

**PROGRESS IN  
MOLECULAR BIOLOGY AND  
TRANSLATIONAL SCIENCE**

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**VOLUME 90**

**TRANSLATIONAL CONTROL IN  
HEALTH AND DISEASE**

**EDITED BY  
JOHN W.B. HERSHEY**





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Translational Science**  
**Translational Control in  
Health and Disease**

*edited by*

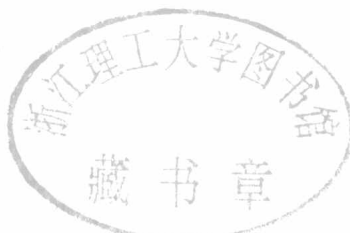
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## Preface

Protein synthesis, a key pathway in overall gene expression, contributes to the establishment of specific protein levels in cells. Many such proteins, such as enzymes, are involved in various aspects of cell metabolism, making the process of translation important in the overall coordination of cellular events. Aberrations in protein synthesis may result in disease states, such as cancer and diabetes. However, substantial defects in the process of protein synthesis are expected to be embryonic lethal, such that only mild and barely detectable changes are seen in actual human disease. This fact generates a challenging situation for the researcher and medical practitioner: to understand diseases involving protein synthesis, a precise and detailed understanding of translation is required in order to perceive the subtle changes involved. This volume focuses on the regulation of protein synthesis, with emphasis on those features encountered in disease states. It attempts to provide the necessary understanding of the details of protein synthesis that may enable the reader to comprehend the basis of the disease state and to conceive of appropriate therapeutic interventions. As most of the regulation of protein synthesis occurs at the initiation stage, we focus primarily on this aspect of the pathway.

The basic mechanism of protein synthesis in bacteria has been elucidated in great detail. In this system, atomic resolution of the structures of the ribosome and its various protein factors has been achieved, and sophisticated kinetic analyses of the various steps have been carried out. There is a general understanding of the translation pathways for human cells since the process is very similar to that in bacteria, except that the initiation phase is much more complex. Unfortunately, our understanding of eukaryotic protein synthesis is less precise, as the structure of the ribosome is not yet known in sufficient detail, and kinetic studies are only just beginning. The current state of our understanding of the molecular mechanism of protein synthesis in human cells is reviewed in Chapter 1, with emphasis on the limitations of our knowledge. This forms the basis of our understanding of the various mechanisms of translational control that follow.

Mechanisms of translational control at the initiation phase are the main topics of this volume. Regulation by phosphorylation of initiation and elongation factors is a common mechanism, and such regulation is determined by a variety of signal transduction mechanisms that affect their activities (Chapters 2 and 3).

A second general mechanism involves microRNAs, which affect protein synthesis either directly or through the degradation of specific mRNAs (Chapter 5). A third general mechanism involves regulating the availability of mRNAs to the translational apparatus, through their sequestration in stress granules or processing bodies (Chapter 4). What is striking about these mechanisms is their coordination with other aspects of cell activity, thereby integrating translation with the overall cell metabolism.

Three examples of such coordination of protein synthesis with cell metabolism concern early development (Chapter 6), synaptic plasticity (Chapter 8) and control of cell proliferation (Chapter 7). How small aberrations in the regulation of protein synthesis can result in disease states are described and emphasized in these and the earlier chapters. For example, only a small (ca. 30%) stimulation of overall protein synthesis greatly enhances the translation of oncogenic mRNAs, leading to cell malignancy. The insights generated by these analyses should be applicable to other areas of medicine as well.

Viruses are small pathogens that rely on the cellular translation machinery for the synthesis of their proteins. Much insight into the process of protein synthesis has been obtained by studying viral translation, and the results of such efforts are reviewed in Chapter 9. The way viruses take over the cell's translation machinery and the manner in which cells protect themselves also provide new information about basic mechanisms. In the last chapter (Chapter 10), a novel therapeutic approach is proposed based in large part on the fact that the basic machinery of protein synthesis is RNA-driven. The use of RNA aptamers as therapeutic devices to affect protein synthesis rates is one of a number of new approaches to treating disease states.

