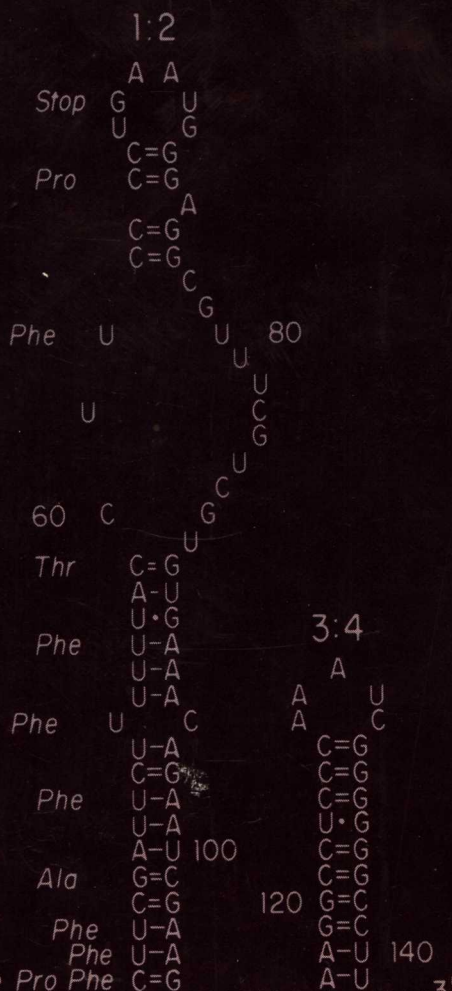


Translational Regulation of Gene Expression

Edited by
Joseph Ilan



AAGUCACUUAAGGAAACAAACAUGAAACACAUACCGUUUUU-ACGAACAAUA-UUUU 3'

Translational Regulation of Gene Expression

Edited by
Joseph Ilan

Case Western Reserve University
Cleveland, Ohio

Plenum Press • New York and London

Library of Congress Cataloging in Publication Data

Translational regulation of gene expression.

Includes bibliographical references and index.

1. Genetic translation. 2. Gene expression. 3. Genetic regulation. I. Ilan, Joseph.

QH450.5.T73 1987

574.87/322

87-15322

ISBN 0-306-42640-4

© 1987 Plenum Press, New York
A Division of Plenum Publishing Corporation
233 Spring Street, New York, N.Y. 10013

All rights reserved

No part of this book may be reproduced, stored in a retrieval system, or transmitted
in any form or by any means, electronic, mechanical, photocopying, microfilming,
recording, or otherwise, without written permission from the Publisher

Printed in the United States of America

Contributors

RICHARD D. ABRAMSON • Department of Biochemistry, Case Western Reserve University, Cleveland, Ohio 44106

MIR F. AHMAD • Department of Chemistry, University of Nebraska, Lincoln, Nebraska 68588

DONALD D. ANTHONY, JR. • Department of Biochemistry, Case Western Reserve University, Cleveland, Ohio 44106

GERARDO ARROYO • Biology Department, University of Puerto Rico, Rio Piedras, Puerto Rico 00931

ANGELA M. CALIENDO • Department of Biochemistry, Case Western Reserve University, Cleveland, Ohio 44106

GRACIELA C. CANDELAS • Biology Department, University of Puerto Rico, Rio Piedras, Puerto Rico 00931

TERESA M. CANDELAS • Biology Department, University of Puerto Rico, Rio Piedras, Puerto Rico 00931

CLARA E. CARRASCO • Biology Department, University of Puerto Rico, Rio Piedras, Puerto Rico 00931

DEBOPAM CHAKRABARTI • Department of Chemistry, University of Nebraska, Lincoln, Nebraska 68588

JOHN CLEMENTS • Department of Biochemistry, University of Rochester School of Medicine and Dentistry, Rochester, New York 14642

THOMAS E. DEVER • Department of Biochemistry, Case Western Reserve University, Cleveland, Ohio 44106

