

CONTENTS

<i>Contributors</i>	<i>xi</i>
<i>Preface</i>	<i>xvii</i>
<i>Volumes in Series</i>	<i>xix</i>
1. Methods for Investigating the UPR in Filamentous Fungi	1
Thomas Guillemette, Arthur F. J. Ram, Neuza D. S. P. Carvalho, Aymeric Joubert, Philippe Simoneau, and David B. Archer	
1. Introduction	2
2. Conditions to Study the UPR in Filamentous Fungi	5
3. Analysis of Expression During the ER Stress	11
4. Modifying the UPR Signaling by Targeted Gene Replacement	14
Acknowledgments	25
References	26
2. Assays for Detecting the Unfolded Protein Response	31
Karen Cawley, Shane Deegan, Afshin Samali, and Sanjeev Gupta	
1. Introduction	33
2. Experimental Approaches for the Detection of ER Stress	36
3. Concluding Remarks	47
Acknowledgments	49
References	49
3. Analysis of the Role of Nerve Growth Factor in Promoting Cell Survival During Endoplasmic Reticulum Stress in PC12 Cells	53
Koji Shimoke, Harue Sasaya, and Toshihiko Ikeuchi	
1. Introduction	54
2. Induction of ER Stress in PC12 Cells	55
3. Measurement of Caspase-3 Activity	62
4. Fragmentation of Caspase-12	63
5. Hyperinduction of GRP78 by NGF	65
Acknowledgments	68
References	68

4. Measuring ER Stress and the Unfolded Protein Response Using Mammalian Tissue Culture System	71
Christine M. Osowski and Fumihiko Urano	
1. Introduction	72
2. ER Stress and the UPR	73
3. Cell Culture System and ER Stress Induction	76
4. Measuring ER Stress	78
5. Studying the UPR Activation	79
6. Studying UPR Downstream Markers and Responses	84
Acknowledgments	88
References	88
5. Real-Time Monitoring of ER Stress in Living Cells and Animals Using ESTRAP Assay	93
Masanori Kitamura and Nobuhiko Hiramatsu	
1. Introduction	94
2. SEAP Reporter System	95
3. Monitoring of ER Stress in Culture Cells by ESTRAP	96
4. Monitoring of ER Stress <i>In Vivo</i> by ESTRAP	101
5. Conclusion	104
References	105
6. HIV Protease Inhibitors Induce Endoplasmic Reticulum Stress and Disrupt Barrier Integrity in Intestinal Epithelial Cells	107
Huiping Zhou	
1. The Pathophysiological Relevance of HIV PI-Induced ER Stress and UPR Activation in IECs	108
2. Analysis of HIV PI-Induced ER Stress and UPR Activation in Cultured IECs	109
3. Analysis of HIV PI-Induced ER Stress and Disruption of Barrier Integrity in the Intestine	114
4. Summary	118
Acknowledgments	118
References	118
7. Dexamethasone Induction of a Heat Stress Response	121
José A. Guerrero, Vicente Vicente, and Javier Corral	
1. Introduction	122
2. Evaluation of the Heat Stress Response in Patients Treated with Dexamethasone	124
3. Heat Stress Response and UPR in Mice and Cellular Models	126
References	134

8. Detecting and Quantitating Physiological Endoplasmic Reticulum Stress	137
Ling Qi, Liu Yang, and Hui Chen	
1. Introduction	138
2. Detecting UPR at the Level of UPR Sensors	139
3. Quantitating ER Stress at the Level of UPR Sensors	141
4. Detecting Levels of Other Common UPR Targets	142
5. Important Tips	143
6. Concluding Remarks	144
Acknowledgments	145
References	145
9. PI 3-Kinase Regulatory Subunits as Regulators of the Unfolded Protein Response	147
Jonathon N. Winnay and C. Ronald Kahn	
1. The Unfolded Protein Response	148
2. PI 3-Kinase Regulatory Subunits as Modulators of the UPR	150
3. Assessing UPR Pathway Activation	151
References	157
10. The Emerging Role of Histone Deacetylases (HDACs) in UPR Regulation	159
Soumen Kahali, Bhaswati Sarcar, and Prakash Chinnaiyan	
1. HDAC Enzymes and Cancer	159
2. HDAC Inhibitors	160
3. HDAC Inhibition and the UPR	162
4. Future Directions	172
References	173
11. Immunohistochemical Detection of Activating Transcription Factor 3, a Hub of the Cellular Adaptive-Response Network	175
Tsonwin Hai, Swati Jalgaonkar, Christopher C. Wolford, and Xin Yin	
1. Introduction	176
2. An IHC Protocol for ATF3	181
Acknowledgments	192
References	192
12. Experimental Approaches for Elucidation of Stress-Sensing Mechanisms of the Ire1 Family Proteins	195
Daisuke Oikawa and Yukio Kimata	
1. Introduction	196

