

## Contents

	<b>Introduction to the Book</b>	<b>1</b>
	Long-Lived Proteins Are Ubiquitous	1
	Aging	1
	Autoimmunity	2
	Age-Related Diseases	3
	Our Lenses in the Vanguard	3
	Brain and Memory	4
<b>1</b>	<b>Long-Lived Cells and Long-Lived Proteins in the Human Body</b>	<b>5</b>
	<i>Roger J.W. Truscott</i>	
1.1	What Constitutes a Long-Lived Cell and a Long-Lived Protein?	5
1.2	Aim of the Chapter	6
1.3	Aging	6
1.4	Location of LLPs Within the Body	7
1.4.1	ECM and Tissue Function	7
1.5	Extracellular LLPs	7
1.5.1	Several ECM Components Are Long Lived	7
1.5.1.1	Elastin	7
1.5.1.2	Structural Glycoproteins and Proteoglycans	8
1.5.1.3	Collagens	8
1.6	Intracellular LLPs and LLCs	10
1.6.1	LLCs and LLPs in the Organs of the Body	10
1.7	Organs and Tissues that Contain LLCs or LLPs	11
1.7.1	Long-Lived Cells	11
1.7.1.1	Eye	11
1.7.1.2	Oocytes	14
1.7.1.3	Kidneys	15
1.7.1.4	Adipose Tissue	15
1.7.1.5	Brain	15
1.7.1.6	Heart	17
1.7.1.7	Lung	17

1.7.1.8	Skeleton	18
1.7.1.9	Teeth	18
1.7.1.10	Hair	18
1.7.1.11	Joints	19
1.7.1.12	Pancreas	19
1.7.1.13	Liver	20
1.7.1.14	Intestine	20
1.7.1.15	Dividing Cells and LLPs	21
1.7.2	Sensory Tissues	21
1.7.2.1	Hearing	21
1.7.2.2	Smell	21
1.8	Protein Changes and DNA Changes with Age	21
1.9	Processes Responsible for the Breakdown of LLPs	22
1.10	Oxidation: Methionine Sulfoxide Reductases and the Glutathione System	23
1.11	Consequences of LLP Decomposition	24
1.11.1	Protein Modification and Cellular Processing	24
1.11.2	Lifelong Proteins and the Consequences	24
1.12	LLPs and Age-Related Disorders	25
1.12.1	Modified LLPs Acting as Novel Antigens: Autoimmune Diseases	25
1.12.2	Defects in Cytosol/Nuclear Communication	25
1.12.3	Defects in Nuclear Transcription	26
1.12.4	Breakdown of Abundant Macromolecules	26
1.12.5	Elastin	26
1.12.6	Collagen	26
1.13	Neurological Diseases Where LLPs May be Implicated	27
1.13.1	Multiple Sclerosis	27
1.13.2	Motor Neuron Disease (MND)/Amyotrophic Lateral Sclerosis (ALS)	27
1.13.3	Alzheimer Disease (AD)	27
1.14	Aging DNA and LLPs	28
1.15	How Can the Role of LLPs in Aging and Disease Be Investigated? What Can Be Done	28
1.15.1	Heterogeneity of Aged LLPs: A Large Hurdle to Overcome	29
1.16	We Will Not Live Forever	29
1.16.1	LLP Degradation and Tissue Function: Is There a Threshold for Decay?	30
1.16.2	Lifelong Proteins May Degrade at Similar Rates	30
1.16.3	Decay in Tissue Function with Age and Its Effect on Fitness, Health, and Mortality	32
1.16.4	LLPs and Life Span	32
1.16.5	Heart	32
1.16.6	Lung	33
1.16.7	Nerves and Brain	33
1.17	Conclusion	33
	Acknowledgments	33
	References	33

