

CONTENTS

<i>Contributors</i>	xiii
<i>Preface</i>	xix
<i>Volumes in Series</i>	xxi

Section I. New Approaches to Studying UPR and Cell Stress **1**

1. CFTR Expression Regulation by the Unfolded Protein Response **3**

Rafal Bartoszewski, Andras Rab, Lianwu Fu, Sylwia Bartoszewska,
James Collawn, and Zsuzsa Bebok

1. Introduction	4
2. Methods	6
Acknowledgments	22
References	22

2. GRP78/BiP Chapter: Modulation of GRP78/BiP in Altering Sensitivity to Chemotherapy **25**

Thomas C. Chen

1. Introduction	26
2. Measurement of ER Stress <i>In Vitro</i> and <i>In Vivo</i>	27
3. Conclusion	36
References	36

3. Targeting the Unfolded Protein Response in Cancer Therapy **37**

Marina V. Backer, Joseph M. Backer, and Prakash Chinnaiyan

1. Introduction	38
2. The UPR and Cancer	38
3. Targeting the UPR	40
4. Combination of EGF-SubA with Other UPR-Targeting Drugs	50
Acknowledgments	54
References	54

4. Large-Scale Analysis of UPR-Mediated Apoptosis in Human Cells	57
Andrew M. Fribley, Justin R. Miller, Tyler E. Reist, Michael U. Callaghan, and Randal J. Kaufman	
1. Introduction	58
2. Monitoring Proliferation and Caspase Activation Following UPR Activation	60
3. Monitoring the Expression of UPR and Cell Death Target Genes	61
4. DNA Fragmentation Analysis	68
Acknowledgments	69
References	69
5. Quantitative Analysis of Amino Acid Oxidation Markers by Tandem Mass Spectrometry	73
Anuradha Vivekanandan-Giri, Jaeman Byun, and Subramaniam Pennathur	
1. Introduction	75
2. Experimental Procedures	76
3. Results	82
4. Conclusions	86
Acknowledgments	86
References	87
6. Animal Models in the Study of the Unfolded Protein Response	91
Hemamalini Bommiasamy and Brian Popko	
1. Introduction	92
2. Activating Transcription Factor 6	93
3. IRE1/X-Box-Binding Protein-1	94
4. PKR-Like ER Kinase	97
5. eIF2 α	98
6. ATF4	99
7. CHOP	100
8. GADD34	101
9. P58IPK	102
10. Transgenic Mouse Models for Monitoring ER Stress	102
11. UPR and Lipid Metabolism	103
12. UPR, Hypoxia, and Cancer	104
13. UPR and Inflammatory-Mediated Demyelination	105
14. Future Challenges	105
References	106

7. Measurement of Fluoride-Induced Endoplasmic Reticulum Stress Using <i>Gaussia</i> Luciferase	111
Ramaswamy Sharma, Masahiro Tsuchiya, Bakhos A. Tannous, and John D. Bartlett	
1. Introduction	112
2. Properties of <i>Gaussia</i> Luciferase	114
3. Materials	115
4. Procedure	118
5. Conclusions	123
References	124
8. Analysis of Nelfinavir-Induced Endoplasmic Reticulum Stress	127
Ansgar Brüning	
1. Introduction	128
2. Obtaining Nelfinavir	129
3. Using Nelfinavir	129
4. Analysis of Nelfinavir-Induced ER Stress	130
5. Immunoblot Analysis	134
6. RT-PCR Analysis	135
7. Conclusions	141
Acknowledgment	142
References	142
9. Using Temporal Genetic Switches to Synchronize the Unfolded Protein Response in Cell Populations <i>In Vivo</i>	143
Alexander Gow	
1. Introduction	144
2. Variable Functions of CHOP in the PERK Signaling Pathway	146
3. Mutations in the <i>PLP1</i> Gene: A Model UPR Disease	147
4. Problems with Current Paradigms Used to Characterize the UPR	149
5. A Novel <i>In Vivo</i> Genetic Switch Solves Many of These Problems	150
6. Generalizing GST to Study Other UPR Diseases	157
Acknowledgments	157
References	158
10. Glycoprotein Maturation and the UPR	163
Andreas J. Hülsmeier, Michael Welte, and Thierry Hennot	
1. Introduction	163
2. N-glycosylation	165
3. O-glycosylation	175
Acknowledgment	181
References	181

