

Translation
In
Eukaryotes

Hans Trachsel

Translation In Eukaryotes

Edited by

Hans Trachsel

*Institute für Biochemie und Molekularbiologie
Universität Berne
Berne, Switzerland*



CRC Press

Boca Raton Ann Arbor Boston London

Library of Congress Cataloging-in-Publication Data

Cataloging information is on file with the Library of Congress

Developed by Telford Press

This book represents information obtained from authentic and highly regarded sources. Reprinted material is quoted with permission, and sources are indicated. A wide variety of references are listed. Every reasonable effort has been made to give reliable data and information, but the author and the publisher cannot assume responsibility for the validity of all materials or for the consequences of their use.

All rights reserved. This book, or any parts thereof, may not be reproduced in any form without written consent from the publisher.

Direct all inquiries to CRC Press, Inc., 2000 Corporate Blvd., N.W., Boca Raton, Florida 33431.

© 1991 by CRC Press, Inc.

International Standard Book Number 0-8493-8816-3

Printed in the United States

PREFACE

The synthesis of a protein is a multi-step biochemical pathway in which numerous components such as RNAs, proteins and small molecules cooperate to translate the nucleotide sequence of an RNA into the corresponding amino acid sequence of the protein. Several steps in this pathway are regulated and for a number of genes regulation at the level of translation contributes significantly to overall regulation of their expression.

Translation in eukaryotes has been studied in many laboratories and for more than twenty years biochemical methods were mainly used. These include the preparation of cell-free extracts competent for *in vitro* translation, fractionation of translational components, and reconstitution of translation systems from purified components. More recently, genes encoding translational components were cloned and genetic and molecular genetic methods were introduced to study the mechanism and regulation of eukaryotic translation. This opened the door for studies of translation at the molecular level *in vivo*. Important contributions to our understanding of eukaryotic translation are also more and more often made by investigators studying the expression of their gene of interest and finding themselves studying a translational phenomenon. These developments have led to rapid accumulation of new data and make it increasingly difficult to keep up with the literature.

This multi-author book was written for students, newcomers in the field, and others interested in obtaining overviews of specific aspects of eukaryotic translation in the form of short reviews. (The authors restrict themselves to the description of concepts and main findings and give references for the study of details.) Therefore this book should serve the reader as a guide to the vast amount of literature in this field. In order to keep the chapters short and to limit their content of information, aspects of mechanism of translation (Part I) were separated from aspects of regulation of translation (Part II) and special aspects such as comparison of eukaryotic and prokaryotic translation, mode of action of inhibitors and nomenclature of initiation factors are treated in Part III.

I would like to thank all the authors for writing their chapters and for making many helpful suggestions. I am specially grateful to my secretary, Mrs. Marianne Berger who did most of the correspondence involved in the preparation of this book.

June 1991

Hans Trachsel

CONTRIBUTORS

J. Bag

*Department of Molecular Biology
and Genetics
College of Biological Science
University of Guelph
Guelph, Ontario, Canada*

Juan P. G. Ballesta

*Centro de Biología Molecular
Universidad Autonoma de Madrid
Campus de Cantoblanco
Madrid, Spain*

Mike J. Clemens

*Department of Cellular and
Molecular Sciences
St. George's Hospital
Medical School
London, England*

Richard Giegé

*Laboratoire de Biochimie
Institute de Biologie Moléculaire
et Cellulaire du CNRS
Strasbourg, France*

Anne-Lise Haenni

*Institute Jacques Monod
Université de Paris VII
Paris, France*

John W. B. Hershey

*Department of Biological
Chemistry
University of California
Davis, California*

Alan Hinnebusch

*National Institutes of Health
Laboratory of Molecular Genetics
Bethesda, Maryland*

Markus Huemmelin

*F. Hoffmann-La Roche, Ltd.
Basel, Switzerland*

Richard J. Jackson

*Department of Biochemistry
University of Cambridge
Cambridge, England*

Jacques Lapointe

*Département de Biochimie
Faculté des Sciences et de Génie
Université Laval
Québec, Canada*