<b>2</b>	<b>Imaging Mass Spectrometry of Long-Lived Proteins</b>	<b>43</b>
	<i>Kevin L. Schey</i>	
2.1	Introduction	43
2.2	Imaging Mass Spectrometry Methods	44
2.2.1	General Considerations	44
2.2.2	MALDI-IMS	44
2.2.3	Desorption Electrospray Ionization (DESI)-IMS	46
2.2.4	Secondary Ion Mass Spectrometry (SIMS)-IMS	46
2.2.5	Other IMS Methods	46
2.3	Protein Identification	47
2.4	LLPs in the Body	48
2.4.1	Lens	48
2.4.2	Optic Nerve	51
2.4.3	Retina	52
2.4.4	Brain and CNS	52
2.4.5	Cartilage	53
2.5	Long-Lived Cells and Structures	53
2.6	Future Directions	54
	References	54
<b>3</b>	<b>Eye Lens Crystallins: Remarkable Long-Lived Proteins</b>	<b>59</b>
	<i>Aidan B. Grosas and John A. Carver</i>	
3.1	Introduction	59
3.2	Eye Lens and Its Transparency	59
3.3	Lens Crystallin Proteins	61
3.3.1	$\alpha$ -Crystallins	61
3.3.2	$\beta$ - and $\gamma$ -Crystallins	63
3.4	Congenital, Early Onset, and Age-Related Cataract	65
3.5	Protein Aggregation and Disease, Particularly Cataract	71
3.5.1	Protein Unfolding and Aggregation and Molecular Chaperones	71
3.5.2	Amyloid Fibril and Amorphous Protein Aggregates	73
3.5.3	Diseases Associated with Protein Aggregation	74
3.5.4	Crystallin Aggregation and Cataract	75
3.6	Concluding Comments	77
	References	78
<b>4</b>	<b>Spontaneous Breakdown of Long-Lived Proteins in Aging and Their Implications in Disease</b>	<b>97</b>
	<i>Michael G. Friedrich</i>	
4.1	Introduction	97
4.2	LLPs Are Found Throughout the Body	98
4.3	Spontaneous Modifications of Aging	99
4.3.1	Deamidation, Racemization, and Isomerization	99
4.3.2	Cross-linking	101

4.3.3	Truncation	102
4.3.4	Age, Disease, and Spontaneous PTMs: General Considerations	103
4.4	LLPs and Onset of Disease: Is Correlation the Only Answer?	105
4.4.1	Eye	106
4.4.1.1	Lens and Age-Related Nuclear Cataract	106
4.4.1.2	Retina, Vitreous Humor, and Sclera	108
4.4.2	Central Nervous System	108
4.4.2.1	Multiple Sclerosis	109
4.4.2.2	Alzheimer's Disease	109
4.4.2.3	Parkinson's Disease	110
4.4.2.4	Amyotrophic Lateral Sclerosis/Motor Neuron Disease	110
4.4.2.5	Systemic Lupus Erythematosus	111
4.4.3	Extracellular Matrix Proteins	111
4.4.3.1	Articular Cartilage, Intervertebral Disc, and Osteoarthritis	112
4.4.3.2	Circulatory System	112
4.4.3.3	Respiratory System	112
4.4.4	Digestive System	112
4.4.4.1	Diabetes	113
4.5	Spontaneous Modifications: Detrimental or Beneficial?	113
4.5.1	NGR Motifs	113
4.5.2	Bcl-x <sub>L</sub>	113
4.6	Protein Turnover Slows with Age	113
4.7	Potential Treatment of Diseases Initiated by LLPs	114
4.8	Future Outlook	114
	Acknowledgments	115
	References	115

## **5 Modifications of Long-Lived Proteins that Affect Protein Solubility** 127

*Larry L. David*

5.1	Introduction	127
5.2	Insoluble Protein Definition	128
5.3	Insolubilization Due to Disulfide Bonding	128
5.3.1	Disulfide Bonding Is Strongly Correlated with Age-Related Cataracts	128
5.3.2	Levels of Disulfide Bonding at Individual Cysteines in Cataractous Lenses	129
5.3.3	Identity of Individual Disulfide Cross-links in Crystallins of Aged Lenses	129
5.4	Insolubilization Due to Nondisulfide Cross-links	130
5.4.1	Cross-links Due to Dehydroalanine Formation	130
5.4.2	Cross-links Due to C-Terminal Anhydrides	130
5.5	Insolubilization Due to Protein Fragmentation	131
5.5.1	Introduction: Protein Hydrolysis and Insolubilization	131
5.5.2	Proteolysis as a Driver of Protein Insolubilization in Animal Lenses	131
5.5.3	Nonenzymatic Hydrolysis as a Driver of Protein Insolubilization in Human Lenses	131
5.6	Insolubilization Due to Deamidation, Isomerization, and Racemization	132
5.7	<i>In vitro</i> Studies of How PTMs Alter Protein Structure and Solubility	133

