Contents

Preface XV 1 A Historyof Protein Biosynthesis and Ribosome Research L Hans-iöraRhe'mberaer 1.1 Introduction 1 The Archaeology of Protein Synthesis - The 1940s: 1.2 Forgotten Paradigms 2 1.3 Basic Mechanisms - The 1950s 5 1.3.1 Steps toward an in vitro Protein Synthesis System 5 Amino Acid Activation and the Emergence of Soluble RNA 1.3.2 7 From Microsomes to Ribosomes 1.3.3 13 1.3.4 Madeis 17 1.4 The Golden Age of Translation - The 1960s 21 From Enzymatic Adaptation to Gene Regulation: Messenger RNA 1.4J21 1.4.2 A Bacterial in vitro System of Protein Synthesis and the Cracking of the Genetic Code 25 1.4.3 The Functional Dissection of Translation 28 IAA The Structural Dissection of the Ribosome 33 1970-1990s: A Brief Synopsis 1.5 35 References 38 2 Structure of the Ribosome 53 Gregor Bloha General Features of the Ribosome and Ribosomal Subunits 2.1 53 2.2 A Special Feature of the 50S Subunit: The Tunnel 54 2.3 Features of the Ribosomal Subunits at Atomic Resolution 59 The Domain Structure of the Ribosomal Subunits 2.4 62 2.5 Interactions of RNA with RNA or Struts and Bolts in the Threedimensioml Fold of rRNA: Coaxial Stacking and A-minor Motifs 65

- 2.5.1 Coaxial Stacking 66
- 2.5.2 A-minor Motifs 69
- 2.5.3 Ribose Zippers and Patches of A-minor Motifs 71
- 2.5.3.1 Canonical Ribose Zipper 71
- 2.5.3.2 Single-base Ribose Zipper 7!

VI Contents

Contents	
2.6	Progress and New Developments in Understanding
	rRNA Structures 72
2.6.1	K-turn 73
2.6.2	Lonepair Triloop 73
2.6.2.1	Classification of Lonepair Triloops 75
2.6.3	Systemizing Base Pairs 76
2.6.4	Systemizing RNA Structural Elements 78
2.7	RNA-protein Interactions 79
2.7.1	Problem of RNA Recognition 79
2.7.2	Chemistry of RNA-protein Interactions SO
2.7.3	rRNA-protein Interaction 8/
	References 82
3	Ribosome Assembly 85
3.1	Assembly Of The Prokaryotic Ribosome 85
5.1	Knud H. Nierhaus
3.1.1	Introduction 85
3.1.2	Processing of rRNAs 86
3.1.2	Precursor Particles and Reconstitution Intermediates 90
3.1.4	Assembly-initiator Proteins 91
3.1.5	Proteins Essential for the Early Assembly: The Assembly Gradient 95
3.1.6	Late-assembly Components 96
3.1.7	Proteins Solely Involved in Assembly 97
3.1.8	Assembly Maps 99
5.1.0	References 104
3.2	Eukaryotic Ribosome Synthesis 107
0.12	Denis L.j. Lafontaine
3.2.1	Introduction 107
3.1.1	Prelude 107
3.2.2	Why so many RRPs? 109
3.2.3	(Pre-)ribosome Assembly, the Proteomic Era 110
3.2.4	Ribosomal RNA Processing, Getting there 113
3.2.5	Ribosomal RNA Modification: A Solved Issue? 118
3.2.5.1	Ribose Mefhylation, Pseudouridines formation and the snoRNAs 119
3.2.5.2	The Emergence of the snoRNAs 121
3.2.5.3	Non-ribosomal RNA Substrates for the snoRNAs \22
3.2.5.4	Possible function(s) of RNA modifications 123
3.2.5.5	Base mefhylation 123
3.2.5.6	U3 snoRNP, the 'SSU Processome', and the Central Pseudoknot 124
3.2.6	SnoRNA Synthesis and Intranuclear TrafBcking 125
3.2.6.1	SnoRNAs Synthesis 125