- RAQUEL E. DOMPENCIEL • Biology Department, University of Puerto Rico, Rio Piedras, Puerto Rico 00931
- DAVID E. DRAPER • Department of Chemistry, Johns Hopkins University, Baltimore, Maryland 21218
- ISAAC EDERY • Department of Biochemistry, McGill University, Montreal, Quebec H3G 1Y6, Canada
- HOWARD M. FRIED • Department of Biochemistry and Nutrition, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27514
- LEE GEHRKE • Harvard-M.I.T. Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139; Department of Anatomy and Cellular Biology, Harvard Medical School, Boston, Massachusetts 02115
- MARIANNE GRUNBERG-MANAGO • Institut de Biologie Physico-Chimique, 75005 Paris, France
- NABA K. GUPTA • Department of Chemistry, University of Nebraska, Lincoln, Nebraska 68588
- G. WESLEY HATFIELD • Department of Microbiology and Molecular Genetics, University of California, Irvine, California 92717
- STUART M. HEYWOOD • Department of Molecular and Cell Biology, University of Connecticut, Storrs, Connecticut 06268
- ALAN G. HINNEBUSCH • Laboratory of Molecular Genetics, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20892
- JOSEPH ILAN • Department of Developmental Genetics and Anatomy, School of Medicine, Case Western Reserve University, Cleveland, Ohio 44106
- MARCELO JACOBS-LORENA • Department of Developmental Genetics and Anatomy, Case Western Reserve University, Cleveland, Ohio 44106
- K. BRUCE JACOBSON • Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831
- DAVID L. KIRK • Department of Biology, Washington University, St. Louis, Missouri 63130
- THOMAS LAZ • Department of Biophysics, University of Rochester School of Medicine and Dentistry, Rochester, New York 14642
- SUSAN LINDQUIST • Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, Illinois 60637

WILLIAM C. MERRICK • Department of Biochemistry, Case Western Reserve University, Cleveland, Ohio 44106

BARBARA MROCZKOWSKI • Department of Molecular and Cell Biology, University of Connecticut, Storrs, Connecticut 06268

PETER P. MUELLER • Laboratory of Molecular Genetics, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20892

NARGIS NASRIN • Department of Chemistry, University of Nebraska, Lincoln, Nebraska 68588

JERRY PELLETIER • Department of Biochemistry, McGill University, Montreal, Quebec H3G 1Y6, Canada

JOEL D. RICHTER • Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts 01545

ERIC ROSENTHAL • Kewalo Marine Lab, Honolulu, Hawaii 96813

ROBERT J. SCHNEIDER • Department of Biochemistry, New York University Medical Center, New York, New York 10016

JANICE A. SHARP • American Biogenetics Corporation, Irvine, California 92715

THOMAS SHENK • Department of Molecular Biology, Princeton University, Princeton, New Jersey 08544

FRED SHERMAN • Departments of Biophysics and Biochemistry, University of Rochester School of Medicine and Dentistry, Rochester, New York 14642

NAHUM SONENBERG • Department of Biochemistry and McGill Cancer Center, McGill University, Montreal, Quebec H3G 1Y6, Canada

MATHIAS SPRINGER • Institut de Biologie Physico-Chimique, 75005 Paris, France

GARY D. STORMO • Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, Colorado 80309

ELIZABETH C. THEIL • Department of Biochemistry, North Carolina State University, Raleigh, North Carolina 27695

FRED WILT • Department of Zoology, University of California, Berkeley, California 94720

DIANE J. ZEZZA • Department of Molecular and Cell Biology, University of Connecticut, Storrs, Connecticut 06268

Preface

Given the accelerated growth of knowledge in the field of gene expression, it seemed timely to discuss current developments in the area of translational regulation of gene expression as well as to evaluate emerging technology.

Translational regulation occurs with prokaryotic as well as with eukaryotic messenger RNA (mRNA) *in vivo* and *in vitro*. In prokaryotes, through genetic manipulations and mutagenesis, the mechanisms are much better understood, as for example the mechanism of attenuation. In bacteria, different translational efficiencies for the same mRNA may vary by 1000-fold. Translational regulation was first observed in 1966 with RNA phages of *Escherichia coli* by Lodish and Zinder. However, translational regulation of proteins from DNA genomes is also well described for bacteria, as for example gene 32 protein of bacteriophage T4 and *E. coli* ribosomal proteins.

In eukaryotes, the utilization of an individual mRNA species with different efficiencies is poorly understood. For example, mRNA for ribosomal proteins is translationally regulated during *Drosophila* oogenesis, without any clue to the mechanism involved. It was observed that ribosomal protein mRNA during *Drosophila* oogenesis and embryogenesis is selectively on or off the polysomes during different developmental stages. In contrast, bacterial ribosomal protein is also translationally regulated by autogenous regulation. The mechanism is well understood and involves binding of the gene product to its transcript in competition with rRNA.

