

CONTENTS

<i>Contributors</i>	<i>xi</i>
<i>Preface</i>	<i>xvii</i>
<i>Volumes in Series</i>	<i>xix</i>

Section I. UPR and Cell Stress in Normal and Diseased Cells **1**

1. The Unfolded Protein Response in Mouse Cerebral Cortex **3**

Nirinjini Naidoo

1. Introduction: The UPR	3
2. Transduction of the UPR Signal by PERK, IRE1, and ATF6	4
3. UPR and Apoptosis	5
4. The UPR in Health and Disease	6
5. Induction of the UPR by Sleep Deprivation	6
6. Experimental Protocols	7
7. Concluding Remarks	18
Acknowledgments	19
References	19

2. Immunohistochemical Detection of the Unfolded Protein Response in Atherosclerotic Plaques **23**

Šárka Lhoták, Ji Zhou, and Richard C. Austin

1. Endoplasmic Reticulum and the Unfolded Protein Response	24
2. Atherosclerosis	24
3. UPR in the Atherosclerotic Lesion	25
4. Mouse Models of Atherosclerosis	27
5. Methods	29
Acknowledgments	44
References	44

3. The Role of PDI as a Survival Factor in Cardiomyocyte Ischemia **47**

Stefano Toldo, Anna Severino, Antonio Abbate, and Alfonso Baldi

1. Introduction	48
2. Identification of PDI as Cardio-Protective Factor	50

3. Conclusions	62
References	62
4. Measurement of the Increase in Endoplasmic Reticulum Stress-Related Proteins and Genes in Adipose Tissue of Obese, Insulin-Resistant Individuals	67
Guenther Boden and Salim Merali	
1. Introduction	68
2. Study Subjects and Fat Biopsies	69
3. Proteomic Analysis	70
4. Western Blot Analysis	79
5. RT-PCR Analysis	80
Acknowledgments	81
References	82
5. Measurement of the Unfolded Protein Response (UPR) in Monocytes	83
Tomás P. Carroll, Catherine M. Greene, and Noel G. McElvaney	
1. Introduction	84
2. Investigating UPR Activation in the Monocyte	86
Acknowledgments	93
References	93
6. Assessment of Endurance Exercise Training in Hepatic Endoplasmic Reticulum Stress	97
Natalie Ann Chapados	
1. Introduction	98
2. Assessment of an Endurance-Training Program	99
3. "Dietary-Inducible" Hepatic ER Stress	104
4. Assessment of Hepatic ER Stress Molecular Markers	105
5. Required Materials	105
Acknowledgments	106
References	106
7. Discovery Approaches to UPR in Athero-Susceptible Endothelium <i>In Vivo</i>	109
Mete Civelek, Elisabetta Manduchi, Gregory R. Grant, Christian J. Stoeckert Jr., and Peter F. Davies	
1. Introduction	110
2. Procedures for the Isolation of Endothelial Cells and Preparation of RNA for Microarray Hybridization	112
3. Microarray Hybridization and Feature Extraction	116

4. Bioinformatics Analysis	117
5. Another Approach to Gene Connectivity: Weighted Gene Coexpression Network Analysis	122
6. Validation and Follow-up	124
Acknowledgments	125
References	125
8. Assessment of Endoplasmic Reticulum Stress and the Unfolded Protein Response in Endothelial Cells	127
Ines Witte and Sven Horke	
1. Introduction	128
2. The Acute Phase of ER Stress	129
3. The Adaptive Reaction Phase: Unfolded Protein Response in Endothelial Cells	131
4. The Final Phase: Cell Death Signaling	140
Acknowledgments	145
References	145
9. Measuring the Impact of Cigarette Smoke on the UPR	147
Hong Zhao, Jin Yang, Lin Shan, and Ellen D. Jorgensen	
1. Introduction	148
2. Preparing Samples for Analysis	149
3. Analysis of Activation of the PERK Pathway	155
4. ATF6 Pathway	158
5. IRE1 Pathway	159
6. Using LSC to Measure UPR Activation	159
Acknowledgments	161
References	162
10. Induction and Measurement of UPR and Osmotic Stress in the Yeast <i>Pichia pastoris</i>	165
Martin Dragosits, Diethard Mattanovich, and Brigitte Gasser	
1. Introduction	166
2. Measuring UPR Induction in <i>P. pastoris</i>	169
3. Inducing and Measuring Osmotic Stress in <i>P. pastoris</i>	179
Acknowledgments	186
References	186
11. Induction of ER Stress Response Leading to Programmed Cell Death in <i>Trypanosoma brucei</i>	189
Hanoch Goldshmidt and Shulamit Michaeli	
1. Introduction	190
2. Induction of ER Stress in Trypanosomes	193

