Methods in ENZYMOLOGY

Volume 448

RNA Turnover in Eukaryotes: Nucleases, Pathways and Analysis of mRNA Decay

Edited by

Lynne E. Maquat

Megerditch Kiledjian

VOLUME FOUR HUNDRED AND FORTY-EIGHT

METHODS IN ENZYMOLOGY

RNA Turnover in Eukaryotes: Nucleases, Pathways and Anaylsis of mRNA Decay

EDITED BY

LYNNE E. MAQUAT
Department of Biochemistry and Biophysics
University of Rochester
School of Medicine and Dentistry
Rochester, NY, USA

MEGERDITCH KILEDJIAN
Department of Cell Biology and Neurosciences
Nelson Biology Labs
Piscataway, NJ, USA

ELSEVIER

AMSTERDAM • BOSTON • HEIDELBERG • LONDON
NEW YORK • OXFORD • PARIS • SAN DIEGO
SAN FRANCISCO • SINGAPORE • SYDNEY • TOKYO
Academic Press is an imprint of Elsevier

Academic Press is an imprint of Elsevier
525 B Street, Suite 1900, San Diego, California 92101-4495, USA
84 Theobald's Road, London WC1X 8RR, UK

For information on all Elsevier Academic Press publications
visit our Web site at elsevierdirect.com

ISBN-13: 978-0-12-374378-7

PRINTED IN THE UNITED STATES OF AMERICA
08 09 10 11 9 8 7 6 5 4 3 2 1

VOLUME FOUR HUNDRED AND FORTY-EIGHT

METHODS IN ENZYMOLOGY

RNA Turnover in Eukaryotes: Nucleases, Pathways and Anaylsis of mRNA Decay

METHODS IN ENZYMOLOGY

Editors-in-Chief

JOHN N. ABELSON AND MELVIN I. SIMON

Division of Biology
California Institute of Technology
Pasadena, California, USA

Founding Editors

SIDNEY P. COLOWICK AND NATHAN O. KAPLAN

Contributors

Bünyamin Akgül
Department of Molecular Biology and Genetics, Izmir Institute of Technology, Izmir, Turkey, and Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, Pennsylvania, USA

Paul Anderson
Division of Rheumatology, Immunology and Allergy, Brigham and Women's Hospital, Boston, Massachusetts, USA

Stuart Archer
Zentrum für Molekulare Biologie der Universität Heidelberg (ZMBH), Heidelberg, Germany

Lionel Bénard
CNRS UPR 9073 (affiliated with Université de Paris 7—Denis Diderot), Institut de Biologie Physico-Chimique, Paris, France

Dmitry Belostotsky
Division of Molecular Biology and Biochemistry, School of Biological Sciences, University of Missouri, Kansas City, Missouri, USA

Chyi-Ying A. Chen
Department of Biochemistry and Molecular Biology, The University of Texas Medical School at Houston, Houston, Texas, USA

Amanda Clark
University of Texas Health Science Center-Houston, Department of Microbiology and Molecular Genetics, Houston, Texas, USA

Christine Clayton
Zentrum für Molekulare Biologie der Universität Heidelberg (ZMBH), Heidelberg, Germany

Michael D. Cleary
University of California, Merced School of Natural Sciences, Merced, California, USA

Jeff Coller
Center for RNA Molecular Biology, Case Western Reserve University, School of Medicine, Cleveland Ohio, USA

Ciarán Condon
CNRS UPR 9073 (affiliated with Université de Paris 7—Denis Diderot), Institut de Biologie Physico-Chimique, Paris, France

Ramona L. Cooperstock
Program in Developmental and Stem Cell Biology, Research Institute, Hospital for Sick Children, Toronto, Ontario, Canada, and Department of Molecular Genetics, University of Toronto, 1 King's College Circle, Toronto, Ontario, Canada

Kevin Czaplinski
Department of Biochemistry and Cell Biology, Stony Brook University, Center for Molecular Medicine 542, Stony Brook, New York, USA

Nader Ezzeddine
Department of Biochemistry and Molecular Biology, The University of Texas Medical School at Houston, Houston, Texas, USA

Niels H. Gehring
Department of Pediatric Oncology, Hematology and Immunology, University of Heidelberg, Heidelberg, Germany, and Molecular Medicine Partnership Unit, EMBL/University of Heidelberg, Heidelberg, Germany

Aaron L. Goldman
Program in Developmental and Stem Cell Biology, Research Institute, Hospital for Sick Children, Toronto, Ontario, Canada, and Department of Molecular Genetics, University of Toronto, 1 King's College Circle, Toronto, Ontario, Canada

Aaron C. Goldstrohm
Department of Biochemistry, University of Wisconsin—Madison, Madison, Wisconsin, USA

Thierry Grange
Institut Jacques Monod du CNRS, Université Paris 7, Paris, France

Jaclyn C. Greimann
Structural Biology Program, Sloan-Kettering Institute, New York, NY, USA

John D. Gross
Department of Pharmaceutical Chemistry, University of California, San Francisco, California, USA