- 3.2.6.2 Non-core snoRNP Proteins required for snoRNA Accumulation 126
- 3.2.6.3 Interactions between Cleavage Factors and Core snoRNP Proteins 128
- 3.2.6.4 SnoRNAs Trafficking 128
- 3.2.6.5 CB/NB are Conserved Sites of Small RNP Synthesis 130
- 3.2.7 Ribosome Intranuclear Movements and Ribosome Export 130
- 3.2.8 The Cytoplasmic Phase of Ribosome Maturation 132
- 3.2.9 Regulatory Mechanisms, all along 134
- 3.2.10 AndNow... What's Next? B4
- 3.2.11 Epilogue 135
- 3.3.12 Useful WWW links 135 References 136
- 4 tRNA and Synthetases 145
- 4.1 tRNA: Strucrure and Function 145 Viter Marquez and Knud H. Nierhaus
- 4.1.1 [ntroduction 145
- 4.1.2 Secondary Strucrure 146
- 4.1.3 Tertiary Strucrure 149
- 4.1.4 tRNA Modifications 154
- 4.1.5 Recognition of tRNA by tRNA synthetase: Identity Elements 154
- 4.1.6 Is the tRNA Cloverleaf Structure a Pre-requisite for the L-shape? *160*
- 4.1.7 Other Functions of tRNA outside the Ribosomal Elongation Cyde 161
- 4.1.8 Human Neurodegenerative Disorders Associated with Mitochondrial tRNAs 162

References 166

- 4.2 Aminoacylations of tRNAs: Record-keepers for the Genetic Code 169 Uu's Ribas de Poupiana and Paul Schimmel
- 4.2.1 Introduction 169
- 4.2.2 The Operational RNA Code 170
- 4.2.3 Extant Aminoacyl-tRNA Synthetases 172
- 4.2.4 **The** Origin of Aminoacyl-tRNA Synthetase Classes: Two Proteins bound to one tRNA *174*
- 4.2.5 A Common Genetic Origin for all Aminoacyl-tRNA Synthetases? 177
- 4.2.5.1 Evolution of Extant Enzymes prior to LUCA 179
- 4.2.5.2 **Changes** in Acceptor Stem Identity Elements Correlate with Changes in die Code *ISO* References *183*
- 5 mRNA Decay and RNA-degrading Machines in Prokaryotes and Eukaryotes 185 Agamemnon]. Carpousis and Marc Dreyfus
- 5.1 Summary 185

5.2	Introduction 185
5.3	raRNA Decay in E. coli 186
5.4	mRNA Decay in S. cerevisiae 188
5.5	A Comparison of mRNA Decay in E. coli and S. cerevisiae 188
5.6	RNasc E Specifkity: A Role in Translation Arrest? 189
5.7	The E. coli RNA degradosome 192
5.8	The Autoregulation of RNase E and PNPase Synthesis: A Link
	between Bulk Translation and mRNA Stability 195
5.9	RNA-degrading Machines in other Organisms 197
5.10	DEAD-box ATPases 201
5.11	Perspective 202
	References 204
6	tRNA Locations on the Ribosome 207
	Knud H. Nierhaus
6.1	tRNAs Move through Functional Sites on the Ribosome 207
6.2	Visualization of tRNAs on the Ribosome 209
6.3	tRNA-ribosome Contacts 215
	References 216
7	Initiation of Protein Synthesis 219
7.1	Initiation of Protein Synthesis in Eubacteria 219
	Daniel N. Wilson
7.1.1	Overview of Initiation in Eubacteria 219
7.1.2	Specialized initiation events: translational coupling, 70S initiation
	and leaderless mRNAs 222
7A3	Initiation Factor 1 Binds to the Ribosomal A-site 224
7.1.4	The Domain Structure of Bacterial 1F2 227
7.1.5	Interaction Partners of IF2 230
7.1.6	The Role of the IF2-dependent GTPase Activity 232
7.1.7	The Mystery of the IF3-binding Site on the 30S Subunit 233
	References 236
7.2	Mechanism and Regulation of Protein Synthesis Initiation
	in Eukaryotes 241
	Alan C. Hinnebusch, Thomas E. Dever, and Nahum Sonenberg
7.2.1	Introduction 241
7.2.1.	5
	and Prokaryotes 241
7.2.1.	
7.0.1	bacteria, archaea and eukaryotes 244
7.2.1	
7.2.2	Generation of Free 40S Subunits and 40S Binding of Met-tRNA 251

