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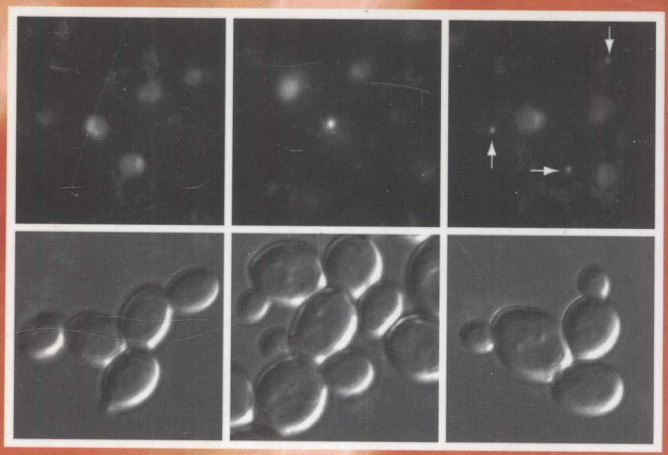
Post-Transcriptional Gene Regulation

Edited by

Jeffrey Wilusz



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Post-Transcriptional Gene Regulation

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Preface

This volume in the *Methods in Molecular Biology*™ series is organized into three sections. First, a series of bioinformatic approaches are presented in Chapters 1–3 to address the use of RNA databases and algorithms in the study of post-transcriptional regulation involving untranslated regions of transcripts. In the second section, a series of methods applicable to fundamental issues in mRNA biology are presented. These include RNA structure/function (Chapter 4), mRNP analysis (Chapters 5–8), and novel methods for mRNA labeling and isolation (Chapters 9–10). The third section of this volume presents 11 chapters that outline methodologies to study particular aspects of post-transcriptional control. This section includes methods for the study of alternative splicing and 3'-end processing (Chapters 11 and 12), mRNA localization (Chapters 13 and 14), mRNA translation (Chapters 15 and 16), mRNA stability (Chapters 17–19), and si/miRNA regulation (Chapters 20 and 21). Collectively, therefore, this volume strives to present current technical approaches to most aspects of post-transcriptional control and provide the reader with a useful and versatile laboratory bench resource.

Section I: Bioinformatics

Given the plethora of data available to the molecular biologist from available databases and high-throughput experimental techniques, bioinformatics and computational biology approaches are vital to the analysis of post-transcriptional control. This volume, therefore, contains three chapters that deal with aspects of bioinformatic analysis of mRNA processes. First, Bagga presents a chapter containing a very useful overview of the software tools and databases currently available that can be effectively applied to study the function of untranslated regions of mRNAs. As the untranslated regions (UTRs) of mRNAs undoubtedly contain numerous regulatory motifs that influence gene expression—many of which remain to be characterized—this chapter should prove to be a useful starting point for such experiments. Second, Lee and colleagues present a chapter detailing their computational approach for

assigning mRNA polyadenylation sites. Given the high occurrence of alternative polyadenylation in mRNAs, this methodology is important to obtain a clear picture of the complexity of the ribonome. The methods and approaches outlined in this chapter are also readily adaptable to other areas of molecular biology as well. Finally, Doyle and colleagues present a chapter that outlines the University of Albany Training UTR database. This collection of validated and well-defined regulatory motifs represents a very practical resource that will allow the evaluation and fine tuning of new software programs to facilitate the development of tools to discover RNA regulatory motifs.

Section II: Fundamental Aspects of the Study of RNA Biology

RNA structure plays an important role in a variety of biological processes. Regulski and Breaker present a chapter on a technique called in-line probing of RNA structure. Although their assay is presented as a powerful way to evaluate the ligand-binding characteristics of riboswitches, the technique is readily adaptable to the study of structural changes in other RNAs as well.

Messenger RNAs clearly function as dynamic ribonucleoprotein (RNP) complexes rather than naked nucleic acids. RNPs, for example, are hypothesized to play a key role in defining post-transcriptional operons. However, our understanding of the dynamics of protein–RNA interactions in the cell is still rather rudimentary. Four chapters in this volume provide exciting methodologies to address this problem from both the RNA and RNA-binding protein perspectives. Beach and Keene describe a ribo-trap method to specifically purify targeted native RNPs through immunoaffinity technology and identify associated RNA-binding proteins. Baroni and colleagues present a chapter on ribonomic profiling using RNA-binding protein Immunoprecipitation-microarray/Chip (RIP-Chip) to identify mRNAs associated with a selected RNA-binding protein. These complementary chapters provide the latest and most effective methodologies currently available to unravel the dynamics of mRNP structure on a global scale. The study of reconstituted RNA–protein interactions also provides a great deal of insight into structure–function and biochemical relationships. The chapter by Tombelli and coworkers describes current methodologies to study the interaction of RNA aptamers and RNA-binding proteins using surface plasmon resonance. Finally, artificially tethering proteins to transcripts has become a powerful way to assess the influence of proteins on post-transcriptional processes. Clement and Lykke-Andersen present a chapter containing their detailed methods for tethering proteins to mRNAs through the MS2 coat protein. Although the chapter focuses on an application of protein tethering to the study

of mRNA decay, the reader should note that the tethering technology is fully applicable to the study of a variety of post-transcriptional processes.