I am grateful to the authors, without whom this book would not have been possible. Each has written a superb, authoritative chapter that I am confident will be useful to a wide audience of researchers and physicians. I am also indebted to the staff of Elsevier, and Delsy Retchagar in particular, for their constant help and patience. I hope that the readers of this volume find its content stimulating and enlightening.

JOHN W. B. HERSHEY

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# The Molecular Basis of Translational Control

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Our current understanding of eukaryotic protein synthesis has emerged from many years of biochemical, genetic and biophysical approaches. Significant insight into the molecular details of the mechanism has been obtained, although there are clearly many aspects of the process that remain to be resolved. Importantly, our understanding of the mechanism has identified a number of key stages in the pathway that contribute to the regulation of general and gene-specific translation. Not surprisingly, translational control is now widely accepted to play a role in aspects of cell stress, growth, development, synaptic function, aging, and disease. This chapter reviews the mechanism of eukaryotic protein synthesis and its relevance to translational control.

## I. Introduction

Ribosomes, like all enzymes, function by lowering the activation energy for the reaction they catalyze. For protein synthesis, the peptidyl transferase reaction results in the formation of a peptide bond between amino acids, enabling the assembly of a polypeptide chain. The ribosome uses its RNA component as the catalytic center for this reaction, making it a *bona fide* ribozyme.<sup>1</sup> Protein synthesis in all three kingdoms of life can be separated into four individual steps: initiation, elongation, termination, and ribosome recycling. The initiation event consists of mRNA recruitment to the small ribosomal subunit, recognition of the start codon, and subsequent joining of the large ribosomal subunit. Elongation proceeds by repeated cycles of three distinct steps: (1) recruitment of the aminoacyl-tRNA corresponding to the codon located in the aminoacyl (A) site of the small ribosomal subunit; (2) peptide bond formation between the aminoacyl-tRNA and the peptidyl-tRNA (or the initiator met-tRNA<sub>i</sub>) located in the peptidyl (P) site; and (3) translocation of mRNA and tRNAs through the ribosome so that the next codon of the mRNA enters the A-site of the small ribosomal subunit. Termination takes place when a stop codon enters the A-site, prompting a sequence of events that promote the release of the polypeptide chain from the ribosome. Finally, recycling involves the dissociation of mRNA and the deacylated tRNA from the P-site so that the ribosome can enter another round of protein synthesis. The ribosome, together with many accessory factors, coordinates these steps to ensure that each mRNA is translated accurately and efficiently into protein. All ribosomes share a common core structure, likely indicating the conservation of peptide bond formation throughout evolution. In contrast, significant variation in the mechanism of initiation is particularly evident between the kingdoms of life, with the eukaryotic ribosome employing roughly an order of magnitude more initiation factor mass than bacteria (reviewed in Refs. 2,3). This divergence appears to have enabled eukaryotic cells to evolve intricate regulatory mechanisms to control protein synthesis.

Our current view of eukaryotic protein synthesis has emerged from many years of biochemical, genetic, and biophysical approaches. Recently, structural biology has provided us with many high-resolution images of translation components both individually and as complexes, helping us to build a more detailed molecular understanding of their function. However, high-resolution structures of components associated with the eukaryotic ribosome during intermediates of the pathway are still lacking. Biochemical and genetic studies have identified many important interactions between components in the pathway together with their contributions to regulatory mechanisms. In addition, emerging studies using highly purified reconstituted systems are beginning to

provide essential thermodynamic and kinetic frameworks for important intermediates in the eukaryotic protein synthesis pathway. The number of genes appearing to be controlled at the translational level has increased rapidly in the postgenomic era. In particular, the discovery that translation is regulated by miRNAs has emphasized the importance of translational control as a fundamental regulator of gene expression. Perhaps not surprisingly, translational control is now widely accepted to play a role in aspects of developmental regulation, neuroscience, and disease. This chapter reviews the mechanism of eukaryotic protein synthesis and its relevance to translational control.

