Methods in ENZYMOLOGY

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RNA Turnover in Bacteria, Archaea and Organelles

Edited by

Lynne E. Maquat

Cecilia M. Arraiano

VOLUME FOUR HUNDRED AND FORTY-SEVEN

METHODS IN ENZYMOLOGY

RNA Turnover in Bacteria, Archaea and Organelles

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PREFACE

The cellular cleavage of RNA has remarkably diverse biologic consequences. With growing recognition that RNA turnover has a profound impact on gene expression and that rates of RNA decay can be modulated in response to environmental and developmental signals, significant progress has been made toward explaining cleavage mechanisms and how such mechanisms are regulated.

In view of research developments over the past 20 years, it is surprising that a *Methods in Enzymology* on RNA turnover is not a well-worn tome on our bookshelves. To fill the void, this volume, which addresses RNA turnover in bacteria, archaea, and organelles, and two companion volumes (Volumes 448 and 449), which focus on RNA turnover in eukaryotes, are hoped to serve both as useful references for specialists in the field and as a helpful guides for the broader community of research scientists wishing to initiate studies of RNA decay. Fundamental principles govern RNA decay and how to study it. By incorporating this information for a diverse array of organisms, we hope to stimulate the cross-fertilization of concepts and techniques.

A wide range of methods and reagents are presented, often in the context of answering important biologic questions. The authors have offered detailed rationalizations for and descriptions of their work, endeavoring to ensure that important technical points are made clearly. We wish to express our appreciation to the authors for their thoughtful contributions and willingness to share expertise.

Most chapters provide not only methodologic recipes but also short reviews that place the methods in a proper biologic framework, taking into account the interplay between RNA turnover and other cellular processes. Some degree of overlap between chapters on related topics is unavoidable. This allows each chapter to be read and understood as an independent unit while permitting the expression of diverse viewpoints on alternate methods.

Chapters are written to emphasize the characterization of RNA elements, protein factors, and cellular processes that mediate and/or regulate RNA turnover. RNA elements, including primary sequences and, frequently, higher-order structures, can influence the decay functions of a variety of protein factors such as exoribonucleases, endoribonucleases, helicases, and other types of RNA-binding proteins, all of which often exist in multiprotein complexes. Cellular processes having the potential to affect RNA turnover

include transcription, translation, and polyadenylation. Many experimental approaches for analyzing RNA decay are presented, each generally focusing on a specific organism and a particular class of RNA such as mRNA, a stable RNA, or a type of small noncoding RNA. Techniques presented include isolating total-cell or organelle RNA by different methods, determining RNA half-lives, establishing *in vitro* and *in vivo* RNA degradation assays, mapping full-length RNAs and decay intermediates, and purifying and characterizing ribonucleases and RNA binding proteins. Global approaches are also discussed, such as genomic analyses of RNA decay with DNA microarrays and proteomic analyses of the RNA degradosome with two-dimensional chromatography coupled to tandem mass spectrometry.

The knowledge collected makes clear how far our understanding of RNA degradation has come in the past few years and how much about this important regulatory process remains to be discovered. This volume should be of lasting value in providing techniques and tools for studying RNA turnover mechanisms. Applications of this knowledge to medicine and biotechnology are underway.

It is our hope that this volume will reflect the excitement currently held by RNA turnover specialists and will serve as a source of inspiration for scientists entering this rapidly moving and important field.

Lynne E. Maquat and Cecilia M. Arraiano

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