Regulation of specific mRNA translation in eukaryotes has been described for many diverse systems. Specific mRNA translation is known to be regulated during embryogenesis of invertebrates as well as vertebrates. It is regulated in specialized differentiated cells such as hepatocytes, myocytes, and reticulocytes. It is regulated during heat shock, fibroin production, and during photomorphogenesis.

A significant site of regulation of mRNA translation is at the level of initiation of protein synthesis. Initiation of protein synthesis in eukaryotes is very different from that in prokaryotes. Eukaryotic mRNA is monocistronic, and eukaryotic ribosomes do not initiate at internal AUG codons as in bacteria. The first AUG encountered by the ribosome is the functional initiation codon. Almost all eukaryotic cellular mRNAs carry a methylated cap structure that is recognized specifically by cap-binding proteins. These proteins are postulated to unwind a secondary structure at the 5' end of the eukaryotic mRNA. In eukaryotic mRNA, there is no sequence homology at the 5' noncoding region to serve as a ribosome binding site such as the Shine–Dalgarno sequence in bacteria.

At the level of initiation, the proposed mechanism for different translational efficiencies of mRNA is variation of secondary structure among mRNA species. However, this hypothesis has not been thoroughly tested. For such testing, the availability of highly purified mRNA is essential for structural analysis. With eukaryotes, this analysis was possible for very limited special messages such as α - and β -globin. The introduction of plasmid vectors containing phage RNA promoter (SP6, T7, and T3), which transcribe micrograms of pure message, has revolutionized the experimental analysis of eukaryotic mRNA. With such systems, one can increase or decrease secondary structure of a defined mRNA by *in vitro* mutagenesis and analyze structure–function relationships and the protein factors involved.

However, initiation of mRNA translation in eukaryotes is not the only point of potential discrimination in translational efficiency. A prime example is the translational control of a transcriptional activator in the regulation of amino acid biosynthesis in yeast. In addition, there are many examples of regulation of specific mRNA translation at the elongation step level following hormone treatment *in vivo* as well as regulation of specific mRNA translation by transfer RNA. In this book, we try to put forward the current development, the excitement, and the emergence of new ideas and methodology as applied to the translational regulation of gene expression.

Joseph Ilan

Cleveland

Contents

Chapter 1

Translational Regulation of Ribosomal Proteins in *Escherichia coli*: Molecular Mechanisms

DAVID E. DRAPER

1. Evidence for Autoregulation of Ribosomal Proteins	1
2. Regulation of the α Operon by S4	2
2.1. Introduction	2
2.2. Thermodynamics of S4-Messenger RNA Complex Formation	3
2.3. Structure of the α Messenger RNA Leader	5
3. Regulation of the L11 Operon by L1	6
4. Regulation of the <i>rif</i> Operon by L10	7
5. Other Ribosomal-Protein Repressors	10
6. Common Themes in Ribosomal-Protein Autoregulation	10
7. Thermodynamics of Translational Repression	12
7.1. Thermodynamics of Translation	12
7.2. Translation and Repression <i>in Vivo</i>	14
8. Predictions of Different Translational Repression Models	15
8.1. Displacement Model	15
8.2. Entrapment Model	16
8.3. Influences of Protein Binding on Messenger RNA Turnover ..	17
8.4. Prediction of Gene-Dosage Effects	19
9. Influence of Messenger RNA Secondary Structure on Translation ..	21

10. Future Directions	23
References	23

Chapter 2

Translational Regulation in Bacteriophages

GARY D. STORMO

1. Introduction	27
2. RNA Phage	28
2.1. Repression by Replicase	28
2.2. Repression by Coat Protein	29
3. T4 Gene 32	31
3.1. Autogenous Translational Repression	32
3.2. Binding Parameters	32
3.3. Quantitative Model of Repression	34
3.4. Tests of the Model	37
4. f1 Gene V	37
5. P22 Gene 8	41
6. T4 RegA Protein	42
7. Structural Repression and Activation	43
8. Conclusions	45
References	48

Chapter 3

Escherichia coli Threonyl-Transfer RNA Synthetase as a Model System to Study Translational Autoregulation in Prokaryotes