I

- 7.2.2.1 Dissociation of ldle 80S Ribosomes 251
- 7.2.2.2 Components of the eIF2/GTP/Met-tRNA Temary Complex 252
- 7.2.2.3 The GEF eIF2B regulates ternary complex formation 263
- 7.2.2.4 Binding of Ternary Complex and mRNA to the 40S Ribosome is Stimulated by eIF3 269
- 7.2.2.5 elFlA Stimulates Ternary Complex Binding to 40S Subunits and Participates in AUG Selection During Scanning 275
- 7.2.3 Binding of Ribosomes to mRNA 279
- 7.2.3.1 The Ends of Eukaryotic mRNAs Contain Distinctive Conserved Structures 279
- 7.2.3.2 Ribosome Binding to mRNA is Stimulated by the eIF4 Factors 279
- 7.2.3.3 Circularization of mRNA via eIF4G-PABP Interaction 290
- 7.2.4 Translational Control by mRNA Circularization 291
- 7.2.5 Regulation of eIF4 Function by Phosphorylation 292
- 7.2.5.1 eIF4E Phosphorylation 292
- 7.2.5.2 eIF4E-4E-BPs 292
- 7.2.5.3 eIF4G Phosphorylation 294
- 7.2.5.4 eIF4B Phosphorylation 295
- 7.2.6 Translational Control by Paips PABP Interacting Proteins 295
- 7.2.7 AUG Recognition during Scanning 296
- 7.2.7.1 AUG is the Predominant Signal for Initiation and is Sck-cted liy Proximity to the 59-end by the Scanning Mechanism 296
- 7.2.7.2 The Anticodon of tRNA, **eIF2** Subunits, **eIF1**, and **eIF5** are Determinants of AUG Selection during Scanning 299
- 7.2.7.3 eIFl plays a role in TC binding, scanning, and AUG selection 299
- 7'.2.7'A eIF5 Functions as a GTPase Activating Protein for eIF2 in AUG Selection and Subunit loining 300
- 7.2.8 Joining of 60S Subunits to 40S Ribosomal Complexes 302
- 7.2.8.1 elF5B Catalyzes a Second GTP-dependent Step in Translation Initiation 303
- 7.2.8.2 GTPase Switch Regulates Ribosome Affinity of elF5B and Governs Translational Efficiency 304
- 7.2.9 IRES-mediated Translation Initiation 308
- 7.2.10 Future Prospects 310 References 313
- 8 The Elongation Cycle 323 Knud H. Nierhaus
- 8.1 Models of the Elongation Cycle *326*
- 8.1.1 The Hybrid-site Model for Elongation 326
- 8.1.2 The Allosteric Three-site Model (a-e Model; Reciprocal Coupling berween the A- and E-sites) 329

X Contents

- 8.2 Decoding and A-site Occupation 333
- 8.2.1 Some General Remarks about Proofreading 333
- 8.2.2 Discrimination against Noncognate aa-tRNAs 333
- 8.2.3 Decoding of an aa-tRNA fCognate versus Near-cognate aa-tRNAs} 337
- 8.2.4 RolesofEF-Tu 341
- 8.2.5 Mimicryat the Ribosomal A-site 341
- 8.2.5 Translational Errors 342
- 8.3 The PTF Reaction 345
- 8.3.1 A Short Intermission: Two Enzymatic Principles of PTF Activity 348
- 8.3.1.1 Chemical Concept: A Transient Covalent Bond between Active Center and Substrate(s) 348
- 8.3.1.2 Physical Concept: The Template Model 350
- 8.3.2 Data from the Crystal Structures 352
- 8.3.3 **Why** both the Physical and Chemical Concepts for Peptide-bond Formation? *355*
- 8.4 The Translocation Reaction 355
- 8.4.1 Conservation in the Elongaüon Factor-G Binding Site 356
- 8.4.2 Dynamics within the Ribosome 359 References 363
- 9 Termination and Ribosome Recycling 367 Daniel N. Wilson
- 9.1 Introduction 367
- 9.2 Stop Codon Recognition and Release of the Nascent Polypeptide Chain 368
- 9.3 The Bacterial Class I Decoding Release Factors 369
- 9.3.1 The Structure of RF2 and Translational Mimicry 369
- 9.3.2 The Two-domain Functional Model for RF2 371
- 9.3.3 Identifying Functional Important Regions within the Decoding RFs 371
- 9.3.4 Codon Recognition Domain of Bacterial RFs: the Termination Signal *374*
- 9.3.5 Codon Recognition Domain of Bacterial RFs: the "Tripeptide Motif" 375
- 9.3.6 Peptidyl-tRNA hydrolase function of bacterial RFs: domain III and the GGQ motif 376
- 9.3.7 Large Conformational Changes Associated with RF2 Binding to the Ribosome 379
- 9.3.8 The Trigger for RF-mediated Release of the Nascent Chain and the Outcome 383
- 9.4 Eukaryotic Class I Termination Factors 384
- 9.4.1 Stop-codon Recognition is Associated with Domain I of eRFl 386

