Table of contents

Plastid biogenesis and differentiation 1
Kevin Pyke 1
Abstract 1
1 Introduction 1
2 Proplastids 2
3 The morphology and structure of different plastid types 3
3.1 Chloroplast structure and morphology 4
3.2 Amyloplast structure and morphology 6
3.3 Chromoplast structure and morphology 8
3.4 Leucoplasts and root plastids 11
3.5 Other types of storage plastids 12
4 The control of plastid differentiation 13
4.1 Plastid interconversions 14
5 Plastid division 16
6 Stromules 19
7 Conclusion 20
Acknowledgement 21
References 21

Structure, function, and inheritance of plastid genomes 29
Ralph Bock 29
Abstract 29
1 Introduction 29
2 Physical properties of plastid genomes 35
2.1 Copy number of plastid genomes 35
2.2 Organization of plastid genomes in nucleoids 36
2.3 Structural conformations of plastid genomes 37
3 Fine structure of plastid genomes 38
3.1 Inverted repeats and single-copy regions 38
3.2 Information content of plastid genomes 39
4 Inheritance of plastid genomes 45
4.1 Maternal inheritance 47
4.2 Biparental inheritance 50
4.3 Paternal inheritance 51
4.4 Paternal leakage 51
4.5 Biotechnological implications of plastid inheritance 52
Acknowledgement 53
References 53
List of abbreviations 63

DNA replication, recombination, and repair in plastids 65
Anil Day and Panagiotis Madesis 65
Abstract 65
1 The importance of DNA replication, recombination, and repair pathways in plastids 65
1.1 Proteins and DNA targets of plastid DNA-RRR pathways 67
2 Plastid DNA polyploidy, packaging, and segregation 67
2.1 Plastid DNA copy number 67
2.2 Packaging of plastid DNA 69
2.3 Segregation of plastid genomes 70
3 Topological forms of plastid DNA 70
3.1 Linear hairpin DNA molecules in plastids 74
3.2 Linear plastid DNA molecules with discrete ends in WT plastids 75
4 A replicon model for plastid genome maintenance 77
4.1 Replication origins mapped to the large inverted repeat 77
4.2 Replication origins located in the single copy regions 80
5 Maintenance of small DNA molecules in plastids 81
6 Deletion mapping delimits DNA sequences capable of self-replication in plastids 82
7 A recombination-dependent DNA replication model of plastid DNA 82
8 DNA recombination in plastids 84
8.1 Integration of foreign genes by homologous recombination 86
8.2 Homologous recombination between short DNA repeats 88
9 Recombination and plastid genome stability 92
10 Homeologous recombination in plastids 93
11 Replication slippage in plastids 94
12 DNA repair in plastids 96
13 Identification of proteins involved in plastid DNA RRR-pathways 97
13.1 Plastid DNA polymerases 98
13.2 DNA primase activities in plastids 100
13.3 Plastid localised RecA 100
13.4 DNA topoisomerases 101
13.5 DNA helicases 103
14 Identifying DNA-RRR proteins by complementation of *E. coli* mutants 103
15 Conclusions and outlook 105
Acknowledgement 107
References 107
List of abbreviations 119

Transcription and transcriptional regulation in plastids 121
Karsten Liere and Thomas Börner 121
Abstract 121
1 Introduction 121
2 RNA polymerases 122

2.1 NEP: nuclear-encoded plastid RNA polymerase 122
2.2 PEP: plastid-encoded plastid RNA polymerase 128
3 Plastidial Promoters 129
3.1 NEP promoters 129
3.2 PEP promoters 132
3.3 Internal promoters of tRNAs 133
4 Regulation of transcription in plastids 133
4.1 Role of multiple and diverse promoters 135
4.2 Transcription factors involved in promoter recognition in plastids 139
4.3 Exogenous and endogenous factors controlling plastidial transcription 148
Acknowledgement 154
References 154