MATHIAS SPRINGER AND MARIANNE GRUNBERG-MANAGO

1. Introduction	51
2. Structure of the <i>Escherichia coli</i> Genome around the Gene for Threonyl-Transfer RNA Synthetase	52
3. The Expression of the Gene for Threonyl-Transfer RNA Synthetase Is Negatively Autoregulated at the Translational Level	53
3.1. <i>In Vitro</i> Studies	53
3.2. <i>In Vivo</i> Studies	53
4. Genetic Definition of the Translational Operator	56
4.1. Isolation of Operator Constitutive Mutants	57
4.2. Nucleotide Sequence of the Operator Constitutive Mutants	59
4.3. Homologies between the <i>thrS</i> Translational Operator and the Threonine-Specific Transfer RNAs	59
References	61

Chapter 4

Translational Regulation of Ribosomal Protein Gene Expression in Eukaryotes

MARCELO JACOBS-LORENA AND HOWARD M. FRIED

1. Introduction	63
2. Translational Regulation of Yeast Ribosomal Protein	
Synthesis	64
2.1. Genetics of Yeast Ribosomal Proteins	64
2.2. Is There Life after Transcription?	66
2.3. Evidence for Translational Regulation of Yeast Ribosomal	
Protein Synthesis	67
2.4. Other Aspects of Ribosomal Protein Messenger RNA	
Translation	70
2.5. Future Directions	73
3. Translational Regulation of Ribosomal Protein Synthesis during	
<i>Drosophila</i> Development	75
4. Translational Regulation of Ribosomal Protein Synthesis during	
<i>Xenopus</i> Development	77
5. Translational Regulation of Ribosomal Protein Synthesis during	
Mammalian Development	79
6. Translational Regulation of Ribosomal Protein Synthesis in Other	
Eukaryotic Cells	79
7. Conclusions and Prospects	81
References	82

Chapter 5

Selective Messenger RNA Translation in Marine Invertebrate Oocytes, Eggs, and Zygotes

ERIC ROSENTHAL AND FRED WILT

1. Introduction	87
2. Translational Control in Sea Urchin Eggs and Embryos	88
2.1. Role of Changes in the Translational Machinery	90
2.2. Role of Changes in the Availability of Messenger RNA	93
3. Qualitative Changes in Other Organisms	97
3.1. Qualitative Changes in Protein Synthesis	97
3.2. Mechanisms of Selective Translation	100
3.3. Regulation of Message Availability through the Association of	
the Maternal Messenger RNA with Other Macromolecules	100
3.4. Changes in Messenger RNA Structure Related to Changes in	
the Translation of Different Messenger RNAs	101

3.5. Role of Messenger RNA Competition in Changing Relative Rates of Messenger RNA Utilization	103
3.6. Role of Messenger RNA Localization in Selective Translation	104
4. Conclusions	105
References	107

Chapter 6

Molecular Mechanisms of Translational Control during the Early Development of *Xenopus laevis*

JOEL D. RICHTER

1. Introduction	111
2. Oogenesis and Embryogenesis in <i>Xenopus laevis</i>	112
2.1. RNA and Protein Synthesis during Oogenesis	112
2.2. Messenger RNA Recruitment during Oocyte Maturation	114
2.3. Messenger RNA Recruitment during Embryogenesis	116
3. Compartmentalization of Messenger RNAs	117
3.1. Localized Messenger RNAs	117
3.2. Membrane-Bound Messenger RNAs	118
4. Special Features of Translational Control	123
4.1. Translational Capacity of Oocytes	123
4.2. RNA Binding Proteins	124
4.3. Interspersed RNAs	129
4.4. Heat-Shock Response	133
4.5. Role of Polyadenylation	134
5. Conclusions	135
References	136

Chapter 7

Storage and Translation of Ferritin Messenger RNA

ELIZABETH C. THEIL

1. Introduction	141
2. Ferritin Structure	143
2.1. Protein Shell	143
2.2. Iron Core and Iron-Protein Interactions	145
3. Storage of Ferritin Messenger RNA	145
3.1. Ferritin Messenger RNA Encoding a Luxury Protein	146
3.2. Ferritin Messenger RNA Encoding a Housekeeping Protein	150
3.3. Significance of Ferritin Messenger RNA Storage	151
4. Translational Efficiency of Ferritin Messenger RNA	152
4.1. Translational Competition in Whole Cells	152