## II. Initiation Pathway Overview

Regulation of eukaryotic protein synthesis predominantly takes place during initiation, which is generally regarded as the rate-limiting step of the pathway.<sup>4,5</sup> A schematic representation of the initiation pathway is presented in Fig. 1. This pathway is depicted as a series of stages that are individually promoted by many eukaryotic initiation factors (eIFs; Table I). Initiation factors accelerate the rate of these events by lowering the required activation energy to carry out each step. The evolution of initiation factor structure and function in the three kingdoms of life has recently been reviewed in detail.<sup>2</sup> The steps of the pathway indicate a general order of binding, but it is important to note that little data actually exists to explain the true kinetic order of these events. During the dissociation of the 80S ribosome into its two subunits, the 40S subunit is bound by eIF1, eIF1A, eIF2–Met–tRNA<sub>i</sub>, and eIF3 to form what is often named the 43S complex. The cap-structure of the mRNA is recognized by the eIF4F cap-binding complex, which in turn binds to the 43S complex to form the 43S–mRNA complex. The 40S subunit then migrates along the mRNA in a 5'–3' direction until it reaches the initiation codon. This scanning mechanism requires energy in the form of ATP to enable unwinding of RNA secondary structure and explains initiation on the overwhelming majority of mRNAs.<sup>6,7</sup> An alternative mechanism involving cap-independent initiation, utilized by many virus-encoded mRNAs is described in detail in the chapter by Willis (this volume). Initiation codon recognition by the initiator tRNA promotes the release of inorganic phosphate after GTP hydrolysis by eIF2.<sup>8</sup> A second GTP hydrolysis step involving eIF5B enables 60S subunit binding to form a competent 80S ribosome ready for the elongation phase of protein synthesis.<sup>9</sup>