5.7.1	<i>In vitro</i> Studies of Disulfide Bonding	133
5.7.2	<i>In Vitro</i> Studies of Deamidation	135
5.8	Proteomics Methods to Detect Post-translation Modifications Contributing to Protein Insolublization	135
5.8.1	Crystallins as Ideal Proteins to Detect Age-Related PTMs	135
5.8.2	Two-Dimensional Liquid Chromatography/Mass Spectrometry to Detect PTMs	136
5.8.3	Searches for Known PTMs	136
5.8.4	Searches for Unknown PTMs	137
5.8.5	Identifying Disulfide Cross-links	138
5.8.6	Identifying Deamidation Sites	139
5.8.7	Identifying Isomerization Sites	142
5.8.8	Identifying Racemization Sites	143
5.8.9	Peptide Standards to Study Deamidation, Isomerization, and Racemization	145
5.9	Future PTM Studies of Long-Lived Proteins	145
5.10	Concluding Remarks	148
	Acknowledgments	150
	References	150
<b>6</b>	<b>Degradation of Long-Lived Proteins as a Cause of Autoimmune Diseases</b>	<b>159</b>
	<i>Roger J.W. Truscott</i>	
6.1	Introduction	159
6.1.1	Background	159
6.1.2	Autoimmunity: Long-Lived Proteins and Long-Lived Cells	159
6.1.3	Focus of this Chapter	159
6.2	Long-Lived Cells Are Widespread in the Body	160
6.3	Long-Lived Proteins Are Present in Many Tissues	160
6.4	Long-Lived Proteins Decompose Over Time	161
6.5	Defenses Against LLP Decomposition	162
6.5.1	Rebuilding Degraded Asp and Asn Sites Within a Protein	162
6.5.2	Oxidation-Related Modification Repair Enzymes and Antioxidants	163
6.6	Consequences of Long-Lived Protein Decomposition	163
6.7	Individual Autoimmune Diseases	165
6.7.1	Pancreas	165
6.7.2	Nerves	165
6.7.3	Stomach	166
6.7.4	Blood Vessels	166
6.7.5	Gastrointestinal Tract	166
6.7.6	Liver	166
6.7.7	Thyroid Gland	166
6.7.8	Adrenal Gland	166
6.7.9	Joints	167
6.7.10	Multiple Sites	167
6.7.11	Skin	167
6.7.12	Moisture-Secreting Glands	167

- 6.7.13 Blood 167
- 6.7.14 Muscles 168
- 6.7.15 Heart 168
- 6.8 Person-to-Person Variability in Breakdown of LLPs: Multiple Sclerosis 168
- 6.8.1 Why Do Not All Adults Develop Autoimmune Disorders? 168
- 6.8.2 Widespread LLPs and Modulation of an Immune Response 169
- 6.9 Conclusions and Future Research 169
- Acknowledgments 170
- References 170

## **7 How Isomerization and Epimerization in Long-Lived Proteins Affect Lysosomal Degradation and Proteostasis**

- Ryan R. Julian* 175
- 7.1 Proteostasis 175
- 7.2 Invisible Modifications 176
- 7.3 Repair 179
- 7.4 Identification 180
- 7.5 Protein Turnover 180
- 7.6 Mechanistic Considerations 181
- 7.7 Prevention 182
- 7.8 Conclusion 184
- Acknowledgments 184
- References 184

## **8 The Maillard Reaction: Protein Modification by Ascorbic Acid 189**

- Vincent M. Monnier, David R. Sell, Grant Hom, Shiyuan Dong, Benlian Wang and Xingjun Fan*
- 8.1 Introduction 189
- 8.2 Ascorbic Acid Homeostasis in the Lens: A Dual Sword 190
- 8.3 Ascorbic Acid as a Source of Age-Related Damage to the Lens 190
- 8.4 Chemical Pathways of Ascorbic Acid Degradation *In Vitro* and the Human Lens 191
- 8.5 Advanced Glycation End Products that have been Detected in the Human Lens 192
- 8.6 Glucose vs. Ascorbic Acid as a Source of Advanced Glycation End Products in the Lens 193
- 8.7 Ascorbic Acid as a Major Source of Oxoaldehydes in Lens and Brain 195
- 8.8 Significance of Advanced Glycation/Ascorbylation Products in the Lens and Brain 196
- 8.9 Conclusions 197
- Acknowledgments 197
- References 197