Processing, degradation, and polyadenylation of chloroplast transcripts 175
Thomas J. Bollenbach, Gadi Schuster, Victoria Portnoy, and David B. Stern 175
Abstract 175
1 Introduction 175
2 The enzymes of RNA degradation and maturation 177
2.1 Endoribonucleases 177
2.2 Exoribonucleases 181
3 Polyadenylation 185
3.1 Historical perspective on polyadenylation 185
3.2 The polyadenylation-stimulated degradation pathway in bacteria .. 186
3.3 PNPase as the major polyadenylating enzyme: variations from *E. coli* 187
3.4 Polyadenylation in the chloroplast 189
4 RNA maturation 191
4.1 5' end maturation 191
4.2 Intercistronic processing 192
4.4 Non-coding RNAs 195
5 Regulatory factors 195
5.1 Mutations affecting single chloroplast loci 196
5.2 Pleiotropic mutations 197
5.3 The PPR/TPR protein superfamilies 198
6 Conclusions 198
Acknowledgements 199
References 199

RNA splicing and RNA editing in chloroplasts 213
Christian Schmitz-Linneweber and Alice Barkan 213
Abstract 213
1 Introduction 213
2 Plastid RNA splicing 213

2.1 Intron classes and splicing mechanisms 214
2.2 Intron distribution 215
2.3 Proteins involved in the splicing of chloroplast introns 216
2.4 The regulation of chloroplast RNA splicing 223
2.5 Perspective 225
3 Plastid RNA editing 226
3.1 Editing sites impact protein function 227
3.2 Mechanism of RNA editing 227
3.3 *cis*-elements involved in plastid RNA editing 230
3.4 *trans*-factors involved in plastid RNA editing 232
3.5 Models for the editosome 234
3.6 Function and evolution of plastid RNA editing 236
3.7 Perspectives 238
Acknowledgement 238
References 238

Translation and translational regulation in chloroplasts 249
Hadas Peled-Zehavi and Avihai Danon 249
Abstract 249
1 Introduction 249
2 Chloroplast translation machinery 251
3 Mechanisms of translation initiation 253
4 Translation initiation regulation – intricate interplay between cis- and trans-acting elements 258
4.1 Cis-elements in chloroplast 5'UTRs 258
4.2 Structural elements in 5'UTRs 259
4.3 General and specific translation factors 260
4.4 Multiple proteins interact with single mRNA 261
5 Translation regulation examples 262
5.1 Translation regulation of D1 synthesis 262
5.2 Negative feedback loops: assembly-controlled regulation of translation 265
6 Regulation of translation elongation 266
7 Interactions of 5' and 3' ends of chloroplast mRNA 267
8 Subchloroplast location of translation 268
9 Concluding remarks 269
Acknowledgement 270
References 271

Assembly of protein complexes in plastids 283
Eira Kanervo, Marjaana Suorsa, and Eva-Mari Aro 283
Abstract 283
1 Introduction 283
2 Assembly of the protein complexes 284
2.1 Assembly of PSII 284
2.2 Assembly of the PSI complex 293

2.3 Assembly of the Cyt b_6f complex 295
2.4 Assembly of soluble complexes 296
3 Insertion of proteins to the thylakoid membrane - thylakoid translocase complexes and chaperones 297
3.1 Thylakoid translocases 297
3.2 Chaperones 299
4 Posttranslational modifications of chloroplast proteins 300
4.1 N-terminal methionine excision 300
4.2 Protein phosphorylation 301
5 Concluding remarks 302
Acknowledgements 302
References 303

Protein stability and degradation in plastids 315
Zach Adam 315
Abstract 315
1 Introduction 315
2 Major chloroplast proteases 316
2.1 Clp protease 317
2.2 FtsH protease 319
2.3 Lon protease 321
2.4 Deg protease 322
2.5 Intramembrane proteases 322
3 Proteolytic processes in chloroplasts and the enzymes involved 323
3.1 Maturation of pre-proteins 323
3.2 Adaptation to changing light intensities 325
3.3 Protein quality control 325
3.4 Oxidatively damaged proteins 327
4 Other functions 328
4.1 Nutrient stress and senescence 328
4.2 Thermotolerance 329
5 Identification of specific substrates 329
6 Determinants of protein instability 329
7 Future prospects 330
Acknowledgement 331
References 332

