Transient Assay Applications 304
- II. Transient Assays in Plant Cells 304
- III. Transient Expression after Electroporation-Mediated Gene Transfer 305
- IV. Discussion 308
- V. References 309
- Chapter 14 **Plastid Transformation: A Progress Report**
M. J. Cornelissen, M. De Block, M. Van Montagu, J. Leemanns, P. H. Schreier, and J. Schell, Köln, Federal Republic of Germany, and Gent, Belgium
- I. Introduction 311
- II. Construction of Vectors for the Transformation of Plastids 313
- III. General Conclusions 318
- IV. References 320

Chapter 15 Targeting Nuclear Gene Products into Chloroplasts
L. J. Szabo and A. R. Cashmore, New York, N. Y., U.S.A.

- I. Introduction 321
 - II. Binding of Precursors to the Outer Membrane of the Chloroplast 323
 - III. Translocation of Polypeptides Across the Envelope Membranes 324
 - IV. Processing of Precursors to the Native Polypeptide 325
 - V. The Transit Peptide Itself can Mediate Import of Foreign Polypeptides 327
 - VI. Structural Analysis of Chloroplast Transit Peptides 329
 - VII. Experimental Analysis of Transit Peptides 330
 - VIII. Future Prospects 334
 - IX. References 334
- Subject Index 341**