

Contents

About the Series Editors *xvii*

1	Platform Technology for Therapeutic Protein Production	1
	<i>Tae Kwang Ha, Jae Seong Lee, and Gyun Min Lee</i>	
1.1	Introduction	1
1.2	Overall Trend Analysis	3
1.2.1	Mammalian Cell Lines	3
1.2.2	Brief Introduction of Advances and Techniques	5
1.3	General Guidelines for Recombinant Cell Line Development	6
1.3.1	Host Selection	6
1.3.2	Expression Vector	7
1.3.3	Transfection/Selection	7
1.3.4	Clone Selection	8
1.3.4.1	Primary Parameters During Clone Selection	8
1.3.4.2	Clone Screening Technologies	9
1.4	Process Development	9
1.4.1	Media Development	10
1.4.2	Culture Environment	10
1.4.3	Culture Mode (Operation)	10
1.4.4	Scale-up and Single-Use Bioreactor	11
1.4.5	Quality Analysis	12
1.5	Downstream Process Development	12
1.5.1	Purification	12
1.5.2	Quality by Design (QbD)	13
1.6	Trends in Platform Technology Development	14
1.6.1	Rational Strategies for Cell Line and Process Development	14
1.6.2	Hybrid Culture Mode and Continuous System	15
1.6.3	Recombinant Human Cell Line Development for Therapeutic Protein Production	16
1.7	Conclusion	17
	Acknowledgment	17
	Conflict of Interest	17
	References	17

2	Cell Line Development for Therapeutic Protein Production	23
	<i>Soo Min Noh, Seunghyeon Shin, and Gyun Min Lee</i>	
2.1	Introduction	23
2.2	Mammalian Host Cell Lines for Therapeutic Protein Production	25
2.2.1	CHO Cell Lines	25
2.2.2	Human Cell Lines	26
2.2.3	Other Mammalian Cell Lines	27
2.3	Development of Recombinant CHO Cell Lines	27
2.3.1	Expression Systems for CHO Cells	28
2.3.2	Cell Line Development Process Using CHO Cells Based on Random Integration	28
2.3.2.1	Vector Construction	29
2.3.2.2	Transfection and Selection	30
2.3.2.3	Gene Amplification	30
2.3.2.4	Clone Selection	31
2.3.3	Cell Line Development Process Using CHO Cells Based On Site-Specific Integration	32
2.4	Development of Recombinant Human Cell Lines	34
2.4.1	Necessity for Human Cell Lines	34
2.4.2	Stable Cell Line Development Process Using Human Cell Lines	35
2.5	Important Consideration for Cell Line Development	36
2.5.1	Clonality	36
2.5.2	Stability	36
2.5.3	Quality of Therapeutic Proteins	37
2.6	Conclusion	38
	References	38
3	Transient Gene Expression-Based Protein Production in Recombinant Mammalian Cells	49
	<i>Joo-Hyoung Lee, Henning G. Hansen, Sun-Hye Park, Jong-Ho Park, and Yeon-Gu Kim</i>	
3.1	Introduction	49
3.2	Gene Delivery: Transient Transfection Methods	50
3.2.1	Calcium Phosphate-Based Transient Transfection	50
3.2.2	Electroporation	51
3.2.3	Polyethylenimine-Based Transient Transfection	52
3.2.4	Liposome-Based Transient Transfection	52
3.3	Expression Vectors	53
3.3.1	Expression Vector Composition and Preparation	53
3.3.2	Episomal Replication	53
3.3.3	Coexpression Strategies	54
3.4	Mammalian Cell Lines	54
3.4.1	HEK293 Cell-Based TGE Platforms	55
3.4.2	CHO Cell-Based TGE Platforms	56
3.4.3	TGE Platforms Using Other Cell Lines	58
3.5	Cell Culture Strategies	58
3.5.1	Culture Media for TGE	58