Protein import into plastids 339
Birgit Agne and Felix Kessler 339
Abstract 339
1 Plastids 339
1.1 Plastid biogenesis 340
2 Chloroplast targeting signals 340
2.1 Structure of transit peptides 341
3 Energy requirements of *in vitro* chloroplast protein import 341
3.1 Precursor protein recognition at the chloroplast surface 341

3.2 The early translocation intermediate 341
3.3 The late translocation intermediate 342
4 Identification of components of the translocation machinery 343
4.1 Components of the Toc complex 343
4.2 Components of the Tic complex 346
5 Regulation at the Toc and Tic complexes 349
5.1 GTP-regulated protein recognition at the Toc complex 349
5.2 Regulation by phosphorylation 351
5.3 Redox-regulation 352
5.4 Calcium/calmodulin regulation 352
6 Functional specialization in the general import pathway 354
6.1 Plastid protein import mutants and phenotypes 354
6.2 Expression patterns of Toc GTPases 358
6.3 Biochemical evidence for functional specialization of chloroplast import receptors 358
6.4 Substrate specificity of Toc-GTPase sub-pathways 358
7 Toc/Tic independent “alternative” import pathways into the chloroplast 359
7.1 Import depending on internal targeting sequences 359
7.2 Substrate dependent import 360
7.3 Protein import via the secretory pathway 360
Acknowledgements 362
References 362

Insights into chloroplast proteomics: from basic principles to new horizons 371
Bianca Naumann and Michael Hippler 371
Abstract 371
1 The art of proteomics 371
1.1 Prerequisite for biomolecular mass spectrometry: MALDI and ESI Ionization 372
1.2 Peptide mass finger printing and tandem mass spectrometry 372
1.3 Database searching 374
1.4 *De Novo* sequencing 375
1.5 Linking database searching and *de novo* sequencing 375
1.6 Strategies for the analysis of proteome dynamics 377
2 Proteomics of the chloroplast and its compartments 379
2.1 Envelope membranes 379
2.2 Stroma and chloroplast ribosome 381
2.3 Thylakoid membrane 384
2.4 Thylakoid lumen 388
4 Comparative proteomics 392
4.1 Plant and chloroplast development 392
4.2 Biotic stress 393
4.3 Abiotic stress 394
5 Conclusion 398
Acknowledgement 398

References 398
List of abbreviations 406

Plastid-nucleus communication: anterograde and retrograde signalling in the development and function of plastids 409
Katharina Bräutigam, Lars Dietzel, and Thomas Pfannschmidt 409
Abstract 409
1 Introduction 409
2 Major problems of coordination and communication between plastids and nucleus 410
2.1 Tissue specificity of plastid development 411
2.2 The gene copy number problem 411
2.3 Integration of plastid responses within the cell 413
3 Anterograde signalling 413
3.1 The nuclear control principle 413
3.2 Developmental signals 418
3.3 Environmental control of plastid development 422
4 Retrograde signalling 423
4.1 Signals depending on plastid gene expression 423
4.2 Retrograde signals depending on pigment synthesis 425
4.3 Redox signals from chloroplasts 431
4.4 Plastid signals controlling tissue development 441
5 Conclusions and perspectives 442
Acknowledgements 443
References 443
List of abbreviations 455

The genetic transformation of plastids 457
Hans-Ulrich Koop, Stefan Herz, Timothy J Golds, and Jörg Nickelsen 457
Abstract 457
1 Introduction 457
1.1 Plastid biology in *Chlamydomonas* and tobacco 458
2 General procedures 462
2.1 Gene transfer methods 462
2.2 Transformation vectors 463
2.3 Marker gene removal 465
3 Plastid transformation in algae 467
3.1 Expression control elements 467
3.2 Resistance marker genes 468
3.3 Targeted inactivation 468
3.4 Introduced genes, expressed proteins 471
3.5 Transformed species 471
4 Plastid transformation in higher plants 472
4.1 Expression control elements 472
4.2 Inducible gene expression 474
4.3 Resistance marker genes and selection schemes 475

4.4 Targeted inactivation 477
4.5 Introduced genes, expressed proteins 479
4.6 Transformed species 489
5 Perspectives 493
References 494

Index 511