Richard D. Klausner

*National Institutes of Health
Cell Biology and Metabolism
Branch
Bethesda, Maryland*

Karen Meerovitch

*Department of Biochemistry
McGill University
Montreal, Québec, Canada*

Virginia M. Pain

*School of Biological Sciences
University of Sussex
Falmer, Brighton, England*

Jerry Pelletier

*Department of Cancer Research
Massachusetts Institute of
Technology
Cambridge, Massachusetts*

Robert E. Rhoads

*Department of Biochemistry
University of Kentucky
College of Medicine
Lexington, Kentucky*

Alexey G. Ryazanov

*Institute of Protein Research
Academy of Sciences of the
U.S.S.R.
Pushchino, Moscow Region,
U.S.S.R.*

Brian Safer

*Section on Protein Biosynthesis
National Institutes of Health
Bethesda, Maryland*

Lawrence I. Slobin

*Department of Biochemistry
University of Mississippi
School of Medicine
Jackson, Mississippi*

Nahum Sonenberg

*Department of Biochemistry and
McGill Cancer Center
McGill University
Montreal, Québec, Canada*

Alexander S. Spirin

*Institute of Protein Research
Academy of Sciences of the
U.S.S.R.
Pushchino, Moscow Region,
U.S.S.R.*

George Thomas

*Fredrich Miescher Institut
Basel, Switzerland*

Rosaura P. C. Valle

*Institut Jacques Monod
Université de Paris VII
Paris, France*

Harry O. Voorn

*Department of Molecular Cell
Biology
University of Utrecht
Utrecht, The Netherlands*

Ira G. Wool

*Department of Biochemistry and
Molecular Biology
The University of Chicago
Chicago, Illinois*

CONTENTS

<i>Preface</i>	v
<i>Contributors</i>	vii
 PART I: MECHANISM OF TRANSLATION	
 Chapter 1	
EUKARYOTIC RIBOSOMES: STRUCTURE, FUNCTION, BIOGENESIS, AND EVOLUTION	3
<i>Ira G. Wool</i>	
1.1. Introduction	3
1.2. Structure of Eukaryotic Ribosomes	4
1.3. Evolution of Eukaryotic Ribosomes	11
1.4. The Biogenesis of Ribosomes: The Regulation of the Synthesis of the Molecular Components and the Assembly of the Particles	15
1.5. RNA-Protein Interaction: An Analysis of the Recognition by α -Sarcin of a Ribosomal Domain Critical for Function	18
1.6. CODA	26
Acknowledgments	26
References	26
 Chapter 2	
TRANSFER RNAs AND AMINOACYL-tRNA SYNTHETASES	35
<i>Jacques Lapointe and Richard Giegé</i>	
2.1. Transfer Ribonucleic Acids	35
2.1.1. The Biological Necessity of tRNA	35
2.1.2. Cytoplasmic and Organellar tRNA Structure	36
2.1.3. tRNA Genes and Operons	38
2.1.4. tRNA Processing	39
2.1.5. tRNA Population and Codon Usage	40
2.2. Aminoacyl-tRNA Synthetases	40
2.2.1. An Overview	40
2.2.2. Structural Diversity of the Aminoacyl-tRNA Synthetases and Some Unifying Principles	42
2.2.3. Search for the Core Aminoacyl-tRNA Synthetases and Evolutionary Relationships Between Them	45
	ix