3.5.2	Optimization of Cell Culture Processes for TGE	59
3.5.3	q_p -Enhancing Factors in TGE-Based Culture Processes	59
3.5.4	Culture Longevity-Enhancing Factors in TGE-Based Culture Processes	59
3.6	Large-Scale TGE-Based Protein Production	60
3.7	Concluding Remarks	62
	References	62
4	Enhancing Product and Bioprocess Attributes Using Genome-Scale Models of CHO Metabolism	73
	<i>Shangzhong Li, Anne Richelle, and Nathan E. Lewis</i>	
4.1	Introduction	73
4.1.1	Cell Line Optimization	73
4.1.2	CHO Genome	75
4.1.2.1	Development of Genomic Resources of CHO	75
4.1.2.2	Development of Transcriptomics and Proteomics Resources of CHO	75
4.2	Genome-Scale Metabolic Model	76
4.2.1	What Is a Genome-Scale Metabolic Model	76
4.2.2	Reconstruction of GEMs	77
4.2.2.1	Knowledge-Based Construction	77
4.2.2.2	Draft Reconstruction	77
4.2.2.3	Curation of the Reconstruction	77
4.2.2.4	Conversion to a Computational Format	79
4.2.2.5	Model Validation and Evaluation	79
4.3	GEM Application	80
4.3.1	Common Usage and Prediction Capacities of Genome-Scale Models	82
4.3.2	GEMs as a Platform for <i>Omics</i> Data Integration, Linking Genotype to Phenotype	83
4.3.3	Predicting Nutrient Consumption and Controlling Phenotype	84
4.3.4	Enhancing Protein Production and Bioprocesses	85
4.3.5	Case Studies	86
4.4	Conclusion	86
	Acknowledgments	88
	References	88
5	Genome Variation, the Epigenome and Cellular Phenotypes	97
	<i>Martina Baumann, Gerald Klanert, Sabine Vcelar, Marcus Weinguny, Nicolas Marx, and Nicole Borth</i>	
5.1	Phenotypic Instability in the Context of Mammalian Production Cell Lines	97
5.2	Genomic Instability	99
5.3	Epigenetics	101
5.3.1	DNA Methylation	102

5.3.2	Histone Modifications	102
5.3.3	Downstream Effectors	104
5.3.4	Noncoding RNAs	104
5.4	Control of CHO Cell Phenotype by the Epigenome	105
5.5	Manipulating the Epigenome	107
5.5.1	Global Epigenetic Modification	107
5.5.1.1	Manipulating Global DNA Methylation	107
5.5.1.2	Manipulating Global Histone Acetylation	108
5.5.2	Targeted Epigenetic Modification	109
5.5.2.1	Targeted Histone Modification	110
5.5.2.2	Targeted DNA Methylation	112
5.6	Conclusion and Outlook	113
	References	114
6	Adaption of Generic Metabolic Models to Specific Cell Lines for Improved Modeling of Biopharmaceutical Production and Prediction of Processes	127
	<i>Calmels Cyrielle, Chintan Joshi, Nathan E. Lewis, Malphettes Laetitia, and Mikael R. Andersen</i>	
6.1	Introduction	127
6.1.1	Constraint-Based Models	127
6.1.2	Limitations of Flux Balance Analysis	131
6.1.2.1	Thermodynamically Infeasible Cycles	131
6.1.2.2	Genetic Regulation	131
6.1.2.3	Limitation of Intracellular Space	132
6.1.2.4	Multiple States in the Solution	132
6.1.2.5	Biological Objective Function	133
6.1.2.6	Kinetics and Metabolite Concentrations	133
6.2	Main Source of Optimization Issues with Large Genome-Scale Models: Thermodynamically Infeasible Cycles	134
6.2.1	Definition of Thermodynamically Infeasible Fluxes	134
6.2.2	Loops Involving External Exchange Reactions	134
6.2.2.1	Reversible Passive Transporters from Major Facilitator Superfamily (MFS)	135
6.2.2.2	Reversible Passive Antiporters from Amino Acid-Polyamine-organoCation (APC) Superfamily	136
6.2.2.3	Na ⁺ -linked Transporters	136
6.2.2.4	Transport via Proton Symport	137
6.2.3	Tools to Identify Thermodynamically Infeasible Cycles	138
6.2.3.1	Visualizing Fluxes on a Network Map	138
6.2.3.2	Algorithms Developed	138
6.2.4	Methods Available to Remove Thermodynamically Infeasible Cycles	139
6.2.4.1	Manual Curation	139
6.2.4.2	Software and Algorithms Developed for the Removal of Thermodynamically Infeasible Loops from Flux Distributions	140
6.3	Consideration of Additional Biological Cellular Constraints	144