4.2. Translational Competition in Cell-Free Systems	153
4.3. Ferritin Messenger RNA Structure	155
5. Ferritin Gene Organization	157
6. Summary and Conclusions	158
References	159

Chapter 8

Regulation of Messenger RNA Translation at the Elongation Step during Estradiol-Induced Vitellogenin Synthesis in Avian Liver

LEE GEHRKE AND JOSEPH ILAN

1. Introduction	165
2. Analysis of Polypeptide Chain Elongation in Eukaryotic Systems ...	166
2.1. Examples of Gene Regulation at the Level of Polypeptide Chain Elongation	166
2.2. Methods of Analyzing Rates of Polypeptide Chain Elongation	167
2.3. Polypeptide Chain Elongation in Cockerel Liver following 17 β -Estradiol Stimulation: Analysis of the Average Rate and of Specific Rates for Serum Albumin and Vitellogenin Peptides ..	169
3. Mechanisms of Regulation at the Elongation Step of Protein Synthesis	181
4. Concluding Remarks	183
References	184

Chapter 9

Translational Regulation in the Heat-Shock Response of *Drosophila* Cells

SUSAN LINDQUIST

1. Introduction	187
2. Background	187
2.1. Heat-Shock Proteins	187
2.2. General Features of the <i>Drosophila</i> Response	188
3. Translational Specificity during Heat Shock	190
3.1. General Description of the Change in Translational Specificity	190
3.2. Models of Regulation	191
3.3. Heat-Shock Message-Translation Element	194
3.4. What Cellular Component Discriminates among Messages?	201
4. Translational Regulation during Recovery	202

4.1. Characterization of the Recovery Process.....	202
4.2. Possible Mechanisms of Recovery	202
5. Conclusions	205
References	205

Chapter 10

Strategies of Fibroin Production

GRACIELA C. CANDELAS, CLARA E. CARRASCO, RAQUEL E. DOMPENCIEL,
GERARDO ARROYO, AND TERESA M. CANDELAS

1. Introduction	209
2. Fibroin-Synthesizing Systems	210
2.1. <i>Bombyx mori</i>	210
2.2. Spiders	211
3. <i>Nephila clavipes</i> Model System	212
3.1. Large Ampullate Glands	214
3.2. Cell-Free Translation	217
3.3. Discontinuous Translation	219
3.4. Transfer RNA Functional Adaptation	220
4. Alanine Transfer RNA Isoacceptors	223
5. Alanine Transfer RNA Genes in <i>Bombyx mori</i>	223
6. Relevance to the <i>Nephila</i> System	226
References	227

Chapter 11

Translational Regulation during Photomorphogenesis

DAVID L. KIRK

1. Overview	229
2. Translational Regulation Accompanying Chloroplast Biogenesis	230
3. Translational Regulation Accompanying Cytodifferentiation in <i>Volvox</i>	238
4. Future Studies	240
References	242

Chapter 12

Gene Expression in Muscle: The Role of Small RNAs in the Expression of Muscle-Specific Proteins

DIANE J. ZEZZA, BARBARA MROCZKOWSKI, AND STUART M. HEYWOOD

1. Introduction	245
2. Interaction of Translational Control RNA102 with Messenger RNAs	248
2.1. Interaction <i>in Vivo</i>	248
2.2. Interaction <i>in Vitro</i>	248
2.3. Sequence Homology between Myosin Heavy-Chain Messenger RNA and Translational Control RNA102	253
3. Identification of a Translational Control RNA102 Gene	253
4. Subspecies of Translational Control RNA102	258
5. Conclusion and Prospects	261
References	263

Chapter 13

Involvement of Nucleotides in Protein Synthesis Initiation

WILLIAM C. MERRICK, RICHARD D. ABRAMSON, DONALD D. ANTHONY, JR.,
THOMAS E. DEVER, AND ANGELA M. CALIENDO

1. Introduction	265
2. Requirement for GTP: Eukaryotic Initiation Factor 2	267
3. Other GTP Binding Proteins: Eukaryotic Initiation Factor 5	271
4. GTP Binding Domain	271
5. Requirement for ATP: Messenger RNA Binding	275
5.1. Eukaryotic Initiation Factor 4A	275
5.2. Eukaryotic Initiation Factor 4F	276
6. Interaction of the Messenger RNA Specific Factors	278
7. Mechanism of Binding Messenger RNA	281
8. Control of Protein Synthesis by Nucleotide Binding Proteins	282
References	283