2.2.4. Distinctive Features of Eukaryotic Aminoacyl-tRNA Synthetases	46
2.2.5. Other Functions of Aminoacyl-tRNA Synthetases	47
2.2.6. Biosynthesis of Aminoacyl-tRNA Synthetases	50
2.3. tRNA Aminoacylation	51
2.3.1. Background—specific tRNA Aminoacylation is a Kinetic Process	51
2.3.2. Specific tRNA/aaRS Interactions	52
2.3.3. Mechanism of tRNA Charging, Proofreading, and Editing	58
2.3.4. Associations of Aminoacylated tRNAs with Metabolic Processes	60
References	61
 Chapter 3	
mRNA AND mRNP	71
<i>J. Bag</i>	
3.1. Introduction	71
3.2. Translation and Structure of mRNA	71
3.3. Translational Frameshifting	73
3.4. The 3'-Untranslated Region and Translation	73
3.5. Organellar mRNAs	74
3.6. Stability and mRNA Structure	74
3.7. mRNA-Protein Complexes	77
3.8. Protein Composition of Cytoplasmic mRNP Complexes	79
3.9. Sequence Specific Binding of mRNP Proteins	81
3.10. mRNP Complexes of Specific mRNAs	82
3.11. mRNP Complexes and Translation	84
3.12. Cytoskeleton and mRNA Translation	86
3.13. Small Cytoplasmic RNA-Protein Complexes	89
References	90
 Chapter 4	
INITIATION: MET-tRNA BINDING	97
<i>Harry O. Voorma</i>	
4.1. Introduction	97
4.2. Fractionated Cell-Free Systems	99

4.2.1. Dissociation of 80S Ribosomes	100
4.2.2. Eukaryotic Initiation Factor, eIF-2	100
4.2.3. Ternary Complex Formation	101
4.2.4. Formation of the 43S Preinitiation Complex	101
4.2.5. GDP:GTP Exchange Reaction	103
References	106
 Chapter 5	
INITIATION: mRNA AND 60S SUBUNIT BINDING	109
<i>Robert E. Rhoads</i>	
5.1. Introduction	109
5.2. Formation of the 48S Initiation Complex	111
5.2.1. Structural Features in mRNA Which Affect Formation of the 48S Initiation Complex	112
5.2.2. Proteins Involved in the Formation of the 48S Initiation Complex	119
5.2.3. Models for 48S Initiation Complex Formation	129
5.3. Formation of the 80S Initiation Complex	132
Acknowledgments	135
References	135
 Chapter 6	
POLYPEPTIDE CHAIN ELONGATION	149
<i>Lawrence I. Slobin</i>	
6.1. Introduction	149
6.2. Elongation Rate	149
6.3. The Elongation Cycle	151
6.3.1. EF-1 α Cycle	151
6.3.2. Binding of Aminoacyl tRNA to Ribosomes	153
6.3.3. Peptide Bond Formation	155
6.3.4. Translocation	155
6.4. Structure of Elongation Factors and their Genes	157
6.4.1. EF-1 α	158
6.4.2. EF-1 $\beta\gamma$	160
6.4.3. EF-1	160
6.4.4. EF-1 α -Like Polypeptides	161
6.4.5. EF-2	162
6.5. Elongation Factor Synthesis	163

6.6. Conclusions	165
Acknowledgments	166
References	166

Chapter 7

PEPTIDE CHAIN TERMINATION	177
---------------------------	-----

Rosaura P. C. Valle and Anne-Lise Haenni

7.1. Introduction	177
7.2. Reasons to Terminate: The Basic Process	178
7.3. Other Reasons to "Terminate"	178
7.4. Reasons for Not Stopping	179
7.4.1. Recognition of Termination Codons by tRNAs	179
7.4.2. Frameshifting	183
7.5. What Next?	185
Acknowledgments	185
References	185

PART II: REGULATION OF TRANSLATION

Chapter 8

BINDING OF MET-tRNA	193
---------------------	-----

Richard J. Jackson

8.1. Introduction	193
8.2. Effect of eIF-2 Phosphorylation on Protein Synthesis in Reticulocyte Lysates	194
8.3. Phosphorylation Sites in eIF-2	198
8.4. How Does eIF-2 Phosphorylation Interfere with GEF Activity?	198
8.5. Other Ways of Modulating GEF Activity	201
8.6. Alternative Interpretations	203
8.7. Is Phosphorylation of eIF-2 a Ubiquitous Control Mechanism?	205
8.8. The dsRNA-Activated Protein Kinase	209
8.9. Interferon Treatment Induces (Latent) DAI	211
8.10. DAI and Virus Infection	213
8.11. The Heme-Controlled Kinase, HCR	216
8.12. HCR-like Kinases in Other Cell Types	219
8.13. Dephosphorylation of eIF-2 α (P)	221