6.3.1	Genetic Regulation	144
6.3.1.1	Advantages of Considering Gene Regulation in Genome-Scale Modeling	144
6.3.1.2	Methods Developed to Take into Account a Feedback of FBA on the Regulatory Network	145
6.3.2	Context Specificity	146
6.3.2.1	What Are Context-Specific Models (CSMs)?	146
6.3.2.2	Methods and Algorithms Developed to Reconstruct Context-Specific Models (CSMs)	146
6.3.2.3	Performance of CSMs	148
6.3.2.4	Cautions About CSMs	149
6.3.3	Molecular Crowding	150
6.3.3.1	Consequences on the Predictions	150
6.3.3.2	Methods Developed to Account for a Total Enzymatic Capacity into the FBA Framework	151
6.4	Conclusion	152
	References	153
7	Toward Integrated Multi-omics Analysis for Improving CHO Cell Bioprocessing	163
	<i>Kok Siong Ang, Jongkwang Hong, Meiyappan Lakshmanan, and Dong-Yup Lee</i>	
7.1	Introduction	163
7.2	High-Throughput Omics Technologies	165
7.2.1	Sequencing-Based Omics Technologies	165
7.2.1.1	Historical Developments of Nucleotide Sequencing Techniques	165
7.2.1.2	Genome Sequencing of CHO Cells	166
7.2.1.3	Transcriptomics of CHO Cells	167
7.2.1.4	Epigenomics of CHO Cells	168
7.2.2	Mass Spectrometry-Based Omics Technologies	168
7.2.2.1	Mass Spectrometry Techniques	168
7.2.2.2	Proteomics of CHO Cells	170
7.2.2.3	Metabolomics/Lipidomics of CHO Cells	171
7.2.2.4	Glycomics of CHO Cells	172
7.3	Current CHO Multi-omics Applications	172
7.3.1	Bioprocess Optimization	174
7.3.2	Cell Line Characterization	174
7.3.3	Engineering Target Identification	176
7.4	Future Prospects	177
	References	178
8	CRISPR Toolbox for Mammalian Cell Engineering	185
	<i>Daria Sergeeva, Karen Julie la Cour Karottki, Jae Seong Lee, and Helene Fastrup Kildegaard</i>	
8.1	Introduction	185
8.2	Mechanism of CRISPR/Cas9 Genome Editing	186
8.3	Variants of CRISPR-RNA-guided Endonucleases	187
8.3.1	Diversity of CRISPR/Cas Systems	187

8.3.2	Engineered Cas9 Variants	188
8.4	Experimental Design for CRISPR-mediated Genome Editing	188
8.4.1	Target Site Selection and Design of gRNAs	189
8.4.2	Delivery of CRISPR/Cas9 Components	191
8.5	Development of CRISPR/Cas9 Tools	192
8.5.1	CRISPR/Cas9-mediated Gene Editing	192
8.5.1.1	Gene Knockout	192
8.5.1.2	Site-Specific Gene Integration	194
8.5.2	CRISPR/Cas9-mediated Genome Modification	195
8.5.2.1	Transcriptional Regulation	195
8.5.2.2	Epigenetic Modification	196
8.5.3	RNA Targeting	196
8.6	Genome-Scale CRISPR Screening	197
8.7	Applications of CRISPR/Cas9 for CHO Cell Engineering	197
8.8	Conclusion	199
	Acknowledgment	200
	References	200
9	CHO Cell Engineering for Improved Process Performance and Product Quality	207
	<i>Simon Fischer and Kerstin Otte</i>	
9.1	CHO Cell Engineering	207
9.2	Methods in Cell Line Engineering	208
9.2.1	Overexpression of Engineering Genes	208
9.2.2	Gene Knockout	209
9.2.3	Noncoding RNA-mediated Gene Silencing	209
9.3	Applications of Cell Line Engineering Approaches in CHO Cells	211
9.3.1	Enhancing Recombinant Protein Production	211
9.3.2	Repression of Cell Death and Acceleration of Growth	221
9.3.3	Modulation of Posttranslational Modifications to Improve Protein Quality	227
9.4	Conclusions	233
	References	234
10	Metabolite Profiling of Mammalian Cells	251
	<i>Claire E. Gaffney, Alan J. Dickson, and Mark Elvin</i>	
10.1	Value of Metabolic Data for the Enhancement of Recombinant Protein Production	251
10.2	Technologies Used in the Generation of Metabolic Data Sets	252
10.2.1	Targeted and Untargeted Metabolic Analysis	253
10.2.2	Analytical Technologies Used in the Generation of Metabolite Profiles	253
10.2.2.1	Nuclear Magnetic Resonance	254
10.2.2.2	Mass Spectrometry	255
10.2.3	Metabolite Sample Preparation	256
10.2.3.1	Extracellular Sample Preparation	257
10.2.3.2	Quenching of Intracellular Metabolite Samples	257