Contents

9.4.2	eRFl-mediated Polypeptide Release 388
9.5	Dissociation of the Post-termination Complex 388
9.5.1	Eubacterial RF3 Dissociates the Class I Termination Factors 388
9.5.2	Eukaryotic RF3: Dissociation versus Delivery of eRF1 390
9.6	Ribosome Recycling 39!
9.6.1	RRF Mediates Ribosome Recycling in Eubacteria 391
7.0.1	References 392
	Kerelences 372
10	The Mechanism of Recoding in Pro-and Eukaryotes 397
	Elizabeth S. Poole, Louise L. Major, Andrew C. Cridge, and Warren P. Jäte
10.1	Introduction 397
10.2	Maintaining Decoding Accuracy and the Reading Frame 398
10.3	The Use of a Stop Signal for both Elongation and Termination
	of Protein Synthesis 399
10.4	The Mechanism for See Ineorporation at UGA Sites
	in Bacterial mRNAs 399
10.4.1	The Gene Products 400
10.4.2	The Mechanism of See Incorporation 401
10.4.3	The Competition between See Incorporation and
	Canonical Decoding of UGA by RF2 401
10.5	Mechanism for See Ineorporation at UGA Sites in Eukaryotic
	and Archaeal mRNAs 403
10.5.1	The Gene Products 403
10.5.2	The Mechanism of See Ineorporation at Specific UGA
	Stop Codons 404
10.6	Why does Recoding Occur at Stop Signals? 404
10.6.1	The Stop Signal of Prokaryotic Genomes - Engineered for
	High Efficiency Decoding? 406
10.6.2	The Stop Signal of Eukaryotic Genomes - Diversity Contributes
10 7	to Recoding 411
10.7	Readthrough of a Stop Signal: Decoding Stop as Sense 413
10.8	Bypassing of a Stop Codon: 'Free-wheeling' on the mRNA 415
10.9	Frameshifting Around Stop or Sense Codons 417
10.9.1	Forward Frameshifting: the +1 Event 418
10.9.2	Programed -1 Frameshifting: A Common Mechanism used by
10.10	Many Viruses During Gene Expression 420
10.10	Conclusion 424
	References 426
11	Regulation of Ribosome Biosynthesis in Escherichia coli 429
	Madian laladana Dan D. Orana'' and Kandhi Miadana

- Madina Iskakova, Sean R. Conneii, and Knud H. Nierhaus Overviewof Ribosome Biosynthesis Regulation 429

- 11.1 Regulation of rRNA Synthesis 430
- 11.1.1 Organization of rRNA Operons and Elements of rRNA Promoters 430
- 11.1.2 Models for rRNA Regulation 434
- 11.1.3 Stringent Response 435
- 11.2 Regulation of r-protein Synthesis 438
- 11.2.1 Some General Remarks 438
- 11.2.2 Various Models for r-protein Regulation 441
- **11.2.2.1** spe operon 441
- 11.2.2.2 SlOoperon 441
- 11.2.2.3 a operon 443
- 11.2.2.4 str operon 443
- 11.2.2.5 IF3 operon 444
- 11.3 Conclusion 445
 - References 446