Two chapters in this volume deal with methodologies for labeling and isolating mRNAs in cells. First, the ability to label RNAs in vivo without using radioactivity and without significant biological consequences is a potentially very powerful addition to the toolbox to analyze gene expression. In the chapter by Zeiner et al., a methodology using cell-specific or pulsed expression of a Toxoplasma UPRT enzyme (that is usually missing in mammals) is outlined. This enzyme will specifically incorporate 4-thiouridine into RNA. The ability to biosynthetically tag RNA in a controlled fashion in vivo using this technology has numerous applications, including measurements of RNA decay rates and purification of tagged species. Second, poly(A) tail length is clearly regulated in cells. mRNAs that contain short poly(A) tails are often difficult to isolate using conventional oligo(dT) methods. To circumvent this problem, Bajak and Hagedorn present a chapter outlining a technique where mRNAs can be effectively purified on the basis of their 5' cap structure. This system should also prove very useful in the analysis of RNAs that are capped but are not naturally polyadenylated.

Section III: Techniques for Specific Aspects of RNA Biology

As the number of protein-encoding genes in an organism does not correlate with its cellular complexity, processes such as alternative splicing and polyadenylation must play a large role in defining the complexity of the proteome of a specific cell. Analyses of Expressed Sequence Tag (EST) and microarray data suggest that over two thirds of human genes contain one or more alternative exons. Identifying the full array of alternative splicing in a given gene is often difficult to infer from EST databases (as they are often incomplete), and computer-aided reconstructions of the possible complement of alternatively spliced species from a given gene do not necessarily reflect the array of spliced products that are produced in vivo. To address this problem, a PCR-based methodology to identify alternatively spliced forms from a given gene of interest is presented in the chapter by Venables. This methodology should prove invaluable to any laboratory interested in identifying the full range of alternatively spliced products generated by their gene of interest in a variety of cell types. Recent studies have demonstrated that almost 50% of genes contain more than one polyadenylation site. To effectively address questions of alternative poly(A) site usage, Hague and colleagues present a chapter outlining in vivo methodologies to assess relative usage of polyadenylation signals.

Collectively, these two chapters provide a good foundation for the reader to experimentally address questions of alternative mRNA processing.

mRNA localization in cells is another important and regulated aspect of gene expression. Two chapters in this volume describe interesting methodologies to address this issue. First, Long and Urbinati present a chapter discussing the use of tethered green fluorescent protein to a selected mRNA. In combination with microscopy, this technology allows for the visualization of the dynamics of mRNA localization in live cells. Next, Stephens and coworkers present a chapter discussing the analysis of mRNA partitioning between the cytosol and the endoplasmic reticulum. Given that recent data demonstrate that mRNAs lacking an encoded signal sequence are translated on the endoplasmic reticulum, this technology may prove very useful to obtain a full picture of the localization and translation of numerous mRNAs.

Regulated translation also makes a significant contribution to gene expression. Two chapters in this volume directly address methods for the assessment of relative translation efficiency and localization in cells. First, Peng and colleagues present a step-by-step guide for in vivo and in vitro polysome analysis using sucrose density gradients with a particular focus on the effect of poly(A) tail length on translation. These approaches should prove very useful in the identification of regulatory elements and factors. Second, Eldad and Arava present their methods for assessing the association of ribosomes with specific regions of mRNAs. This technology can be very helpful in assessing regulation at individual stages of the translation process and gives a complete picture of ribosome interactions with a transcript of interest.

Array-based analysis of changes in gene expression in response to changing environmental stimuli suggests that more than 40% are due to alterations in mRNA decay rates. The elucidation of the underlying mechanisms responsible for these mRNA-specific changes in stability will be very important, therefore, to obtain a full understanding of regulated gene expression. This volume, therefore, contains several chapters containing methodologies to assess mRNA stability. Olivas presents a step-by-step guide to assessing mRNA decay rates by northern blotting and real-time PCR. Murray and Schoenberg take the analysis of decay rates one step further and present a detailed discussion of Invader technology and its application to the study of mRNA stability. This highly sensitive signal amplification method appears to be an effective way to assess the relative turnover rates of different portions of an mRNA, allowing for the elucidation of the pathway(s) involved in the decay of selected mRNAs. Finally, the chapter by Sokoloski et al. presents an adaptation of an in vitro mRNA decay system that was first derived in HeLa cells to alternative cell types. The

apparent versatility of this system to reproduce pathways of mRNA decay, often in a regulated fashion, should prove invaluable in understanding tissue-specific and organism-specific nuances in the process of regulated mRNA stability.

Studies involving the role of non-coding RNAs in the regulation of gene expression are revolutionizing our understanding of cell biology. To address this, please note that an entire volume of methodologies for the study of microRNAs was published in this series in April 2006. In this volume, two chapters are presented by Ford and colleagues that address key areas of this field. First, Ford and Cheng present a state-of-the-art approach to studying miRNA function in mammalian cells. Second, applications of siRNAs to assess the biological consequences of knocking down the expression of targeted genes are becoming a routine tool for the molecular biologist. Therefore, in this second chapter, Cheng, Johnson, and Ford present a comprehensive step-by-step protocol for performing such siRNA knockdown experiments.

Finally, I personally thank all of the authors for their contributions to this volume. Science advances very effectively when we share not only our data, insights, and reagents, but also our technical expertise. I applaud the contributors for their willingness to disseminate the tricks of the trade that their laboratories have perfected.

Jeffrey Wilusz

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BIOINFORMATICS

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