11. Monitoring and Manipulating Mammalian Unfolded Protein Response	183
Nobuhiko Hiramatsu, Victory T. Joseph, and Jonathan H. Lin	
1. Introduction	184
2. Monitoring Mammalian UPR	185
3. Chemical-Genetic Manipulation of Mammalian UPR	189
4. Concluding Remarks	194
Acknowledgments	195
References	195
12. A Screen for Mutants Requiring Activation of the Unfolded Protein Response for Viability	199
Guillaume Thibault and Davis T. W. Ng	
1. Introduction	200
2. Screening for Mutants That Require UPR Activation for Viability	202
3. Cloning and Sequencing <i>per</i> Genes	209
4. Monitoring the UPR Activity	212
5. SGA Database	213
6. Media Recipes	214
7. Closing Remarks	214
Acknowledgment	215
References	215
13. Signaling Pathways of Proteostasis Network Unrevealed by Proteomic Approaches on the Understanding of Misfolded Protein Rescue	217
Patrícia Gomes-Alves, Sofia Neves, and Deborah Penque	
1. Introduction	218
2. 2DE-Based Proteomics Approach Analysis to Study Protein Expression Profile	219
3. Experimental Design	220
4. 2D Gel Electrophoresis	221
5. Protein Identification by MS	223
6. Biochemical Validation	225
7. Data Mining and Publication	226
Acknowledgments	231
References	231

14. Decreased Secretion and Unfolded Protein Response Upregulation	235
Carissa L. Young, Theresa Yuraszeck, and Anne S. Robinson	
1. Introduction	236
2. Heterologous Protein Expression	237
3. Quality Control Mechanisms of the Secretory Pathway	238
4. Endoplasmic Reticulum Export and Trafficking	239
5. Experimental Systems to Evaluate Expression, UPR, and Secretory Processing	239
6. <i>S. cerevisiae</i> Strains Used for Optimal Expression	240
7. Plasmid Design	241
8. Evaluation of Heterologous Protein Expression	243
9. Statistical Analysis of Microarray Results	248
Acknowledgment	257
References	257
15. Measuring Signaling by the Unfolded Protein Response	261
David J. Cox, Natalie Strudwick, Ahmed A. Ali, Adrienne W. Paton, James C. Paton, and Martin Schröder	
1. Introduction	262
2. Methods to Induce the UPR	266
3. Measuring Signaling by the UPR in <i>S. cerevisiae</i>	266
4. Measuring Signaling by the UPR in Mammalian Cells	276
Acknowledgments	288
References	289
16. Quantitative Measurement of Events in the Mammalian Unfolded Protein Response	293
Jie Shang	
1. Introduction	294
2. Cell Culture and ER Stress Inducers	295
3. Northern Blots for GRP78/BiP and EDEM	296
4. RT-PCR Analysis of XBP1 mRNA Splicing	299
5. Immunoblotting and Measurement of ATF6	302
6. Measurement of Inhibition of Protein Synthesis	302
7. Measurement of Stimulation of LLO Extension	303
8. Calculations of Transcript Accumulation, Signal Activation, and Correlation	304
9. Conclusion	305
Acknowledgments	306
References	306

17. Regulation of Immunoglobulin Synthesis, Modification, and Trafficking by the Unfolded Protein Response: A Quantitative Approach	309
Adi Drori and Boaz Tirosh	
1. Introduction	310
2. Isolation of Splenic B Cells and Infection Thereof by Retroviruses	312
3. Measurement of Protein Synthesis in Plasma Cells	316
4. Measurement of Ig Mislocalization in Primary B Cells	320
Acknowledgments	324
References	324
18. Use of Chemical Genomics in Assessment of the UPR	327
Sakae Saito and Akihiro Tomida	
1. Introduction	328
2. Assessment of the Activation of UPR Transcriptional Program in Cancer Cell	329
3. Gene Expression Signature-Based Identification of UPR Modulators	334
4. Future Perspective of Chemical Genomics in UPR Research	338
Acknowledgments	339
References	339
19. Small GTPase Signaling and the Unfolded Protein Response	343
Marion Bouchecareilh, Esther Marza, Marie-Elaine Caruso, and Eric Chevet	
1. Introduction	344
2. Materials and Methods	346
3. Monitoring the Unfolded Protein Response	348
4. Monitoring Small GTPase Activity	353
5. Pharmacological and Genetic Modulation of UPR and GTPase Signaling	356
Acknowledgments	358
References	358
20. Inhibitors of Advanced Glycation and Endoplasmic Reticulum Stress	361
Reiko Inagi	
1. Introduction	362
2. Advanced Glycation and Its Pathophysiology	363
3. Measurement of Advanced Glycation	364
4. Link Between Advanced Glycation and ER Stress	369

5. Effects of Advanced Glycation Inhibitor Against ER Stress	373
6. Conclusion	377
Acknowledgments	377
References	377
<i>Author Index</i>	381
<i>Subject Index</i>	399