8.14. Accessibility of eIF-2 to Kinases and Phosphatases	222
Acknowledgments	223
References	223

Chapter 9

COVALENT MODIFICATION OF TRANSLATIONAL COMPONENTS 231

Markus Hümbelin and George Thomas

9.1. Introduction	231
9.2. Phosphorylation of eIF-4F	232
9.3. Phosphorylation of eIF-4B	234
9.4. S6 Phosphorylation	235
9.5. Aminoacyl-tRNA Synthetases	237
9.6. Hypusination of eIF-4D	238
References	238

Chapter 10

EXAMPLES OF EUKARYOTIC TRANSLATIONAL CONTROL: GCN4 AND FERRITIN 243

Alan Hinnebusch and Richard D. Klausner

10.1. Introduction	243
10.2. Gene-Specific Translational Control in <i>S. cerevisiae</i> by Upstream Open-Reading Frames	244
10.2.1. <i>CPA1</i> Expression is Regulated by a Single uORF	245
10.2.2. Translational Control of <i>GCN4</i> Expression by Multiple uORFs	247
10.2.3. Translation Control with Heterologous uORFs	250
10.2.4. Translational Control by uORFs in Higher Eukaryotes	261
10.3. A <i>cis-trans</i> Model for Translation Control	261
10.3.1. Iron Metabolism in Complex Eukaryotic Cells	261
10.3.2. The Iron Responsive Element: The <i>cis</i> -Acting RNA Element	262
10.3.3. The IRE Binding Protein: The <i>Trans</i> -Acting Factor	263
10.3.4. Regulating Both Translation and mRNA Stability	264
10.3.5. A Novel Regulatory Mechanism: The Sulfhydryl Switch	267
10.3.6. A Regulatory Model	268
References	269

Chapter 11	
THE TRANSLATION OF PICORNAVIRUSES	273
<i>Karen Meerovitch, Nahum Sonenberg and Jerry Pelletier</i>	
11.1. Introduction	273
11.2. Shut-Off of Host Protein Synthesis after Poliovirus Infection	274
11.2.1. Early Studies	274
11.2.2. CBP Complex Inactivation	275
11.2.3. eIF-2 α Phosphorylation	277
11.3. Internatal Initiation of Translation	278
11.3.1. Internal Initiation on Non-Picornavirus mRNAs	282
11.3.2. Molecular Mechanism for Internal Ribosome Binding	283
11.4. Future Prospectives	283
Acknowledgments	285
References	285
 Chapter 12	
ADJUSTMENT OF TRANSLATION TO SPECIAL PHYSIOLOGICAL CONDITIONS	293
<i>Virginia M. Pain and Mike J. Clemens</i>	
12.1. Introduction	293
12.2. Regulation of Translation by Cellular Nutrients	294
12.2.1. Amino Acid Starvation	294
12.2.2. Glucose Starvation	296
12.3. Regulation of Translation by Changes in Ionic Environment	297
12.3.1. Effects of Hypertonic Conditions	297
12.3.2. Regulation by Calcium Ions	298
12.3.3. Effects of Heavy Metal Ions	298
12.4. Translation in Relation to Cellular Growth Control	299
12.4.1. Control by Serum	299
12.4.2. The Role of Individual Growth Factors	300
12.4.3. Protein Kinases Involved in Translational Stimulation by Mitogens	301
12.4.4. The Control of Protein Synthesis by Interferons	302
12.5. Regulation of Translation by Heat Shock	303
12.6. Translational Control During Mitosis	306
12.7. Translational Control During Early Development	306
12.8. Concluding Remarks	311