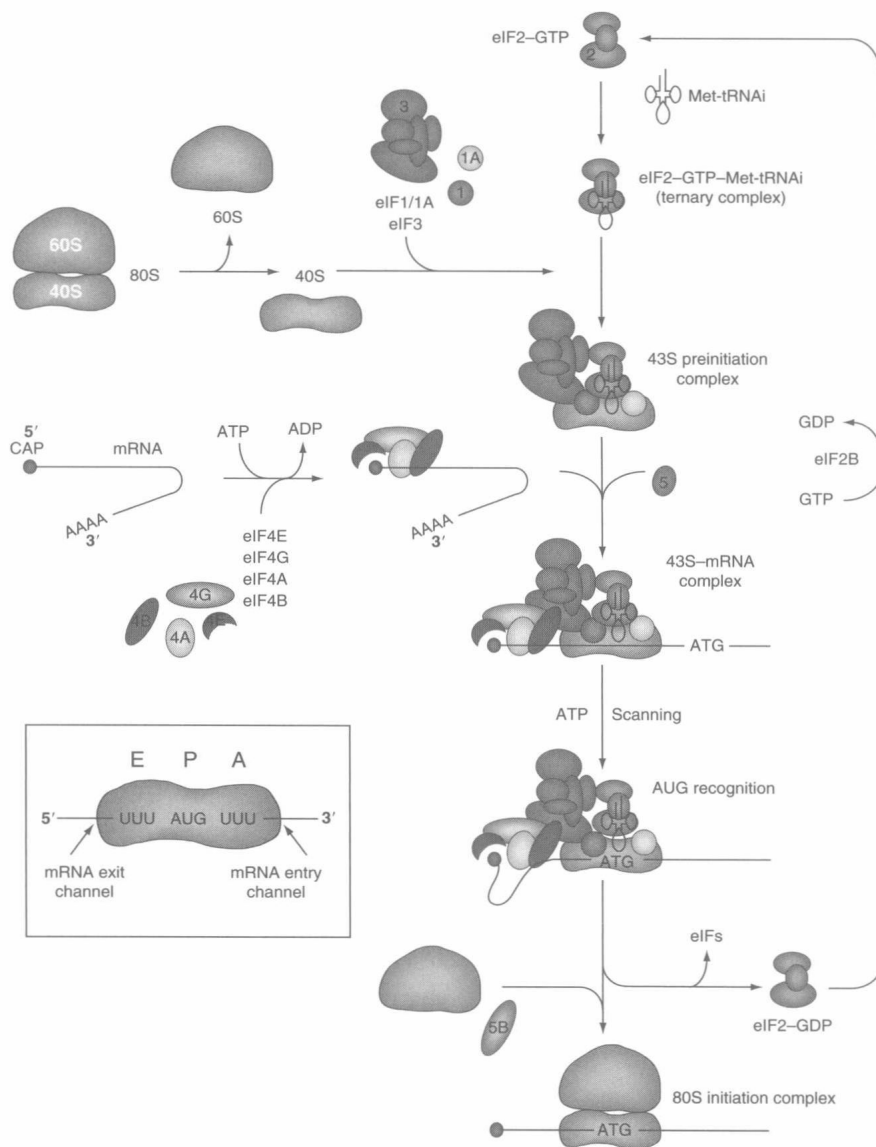


FIG. 1. Cap-dependent translation initiation. The eukaryotic translation initiation pathway is presented. Ribosomal subunits are colored gray and labeled according to their respective sedimentation values. Initiation begins on the 40S subunit, which is composed of an aminoacyl (A) site, peptidyl (P) site, and exit (E) site. The mRNA enters the 40S subunit through the mRNA entry channel, passes through the A, P, and E sites and leaves through the mRNA exit channel (see insert). Initiation factors are shown at the stage they are believed to first participate in the pathway. Following initiation, initiation factors dissociate during the association of the 60S subunit, leaving Met-tRNA<sub>i</sub> in the P-site of the 80S ribosome. The figure assumes that the mRNA cap structure remains associated with the scanning 40S subunit, although this is currently not well understood (see text). In addition, the function of PABP in the circularization of the mRNA is not shown for clarity. (See Color Insert.)

TABLE I  
EUKARYOTIC INITIATION FACTORS

Name	Mass (kDa)	Acc. No.	PDB/EMDB No.
eIF1	12.7	NM_005801	2IF1, 2OGH
eIF1A	16.4	L18960	1D7Q, 1JT8
eIF2 $\alpha$	36.1	NM_004094	1Q8K, 1Q46, 1KL9, 2A1A, 2A19, 1YZ6, 1YZ7, 2AH0
eIF2 $\beta$	38.4	NM_003908	1VB5, 1NEE, 1K8B, 1K8I, 2NXU
eIF2 $\gamma$	51.1	NM_001415	1S0U, 1KK0, 1KK1, 1KK2, 1KK3, 1KJZ, 2AH0, 2PLF, 2PMD
eIF2 $\alpha\beta\gamma$			2QMU, 2QN6, 3CW2
eIF2B $\alpha$	33.7	NM_001414	2QUC, 1VB5
eIF2B $\beta$	39.0	NM_014239	
eIF2B $\gamma$	50.2	AK024006	
eIF2B $\delta$	59.7	NM_172195	1T5O
eIF2B $\epsilon$	80.3	NM_003907	
eIF3a	166.5	NM_003750	
eIF3b	92.5	U78525	2NLW, 2KAQ
eIF3c	105.3	U46025	
eIF3d	64.1	NM_003753	
eIF3e	52.2	NM_001568	
eIF3f	37.5	NM_003754	
eIF3g	35.7	U96074	2CQ0
eIF3h	40.0	NM_003756	
eIF3i	36.5	U39067	
eIF3j	29.0	NM_003758	3BPJ, 2KAQ
eIF3k	25.0	NM_013234	1RZ4
eIF3l	66.7	AF077207	
eIF3m	42.6	NM_006360	
eIF3			EMD-1170
eIF4AI	46.1	D13748	1FUU, 1FUK, 1QDE, 1QVA, 2G9N, 2ZU6, 2VSO, 2VSX
eIF4B	69.3	BC_098437	1WI8, 2J76
eIF4H	27.4	NM_022170	2DNG
eIF4E	24.1	NM_001968	1RF8, 1L8B, 1EJH, 1EJ1, 1EJ4, 2GPQ, 1WKW, 1AP8, 1IPB, 1IPC, 2V8W, 2V8X, 2V8Y, 2IDV, 2IDR
4EHP	28.4	NM_004846	2JGC, 2JGB
4E-BP1	12.6	NM_004095	1WKW, 1EJ4
eIF4GI	175.6	NM_182917	1UG3, 1LJ2, 1LJ2, 2VSO, 2VSX, 1RF8

