Contents

Foreword VII
Preface XV

1 Introduction 1
1.1 Classical Approaches to Protein Modification 1
1.2 Peptide Synthesis, Semisynthesis and Chemistry of Total Protein Synthesis 2
1.3 Chemoselective Ligations Combined with Biochemical Methods 5
1.4 Methods and Approaches of Classical Protein Engineering 5
1.5 Genetically Encoded Protein Modifications – Reprogramming Protein Translation 6
1.6 Basic Definitions and Taxonomy 8
References 10

2 A Brief History of an Expanded Amino Acid Repertoire 13
2.1 The “Golden Years” of Molecular Biology and Triplet Code Elucidation 13
2.2 Early Experiments on the Incorporation of Amino Acid Analogs in Proteins 15
2.3 Test Tube (Cell-free) Synthesis of Proteins and Early Incorporation Experiments 18
2.4 Noncanonical Amino Acids as Tools for Studying Cell Metabolism, Physiology, Protein Processing and Turnover 19
2.5 Problem of Proofs and Formal Criteria for Noncanonical Amino Acid Incorporation 23
2.6 Recent Renaissance – Genetic Code Engineering 26
References 28

3 Basic Features of the Cellular Translation Apparatus 31
3.1 Natural Laws, Genetic Information and the “Central Dogma” of Molecular Biology 31
3.2 Cellular Investments in Ribosome-mediated Protein Synthesis 33
3.3 Molecular Architecture of AARS 34
### Contents

3.4 Structure and Function of the tRNA Molecule 40  
3.5 Aminoacylation Reaction 44  
3.6 AARS:tRNA Interactions – Identity Sets 45  
3.7 Translational Proofreading 48  
3.8 Ribosomal Decoding – A Brief Overview 50  
3.9 Codon Bias and the Fidelity of Protein Synthesis 51  
3.10 Preprogrammed Context-dependent Recoding: fMet, Sec, Pyl, etc. 53  
3.11 Beyond Basic Coding – Posttranslational Modifications 56  
References 59

4 Amino Acids and Codons – Code Organization and Protein Structure 66  
4.1 Basic Features and Adaptive Nature of the Universal Genetic Code 66  
4.2 Metabolism and Intracellular Uptake of Canonical Amino Acids 68  
4.3 Physicochemical Properties of Canonical Amino Acids 69  
4.4 Reasons for the Occurrence of Only 20 Amino Acids in the Genetic Code 73  
4.5 What Properties of Amino Acids are Best Preserved by the Genetic Code? 74  
4.6 Evolutionary Legacy: Dual Nature of Conserved Code and Finite Number of Protein Folds 77  
4.7 Natural Variations in Assignment of Codons of the Universal Genetic Code 79  
4.7.1 Nucleoside Modifications and Codon Reassignments 81  
4.8 Codon Reassignment Concepts Possibly Relevant to Code Engineering 81  
4.8.1 Genome Size, Composition, Complexity and Codon Reassignments 81  
4.8.2 Stop Codon Takeover, Codon Capture and Codon Ambiguity 83  
References 85

5 Reprograming the Cellular Translation Machinery 90  
5.1 Enzyme Specificity of Aminoacyl-tRNA Synthetases (AARS) and Code Interpretation 90  
5.1.1 Living Cells as Platforms for Amino Acid Repertoire Expansion 90  
5.1.2 Uptake, Toxicity and Metabolic Fate of Noncanonical Amino Acids 91  
5.1.2.1 General Considerations 91  
5.1.2.2 Amino Acid Transport 93  
5.1.2.3 Metabolic Conversions and Toxicity of Analogs and Surrogates 94  
5.1.3 Constrains and Levels in Code Engineering 95  
5.1.4 Auxotrophism and Natural AARS with Manipulated Functions 95  
5.1.4.1 Proteome-wide Replacements: “Unnatural Microorganisms” 97  
5.1.4.2 Substitutions at the Level of Single Proteins – Selective Pressure Incorporation (SPI) 100  
5.1.4.3 Kinetic Control – Enhanced System for Protein Translation 102  
5.1.4.4 Extension of the Existing Specificities of AARS 104  
5.1.4.5 AARS with an Attenuated Editing Function 106
6 Implications and Insights: From Reprogrammed Translation and Code Evolution to Artificial Life 184
6.1 Code Engineering and Synthetic Biology 184
6.2 Novel Features of Protein Translation that have Emerged from Research in Code Engineering 185
6.2.1 Code Malleability, Catalytic Promiscuity and the Amino Acid “Identity” Problem 185
6.2.2 A Barrier between Allowable and Nonpermissive Amino Acids – An Index for Entry in the Genetic Code 187
6.2.3 Protein Structural Types and Amino Acid Substitution Capacity 190
6.2.4 Building a Direct Link between Metabolism and Reprogrammed Translation 192
6.3 The Amino Acid Repertoire and its Evolution 193
6.3.1 “Copernican Turn” and the Last Sacrosanct in Biochemistry 193
6.3.2 Spontaneous Terrestrial and Extraterrestrial Generation of Amino Acids 194
6.3.3 Metabolic Routes for Amino Acid Syntheses and Co-evolution Theory 197
6.4 Artificial Genetic Systems and Code Engineering 200
6.4.1 Cells with Chemically Ambiguous Proteomes – Codon Reassignment Issues 200
6.4.2 Is it Possible to Improve the Adaptive Features of the Genetic Code? 201
6.4.3 Possibilities for De Novo Design of Organisms with their “Own” Genetic Codes 203
6.4.4 Code Engineering and Society – Philosophical and Ethical Implications 205
6.4.5 Future Challenges, Chances and Risks 207
References 208

7 Some Practical Potentials of Reprogrammed Cellular Translation 213
7.1 Practical Choice of Methods and Some Controversies in the Field 213
7.2 The Plasticity of the Translation Machinery, Amino Acid Generic Types and Protein Structure 215
7.3 DNA Nucleotide Analogs: From Sequencing to Expanded Code and Therapy 217
7.4 Noncanonical Amino Acids in Material Science 217
7.5 Isomorphous Replacement and Atomic Mutations in Structural Biology and Biophysics 221
7.5.1 Protein X-ray Crystallography 221
7.5.2 Atomic Mutations and Predictable Perturbations in the Protein Structure 223
7.5.3 Proteins Enriched with Chalcogen, Hydroxyl and Aza Analogs and Surrogates of Trp 225
7.6 Translationally Active Amino-Trp Analogs: Novel Spectral Windows and Protein Sensors 228
7.6.1 Providing Proteins with pH Sensitivity 228
7.6.2 Novel “Golden” Class of Autofluorescent Proteins 230
7.7 Fluorinated Amino Acids in Protein Engineering and Design 233
7.7.1 Monofluorinated Amino Acids in Protein Studies, Engineering and Design 233
7.7.2 Nonsticking Eggs and Bio-Teflon – Trifluorinated Amino Acids in Protein Engineering and Design 241
7.8 Protein Processing, Bioorthogonality and Protein Surface Diversifications 245
7.8.1 Chemoenzymatical Control of Protein Processing and Posttranslational Modifications 245
7.8.2 Staudinger–Bertozzi Ligation and “Click” Chemistry on Proteins 246
7.8.3 Tagging, Caging, Crosslinking and Photoswitching at the Protein Surface 247
7.9 Pharmacologically Active Amino Acids 249
7.9.1 Bioisosteric Compounds, Antagonists, Agonists and Antimetabolites 249
7.9.2 Neuroactive Amino Acids and their Derivatives 251

References 253

Epilogue 261

Index 263