10.2.3.3	Metabolite Extraction from Quenched Cells	257
10.2.3.4	Metabolic Flux Analysis	257
10.3	Approaches for Metabolic Data Analysis	257
10.3.1	Data Processing	258
10.3.2	Data Analysis	258
10.3.3	Data Interpretation and Integration	260
10.4	Implementation of Metabolic Data in Bioprocessing	261
10.4.1	Relationship Between Growth Phase and Metabolism	261
10.4.2	Identification of Metabolic Indicators Associated with High Cell-Specific Productivity	263
10.4.3	Utilizing Metabolic Data to Improve Biomass and Recombinant Protein Yield	263
10.4.4	Utilizing Metabolic Understanding to Improve Product Quality	265
10.4.5	Cell Line Engineering to Redirect Metabolic Pathways	265
10.5	Future Perspectives	266
	Acknowledgments	267
	References	267
11	Current Considerations and Future Advances in Chemically Defined Medium Development for the Production of Protein Therapeutics in CHO Cells	279
	<i>Wai Lam W. Ling</i>	
11.1	Introduction	279
11.2	Traditional Approach to Medium Development	279
11.2.1	Cell Line Selection	279
11.2.2	Design and Optimization	280
11.2.3	Process Consideration	282
11.2.4	Additional Considerations in Medium Development	284
11.3	Future Perspectives for Medium Development	284
11.3.1	Systems Biology and Synthetic Biology	284
	Acknowledgment	288
	Conflict of Interest	288
	References	288
12	Host Cell Proteins During Biomanufacturing	295
	<i>Jong Youn Baik, Jing Guo, and Kelvin H. Lee</i>	
12.1	Introduction	295
12.2	Removal of HCP Impurities	295
12.2.1	Antibody Product	296
12.2.2	Non-antibody Protein Product	297
12.2.3	Difficult-to-Remove HCPs	298
12.3	Impacts of Residual HCPs	298
12.3.1	Drug Efficacy, Quality, and Shelf Life	298
12.3.2	Immunogenicity	299
12.3.3	Biological Activity	299
12.4	HCP Detection and Monitoring Methods	300

12.4.1	Anti-HCP Antiserum and Enzyme-Linked Immunosorbent Assay (ELISA)	300
12.4.2	Proteomics Approaches as Orthogonal Methods	302
12.5	Efforts for HCP Control	302
12.5.1	Upstream Efforts	303
12.5.2	Downstream Efforts	304
12.5.3	HCP Risk Assessment in CHO Cells	305
12.6	Future Directions	305
	Acknowledgments	306
	References	306
13	Mammalian Fed-batch Cell Culture for Biopharmaceuticals	313
	<i>William C. Yang</i>	
13.1	Introduction	313
13.2	Objectives of Cell Culture Process Development	314
13.2.1	Yield and Product Quality	314
13.2.2	Glycosylation	314
13.2.3	Charge Heterogeneity	315
13.2.4	Aggregation	316
13.3	Cells and Cell Culture Formats	316
13.3.1	Adherent Cells	316
13.3.2	Suspended Cells	316
13.3.3	Batch Cultures	317
13.4	Fed-batch Cultures	317
13.5	Cell Culture Media	319
13.5.1	Basal Media	319
13.5.2	Feed Media	320
13.6	Feeding Strategies	321
13.6.1	Metabolite Based	321
13.6.2	Respiration Based	323
13.7	Feed Media Design	323
13.8	Process Variable Design	325
13.8.1	Temperature	325
13.8.2	pH and $p\text{CO}_2$	325
13.8.3	Dissolved Oxygen	326
13.8.4	Culture Duration	327
13.9	Cell Culture Supplements	327
13.9.1	Yield	328
13.9.2	Glycosylation	328
13.10	New and Emerging Technologies	329
13.10.1	Analytical Technologies	329
13.10.2	Bioreactor Technologies	331
13.11	Future Directions	332
	References	333