2. Monitoring <i>In Vivo</i> Activity of Mutated Ire1 Family Proteins	199
3. Interaction Between BiP and the Ire1 Family Proteins	206
4. <i>Direct Interaction of Unfolded Proteins with Yeast Ire1 but not with Mammalian IRE1α</i>	210
5. Conclusion and perspective	213
Acknowledgments	214
References	214
13. Measurement and Modification of the Expression Level of the Chaperone Protein and Signaling Regulator GRP78/BiP in Mammalian Cells	217
Wan-Ting Chen and Amy S. Lee	
1. Introduction	218
2. Detection of Total GRP78	220
3. Detection of Cytosolic GRP78 Isoform	224
4. Detection of Cell Surface GRP78	228
Acknowledgments	231
References	232
14. The Endoplasmic Reticulum-Associated Degradation and Disulfide Reductase ERdj5	235
Ryo Ushioda and Kazuhiro Nagata	
1. Introduction	236
2. Productive Folding of Newly Synthesized Proteins in the ER	238
3. ERAD: A Strategy for ERQC Mechanism	242
4. Conclusion	249
References	252
15. Structural Insight into the Protective Role of P58(IPK) during Unfolded Protein Response	259
Jiahui Tao and Bingdong Sha	
1. Endoplasmic Reticulum Stress and Unfolded Protein Response	260
2. P58(IPK) Might be a Dual-Function Protein	261
3. Knocking Out P58(IPK) in Mouse Models	261
4. P58(IPK) is an ER-Resident Hsp40	262
5. Crystal Structure of Mouse P58(IPK) TPR Domain	263
6. P58(IPK) Functions as a Molecular Chaperone to Bind Unfolded Proteins Using Subdomain I	264
7. Structural Basis for P58(IPK) J Domain-BiP Interaction	267
8. Working Model of P58(IPK) During UPR	267
9. Future Research	267
References	268

16. Principles of IRE1 Modulation Using Chemical Tools	271
Kenneth P. K. Lee and Frank Sicheri	
1. Introduction: The Unfolded Protein Response	272
2. Structural Biology of IRE1-XBP1 Signaling	276
3. Chemical Approaches to Modulate IRE1 Function	282
References	292
17. Methods to Study Stromal-Cell Derived Factor 2 in the Context of ER Stress and the Unfolded Protein Response in <i>Arabidopsis thaliana</i>	295
Andrea Schott and Sabine Strahl	
1. Introduction	296
2. <i>Arabidopsis sdf2</i> T-DNA Insertion Lines	297
3. Inducing and Monitoring ER Stress in <i>Arabidopsis sdf2</i> Mutants	299
4. Analyzing the ER Stress-Induced Expression Pattern of <i>SDF2</i> in <i>Arabidopsis</i>	304
5. The Subcellular Localization of SDF2	309
6. Purification of Recombinant SDF2 Protein from <i>E. coli</i>	313
Acknowledgments	316
References	317
18. Nitrosative Stress-Induced S-Glutathionylation of Protein Disulfide Isomerase	321
Joachim D. Uys, Ying Xiong, and Danyelle M. Townsend	
1. Introduction	322
2. Identification and Confirmation of S-Glutathionylated Proteins in Cells	324
3. Identification of Target Cysteine Residues	327
4. Characterization of Structural and Functional Consequences of S-Glutathionylated PDI	329
5. Summary	331
Acknowledgments	331
References	331
19. Methods for Analyzing eIF2 Kinases and Translational Control in the Unfolded Protein Response	333
Brian F. Teske, Thomas D. Baird, and Ronald C. Wek	
1. Introduction to Translational Control in the UPR	334
2. Measuring Activation of the eIF2 Kinase Pathway During ER Stress	338
3. Investigating General and Gene-Specific Translation Control	345

Acknowledgments	353
References	353
<i>Author Index</i>	357
<i>Subject Index</i>	375