12 Antibiotics and the Inhibition of Ribosome Function 449

Daniel N. Wilson

- 12.1 Introduction 449
- 12.1.1 The Inhibition of Protein Synthesis in Bacteria 449
- 12.2 Inhibitors of Initiation 453
- 12.2.1 Kasugamycin 456
- 12.2.2 Edeine 457
- 12.2.3 Pactamycin 459
- 12.2.4 Evernimicin and Avilamycin 460
- 12.2.5 Antibiotic Inhibitors of Ribosome Assembly 462
- 12.3 Inhibitors of the Elongation Cycle 464
- 12.3.1 Antibiotic Action and A-site Occupation 465
- 12.3.1.1 Tetracycline: An Inhibitor of A-site Occupation 465
- 12.3.1.2 Antibiotics Affecting the Fidelity of Translation 468
- 12.3.1.3 Inhibitors of EF-Tu-mediated Reactions 475
- 12.3.2 Inhibitors of Peptide-bond Formation and Nascent Chain Progression 480
- 12.3.2.1 Puromycin and Blasticidin S mimic the CCA end of tRNAs 480
- 12.3.2.2 Sparsomycin Prevents A-site Binding and Stimulates P-site Binding 483
- 12.3.2.3 Antibiotic Overhip in the PTF Center: chloramphenicol, Anisomycin and the Lincosamides 484
- 12.3.2.4 Blocking the Progression of the Nascent Chain by the Macrolidc Antibiotics 4H8
- 12.3.2.5 Streptogramins 494
- 12.3.2.6 New Classes of Translation Inhibitors; the Oxazolidinones and Novel Ribosome Inhibitors 496

- 12.3.3 Translocation Inhibitors 499
- 12.3.3.1 Thiostrepton and Micrococcm 499
- 12.3.3.2 Viomycin Blocks Coupled GTPase Activity 502
- 12.3.3.3 Spectinomycin Interferes with EF-G Binding 503
- 12.3.3.4 Fusidic Acid is the Counterpart of Kirromyän 504
- 12.4 Inhibitors of Termination, Recycling and *trans*-Translation 506
- 12.4.1 Termination 507
- 12.4.2 Recycling 507
- 12.4.3 Trans-translation 508
- 12.5 Mechanisms Causing Drug Resistance 508
- 12.5.1 Modification of the Antibiotic 509
- 12.5.2 Blockage of Transport (without Modification of the Drug) 509
- 12.5.3 Overproduction of the Inhibited Substrate (Target Dilution) 509
- 12.5.4 Bypassing or Replacement of the Inhibited Reaction 510
- 12.5.5 Alteration of the Target Site 510
- 12.5.6 Active Protection of the Target by a Third Component 511
- 12.6 Future Perspectives 512 References 513
- 13 The Work of Chaperones 529 jean-Hetve Alix
- 13.1 From The Levinthal Paradox To The Anfinsen Cage 529
- 13.2 The Folding Machines 532
- 13.2.1 The Trigger Factor (TF) 532
- 13.2.2 The DnaK/DnaJ/GrpE System 532
- 13.2.3 The GroEL/GroFS System 535
- 13.2.4 Other Chaperones 539
- 13.2.4.1 HSP90 539
- 13.2.4.2 Clp/HSPlOO Family 539
- 13.2.4.3 DegP 540
- 13.2.4.4 Periplasmic Chaperones 540
- 13.2.4.5 Pili Chaperones 541
- 13.2.4.6 SmallHSPs 541
- 13.2.4.7 Endoplasmic Reticulum (ER) Chaperones 543
- 13.2.4.8 Intramolecular Chaperones 543
- 13.3 Chaperone Networks 543
- 13.3.1 De novo Protein Folding 543
- 13.3.2 Protein Disaggregation 545
- 13.3.3 Posttranslational Quality Control 545
- 13.4 Chaperones and Stress 547
- 13.4.1 The Heat-shock Response and its Regulation 547

Contents

- 13.4.2 Thermotolerance 548
- 13.4.3 Who Detects Stress? 548
- 13.5 Assembly and Disassembly of Macromolecular Complexes 549
- 13.6 Protein Translocation Across Membranes 550
- 13.7 New Horizons in Chaperone Research 551
- 13.7.1 HSP90 and the Pandora's Box of Hidden Mutations 551
- 13.7.2 Chaperones and Prions 551
- 13.7.3 Chaperones and Ribosome Biogenesis 552
- 13.7.4 RNA Chaperones 55.3
- 13.7.5 Chemical Chaperones 553
- 13.7.6 Medical implications 553
- 13.7.7 Chaperoning the chaperones 553 References 554

Index 563

XIV