14	Continuous Biomanufacturing	347
	<i>Sadettin S. Ozturk</i>	
14.1	Introduction	347
14.2	Continuous Upstream (Cell Culture) Processes	347
14.2.1	Continuous Culture without Cell Retention (Chemostat)	348
14.2.2	Continuous Culture with Cell Retention (Perfusion)	348
14.2.2.1	Cell Retention by Immobilization or Entrapment	349
14.2.2.2	Cell Retention by Cell Retention Device	350
14.2.3	Semicontinuous Culture	351
14.3	Advantages of Continuous Perfusion	351
14.3.1	Higher Volumetric Productivities	351
14.3.2	Better Utilization of Biomanufacturing Facilities	352
14.3.3	Better Product Quality and Consistency	352
14.3.4	Scale-up and Commercial Production	353
14.4	Cell Retention Systems for Continuous Perfusion	354
14.4.1	Cell Retention Devices	354
14.4.1.1	Filtration-Based Devices	354
14.4.1.2	Spin Filters	355
14.4.1.3	Continuous Centrifugation	356
14.4.1.4	Settler	356
14.4.1.5	BioSep Device	357
14.4.1.6	Hydrocyclones	358
14.5	Operation and Control of Continuous Perfusion Bioreactors	358
14.5.1	Feed and Harvest Flow and Volume Control	358
14.5.2	Circulation or Return Pump	359
14.5.3	Control of Perfusion Rate and Cell Density	359
14.5.3.1	Cell Build-up Phase	359
14.5.3.2	Production Phase	360
14.5.3.3	Cell Bleed or Purge	360
14.6	Current Status of Continuous Perfusion	360
14.7	Conclusions	362
	Acknowledgment	362
	References	363
15	Process Analytical Technology and Quality by Design for Animal Cell Culture	365
	<i>Hae-Woo Lee, Hemlata Bhatia, Seo-Young Park, Mark-Henry Kamga, Thomas Reimonn, Sha Sha, Zhuangrong Huang, Shaun Galbraith, Huolong Liu, and Seongkyu Yoon</i>	
15.1	PAT and QbD – US FDA’s Regulatory Initiatives	365
15.2	PAT and QbD – Challenges	365
15.3	PAT and QbD Implementations	366
15.3.1	NIR Spectroscopy	366
15.3.2	Mid-Infrared (MIR) Spectroscopy	367
15.3.3	Raman Spectroscopy	367

15.3.4	Fluorescence Spectroscopy	368
15.3.5	Chromatographic Techniques	368
15.3.6	Other Useful Techniques	369
15.3.7	Data Analysis and Modeling Tools	369
15.4	Case Studies	370
15.4.1	Estimation of Raw Material Performance in Mammalian Cell Culture Using Near-Infrared Spectra Combined with Chemometrics Approaches	370
15.4.2	Design Space Exploration for Control of Critical Quality Attributes of mAb	372
15.4.3	Quantification of Protein Mixture in Chromatographic Separation Using Multiwavelength UV Spectra	372
15.4.4	Characterization of Mammalian Cell Culture Raw Materials by Combining Spectroscopy and Chemometrics	374
15.4.5	Effect of Amino Acid Supplementation on Titer and Glycosylation Distribution in Hybridoma Cell Cultures	375
15.4.6	Metabolic Responses and Pathway Changes of Mammalian Cells Under Different Culture Conditions with Media Supplementations	377
15.4.7	Estimation and Control of N-Linked Glycoform Profiles of Monoclonal Antibody with Extracellular Metabolites and Two-Step Intracellular Models	378
15.4.8	Quantitative Intracellular Flux Modeling and Applications in Biotherapeutic Development and Production Using CHO Cell Cultures	381
15.5	Conclusion	383
	References	383
16	Development and Qualification of a Cell Culture Scale-Down Model	391
	<i>Sarwat Khattak and Valerie Pferdeort</i>	
16.1	Purpose of the Scale-Down Model	391
16.1.1	Development Challenges	391
16.2	Types of Scale-Down Models	392
16.2.1	Power/Volume (P/V) and Air velocity	392
16.2.2	Oxygen Transfer Coefficient ($k_L a$)	392
16.2.3	Gas Entrance Velocity (GEV)	393
16.2.4	Oxygen Transfer Rate (OTR)	393
16.2.5	Model Refinement Workflow	395
16.3	Evaluation of a Scale-Down Model	395
16.3.1	Univariate Analysis	395
16.3.2	Multivariate Analysis	396
16.3.2.1	Statistical Background	396
16.3.2.2	Qualification Data Set	396
16.3.2.3	Observation Level Analysis	397
16.3.2.4	Batch-Level Analysis	397
16.3.2.5	Scores Contribution Plots	398
16.3.3	Equivalence Testing	399

16.3.3.1	Statistical Background	399
16.3.3.2	Considerations for Evaluation and Test Data Sets	399
16.3.3.3	Types of Analysis Outcomes	400
16.4	Conclusions and Perspectives	401
	References	402
	Index	407