(Continues)

TABLE I (Continued)

Name	Mass (kDa)	Acc. No.	PDB/EMDB No.
eIF4GII	176.7	NM_003760	1HU3
eIF5	49.2	NM_001969	2G2K, 2FUL, 2IU1, 2E9H
eIF5A	16.8	NM_001970	1XTD, <i>1EIF</i> , <i>2EIF</i> , <i>1BKB</i> , <i>1IZ6</i> , 3CPF, 3ER0
eIF5B	138.9	NM_015904	<i>1G7R</i> , <i>1G7S</i> , <i>1G7T</i>
eIF6	26.6	AF022229	<i>1G6I</i> , 1G62
PABP	70.7	NM_002568	1CVJ, 1IFW, 1NMR, 1JGN, 1JH4

All sequences are human nucleotides; structures are all eukaryotic or archaeal (*italics*).

Accession numbers are taken from the National Center for Biotechnology Information database (<http://www.ncbi.nlm.nih.gov>).

Protein DataBase (PDB) numbers are taken from the Research Collaboratory for Structural Bioinformatics (RCSB) database (<http://www.rcsb.org/pdb>). Electron Microscopy Data Bank (EMDB) numbers are taken from the European Bioinformatics Institute (<http://www.ebi.ac.uk/pdbe/emdb/>).

### III. Generating a Pool of 40S Ribosomal Subunits

To enter the initiation pathway, a pool of free 40S ribosomal subunits must be established. These can be derived from two sources: (1) dissociation of 80S ribosomes that are not associated with mRNA, and (2) dissociation of post-termination 80S ribosomes that remain bound to mRNA. The mechanism by which the ribosomal subunits dissociate may differ in each case, since extra components are associated with posttermination 80S ribosomes. In general, studies have focused on measuring the shift in equilibrium between associated and dissociated subunits primarily using sucrose density gradient centrifugation. However, this approach does not distinguish between initiation factors actively promoting the dissociation of ribosomal subunits or solely preventing premature joining during the initiation pathway. Moreover, the interactions of some initiation factors do not survive sucrose gradient centrifugation, preventing them from scoring in this assay in the absence of other initiation factors. Nevertheless, monitoring changes in the level of ribosomal species upon the addition of different purified components allows for each component to be tested for its ability to alter the equilibrium.<sup>10–14</sup> A role for the large multi-subunit eIF3 complex in maintaining a pool of free 40S subunits has been identified, but its activity is enhanced significantly by the presence of eIF2–Met-tRNA<sub>i</sub>, eIF1, eIF1A, or single stranded RNA.<sup>11,12,15</sup> As explained in more detail later, eIF2–Met-tRNA<sub>i</sub>, eIF1, and eIF1A are generally believed to reside on the interface surface of the 40S subunit<sup>16–18</sup> (reviewed in Refs. 2,3,19). This likely explains their functions in preventing 60S subunit joining by sterically