3. Measurements of ER Stress in Trypanosomes Cells	194
4. Measurements of ER Stress-Induced Cell Death	201
Acknowledgments	203
References	203
12. Measurement of Activation of the Endoplasmic Reticulum Stress Response in Autoimmune Myositis	207
Heather M. Alger, Sree Rayavarapu, and Kanneboyina Nagaraju	
1. Introduction	208
2. Measuring Endoplasmic Reticulum Stress Response in Skeletal Muscle Tissues Using Western Blotting	209
3. Immunohistochemical Detection of ER Stress Markers in Muscle Tissues	216
4. Determination of mRNA Levels of ER Stress Targets	221
5. Concluding Remarks	223
Acknowledgments	223
References	223
13. Activation of the Unfolded Protein Response in Human Acute Myeloid Leukemia	227
Julian A. Schardt, Beatrice U. Mueller, and Thomas Pabst	
1. Introduction	228
2. Technical Aspects	232
3. Discussion	239
References	241
14. Modulation of the Unfolded Protein Response by GRP78 in Prostate Cancer	245
Gustaaf de Ridder, Rupa Ray, Uma K. Misra, and Salvatore V. Pizzo	
1. Introduction	246
2. Methods for Monitoring UPR Modulation	248
3. Conclusions	256
References	256
15. Endoplasmic Reticulum Stress in Brain Damage	259
Ram Raghubir, Venkata Prasuja Nakka, and Suresh L. Mehta	
1. Introduction	260
2. ER Stress and Unfolded Protein Response	261
3. Cross Talk Between ER and Mitochondria	267
4. Experimental Approaches for the Detection of ER Stress	269
Acknowledgment	271
References	272

16. Methods and Models for Monitoring UPR-Associated Macrophage Death During Advanced Atherosclerosis	277
Edward B. Thorp	
1. Introduction to ER Stress in Atherosclerosis	278
2. Significance of Macrophage ER Stress and Apoptosis in Advanced Atherosclerosis	279
3. Modeling Atherosclerosis-Relevant ER Stress-Induced Apoptosis	281
4. The UPR and Macrophage Death at the Murine Aortic Root	285
5. Conclusion and Future Methodological Advances	293
Acknowledgments	293
References	293
17. Detecting Autophagy in Response to ER Stress Signals in Cancer	297
María Salazar, Sonia Hernández-Tiedra, Sofía Torres, Mar Lorente, Manuel Guzmán, and Guillermo Velasco	
1. Introduction	298
2. Monitoring Autophagy Upon ER Stress	299
3. Monitoring Autophagy Upon ER Stress <i>In Vivo</i>	309
References	317
18. The Unfolded Protein Response Induced by Salt Stress in <i>Arabidopsis</i>	319
Miaoying Wang, Qiangyi Xu, and Ming Yuan	
1. Introduction	319
2. The Treatment of <i>Arabidopsis</i> Seedlings	320
3. The Analysis of Unfolded Protein Response	322
Acknowledgments	327
References	327
19. Measurement of ER Stress Response and Inflammation in the Mouse Model of Nonalcoholic Fatty Liver Disease	329
Ze Zheng, Chunbin Zhang, and Kezhong Zhang	
1. Introduction	330
2. Methods to Measure ER Stress Response and Hepatic Inflammation in the Mouse Model of NAFLD	332
3. Visualizing ER Stress Response Associated with Liver Pathology in the Mouse Model of NAFLD	341
Acknowledgments	345
References	345
<i>Author Index</i>	349
<i>Subject Index</i>	365