Acknowledgments	313
Note Added in Proof	313
References	314
 Chapter 13	
REGULATION OF ELONGATION RATE	325
<i>Alexander S. Spirin and Alexey G. Ryazanov</i>	
13.1. Introduction: Regulation at Elongation Stage is Important in Eukaryotic Translation	325
13.2. Elongation Rate Changes Can Be Revealed from Transit Time Determination and Polyribosome Profile Analyses	326
13.2.1. Transit Time	326
13.2.2. Polyribosome Profile	328
13.2.3. Uncertainty Because of Termination Pause	329
13.3. Elongation Stage of Translation is Shown to be Regulated	330
13.3.1. Overall Elongation Rate in the Cell Can Be Stimulated or Slowed Down	330
13.3.2. Elongation Rates Can Be Differentially Affected on Different mRNAs	331
13.3.3. Regulatable Pauses Occur During Elongation	331
13.4. Overall Elongation Rate Can Be Modulated Through Changes in the Activity of the Elongation Factors	332
13.4.1. Do Aminoacyl-tRNA and GTP Concentrations Control the Elongation Rate?	332
13.4.2. Is the Ribosome a Target for Physiological Regulation of Elongation?	334
13.4.3. Elongation Factors Activities Can Be Regulated	335
13.5. Specific Elongation Rates on Different mRNAs May Be Underlain by Intercytoplasmic Compartmentations and Ribosome Pausing	339
13.6. Mechanisms of Ribosome Pausing and Its Modulation Can Be Different	340
13.6.1. Ribosomes Can Be Retarded at Modulating Codons	340
13.6.2. Structural Barriers May Exist Along mRNA	340
13.6.3. Structure of Nascent Peptide May Serve as a Signal for the Elongation Pause	341
13.7. Can the Regulation at the Elongation Stage Result in Translational Discrimination of Different mRNAs?	341
References	343

PART III: APPENDIX

Chapter 14

COMPARISON OF PROKARYOTIC AND EUKARYOTIC
TRANSLATION

353

John W. B. Hershey

14.1. Introduction	353
14.2. Pathway of Protein Synthesis in Bacteria	354
14.2.1. Overview	354
14.2.2. Initiation	354
14.2.3. Elongation	359
14.2.4. Termination	360
14.2.5. Reinitiation	360
14.3. Comparison of the Translational Machinery	361
14.3.1. mRNA	361
14.3.2. tRNAs and Aminoacyl-tRNA Synthetases	362
14.3.3. Ribosomes	364
14.3.4. Initiation Factors	365
14.3.5. Elongation Factors	367
14.3.6. Termination Factors	368
14.4. Translational Controls	368
14.4.1. Initiator Codon and Context	368
14.4.2. mRNA Secondary Structure	369
14.4.3. <i>Trans</i> -Acting Protein Factors and Antisense RNAs	369
Acknowledgments	370
References	370

Chapter 15

INHIBITORS OF EUKARYOTIC PROTEIN SYNTHESIS

375

Juan P. G. Ballesta

15.1. Introduction	375
15.2. Mode of Action	376
15.2.1. Inhibitors of Initiation	376
15.2.2. Inhibitors of Elongation	378
15.2.3. Translocation	381
15.2.4. Termination	383
15.2.5. Plant and Microbial Toxins	383
15.3. Resistance to Antibiotics	384
15.4. Inhibitors as Research Tools	385

15.5. Future Developments	385
15.6. Techniques Used in Present Antibiotic Research	386
Acknowledgments	386
References	387

Chapter 16

NOMENCLATURE OF INITIATION, ELONGATION AND TERMINATION FACTORS FOR TRANSLATION IN EUKARYOTES

393

Brian Safer

16.1. General Principles of Symbolism	394
16.2. Initiation Factors	394
16.2.1. Main Groups of Initiation Factors	394
16.2.2. New Initiation Factors	394
16.2.3. Subunits of Initiation Factors	395
16.3. Elongation Factors	395
16.4. Termination Factor	395
16.5. List of Known Mammalian Factors	395
16.6. Future Revision	395
References	398
Index	401

Part

I

Mechanism of Translation