Chapter 14

Roles of Eukaryotic Initiation Factor 2 and Eukaryotic Initiation Factor 2 Ancillary Protein Factors in Eukaryotic Protein Synthesis Initiation

NABA K. GUPTA, MIR F. AHMAD, DEBOPAM CHAKRABARTI, AND NARGIS NASRIN

1. Introduction	287
2. Roles of Eukaryotic Initiation Factor 2 and Eukaryotic Initiation Factor 2 Ancillary Protein Factors in Regulation of Protein Synthesis Initiation	288
2.1. Animal Cells	288
2.2. Lower Eukaryotic Cells	321
3. Concluding Remarks	329
References	330

Chapter 15

Role of Eukaryotic Messenger RNA Cap-Binding Protein in Regulation of Translation

ISAAC EDERY, JERRY PELLETIER, AND NAHUM SONENBERG

1. Introduction	335
2. Cap-Binding Proteins Involved in Translation Initiation	336
2.1. Early Studies	336
2.2. ATP-Dependent Cap-Binding Proteins	338
2.3. Inactivation of Cap-Binding Protein Function after Poliovirus Infection and the Discovery of a New Initiation Factor	341
2.4. Structural Analysis of Cap-Binding Proteins and Their Subcellular Distribution	343
3. Messenger RNA Secondary Structure and Cap Recognition	346
3.1. Introduction	346
3.2. ATP and Cap Recognition	348
3.3. Ionic Strength and Cap Function	350
3.4. Poliovirus Infection and Cap-Binding Protein Activity	351
4. Discriminatory Activity of the Cap-Binding Protein Complex	352
5. Role of Cap-Binding Proteins in Regulation of Gene Expression ...	353
5.1. Poliovirus Infection of HeLa Cells	353
5.2. Heat Shock	357
5.3. Involvement of the Cap Structure in Control of Gene Expression in Other Systems	359
6. Concluding Remarks	360
References	361

Chapter 16

Differential Translation of Eukaryotic Messenger RNAs: The Role of Messenger RNA Secondary Structure

LEE GEHRKE

1. Introduction	367
2. Examples of Translational Regulation Mediated through Differential Messenger RNA Translational Efficiencies	368
3. Experimental Analysis of Messenger RNA Secondary Structure	370
4. Analysis of the Cleavage Patterns	372
5. Conclusion	376
References	376

Chapter 17

Translational and Nontranslational Mechanisms of Regulation by Eukaryotic Suppressor Mutants

K. BRUCE JACOBSON

1. Introduction	379
2. Suppressor Mechanisms	379
3. Transcriptional Regulation	380
4. Translational Regulation	383
5. Posttranslational Regulation	386
5.1. Vermilion Mutant	386
5.2. Suppression of Vermilion and Tryptophan Oxygenase	387
5.3. Purple Mutant	391
5.4. Suppression of Purple and 6-Pyruvoyltetrahydropterin Synthase	392
5.5. Suppression of Speck and Phenol Oxidase	394
6. Summary	394
References	395

Chapter 18

Translational Control of a Transcriptional Activator in the Regulation of Amino Acid Biosynthesis in Yeast

ALAN G. HINNEBUSCH AND PETER P. MUELLER

1. Introduction	397
2. General Amino Acid Control	398
3. <i>cis</i> -Acting Transcriptional Signals in General Amino Acid Control	398
4. A Hierarchy of <i>trans</i> -Acting Regulatory Factors in the General Amino Acid Control	400
5. Translational Control of <i>GCN4</i> Expression	402
6. Translational Control of <i>GCN4</i> Is Mediated by Multiple Upstream AUG Codons in <i>GCN4</i> Messenger RNA	404
7. Functional Differentiation of the Upstream AUG Codons in <i>GCN4</i> Messenger RNA	406
8. Translational Control of <i>GCN4</i> and the Scanning Hypothesis	410
References	412

Chapter 19

The Role of Messenger RNA Sequences and Structures in Eukaryotic Translation

THOMAS LAZ, JOHN CLEMENTS, AND FRED SHERMAN