David Grünwald
Albert Einstein College of Medicine, Anatomy and Structural Biology and Gruss-Lipper Singer Biophotonics Center, Bronx, New York, USA

Matthias W. Hentze
European Molecular Biology Laboratory, Heidelberg, Germany, and Molecular Medicine Partnership Unit, EMBL/University of Heidelberg, Heidelberg, Germany

Tyler Hickman
Division of Rheumatology, Immunology and Allergy, Brigham and Women's Hospital, Boston, Massachusetts, USA

Brad A. Hook
Promega Corporation, Madison, Wisconsin, USA

Mandy Jeske
Institute of Biochemistry and Biotechnology, Martin-Luther-University Halle-Wittenberg, Halle, Germany

Xinfu Jiao
Rutgers University, Department of Cell Biology and Neuroscience, Piscataway, New Jersey, USA

Brittnee N. Jones
Program in Chemistry and Chemical Biology, University of California, San Francisco, California, USA

Christian Kambach
Biomolecular Research, Paul Scherrer Institut, Villigen PSI, Switzerland

Nancy Kedersha
Division of Rheumatology, Immunology and Allergy, Brigham and Women's Hospital, Boston, Massachusetts, USA

Megerditch Kiledjian
Rutgers University, Department of Cell Biology and Neuroscience, Piscataway, New Jersey, USA

Jong Heon Kim
Research Institute, National Cancer Center, Goyang, Gyeonggi, South Korea

Andreas E. Kulozik
Department of Pediatric Oncology, Hematology and Immunology, University of Heidelberg, Heidelberg, Germany, and Molecular Medicine Partnership Unit, EMBL/University of Heidelberg, Heidelberg, Germany

Christopher D. Lima
Structural Biology Program, Sloan-Kettering Institute, New York, NY, USA

Howard D. Lipshitz
Program in Developmental and Stem Cell Biology, Research Institute, Hospital for Sick Children, Toronto, Ontario, Canada, and Department of Molecular Genetics, University of Toronto, 1 King's College Circle, Toronto, Ontario, Canada

Shin-Wu Liu
Rutgers University, Department of Cell Biology and Neuroscience, Piscataway, New Jersey, USA

Nathalie Mathy
CNRS UPR 9073 (affiliated with Université de Paris 7—Denis Diderot), Institut de Biologie Physico-Chimique, Paris, France

Stacie Meaux
University of Texas Health Science Center-Houston, Department of Microbiology and Molecular Genetics, Houston, Texas, USA

Elizabeth L. Murray
Department of Molecular and Cellular Biochemistry and The RNA Group, The Ohio State University, Columbus, Ohio, USA

Tracy Nissan
The University of Arizona, Department of Molecular and Cellular Biology and Howard Hughes Medical Institute, Tucson, Arizona, USA

Yuko Oku
Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

Yuichi Otsuka
Department of Molecular and Cellular Biochemistry and The RNA Group, The Ohio State University, Columbus, Ohio, USA

Roy Parker
The University of Arizona, Department of Molecular and Cellular Biology and Howard Hughes Medical Institute, Tucson, Arizona, USA

Dario O. Passos
The University of Arizona, Department of Molecular and Cellular Biology and Howard Hughes Medical Institute, Tucson, Arizona, USA

Olivier Pellegrini
CNRS UPR 9073 (affiliated with Université de Paris 7—Denis Diderot), Institut de Biologie Physico-Chimique, Paris, France

Ger J. M. Pruijn
Department of Biomolecular Chemistry, Nijmegen Center for Molecular Life Sciences, Institute for Molecules and Materials, Radboud University Nijmegen, Nijmegen, The Netherlands

Duc-Uy Quang-Dang
Department of Pharmaceutical Chemistry, University of California, San Francisco, California, USA

Rafael Queiroz
Zentrum für Molekulare Biologie der Universität Heidelberg (ZMBH), Heidelberg, Germany

Joel D. Richter
Program in Molecular Medicine, University of Massachusetts Medical School, Worcester, Massachusetts, USA and Research Institute, National Cancer Center, Goyang, Gyeonggi, South Korea

Daneen Schaeffer
University of Texas Health Science Center-Houston, Department of Microbiology and Molecular Genetics, Houston, Texas, USA

Geurt Schilders
Department of Biomolecular Chemistry, Nijmegen Center for Molecular Life Sciences, Institute for Molecules and Materials, Radboud University Nijmegen, Nijmegen, The Netherlands

Daniel R. Schoenberg
Department of Molecular and Cellular Biochemistry and The RNA Group, The Ohio State University, Columbus, Ohio, USA

Jennifer L. Semotok
Program in Developmental and Stem Cell Biology, Research Institute, Hospital for Sick Children, Toronto, Ontario, Canada, and Department of Molecular Genetics, University of Toronto, 1 King's College Circle, Toronto, Ontario, Canada

Robert H. Singer
Department of Biochemistry and Cell Biology, Stony Brook University, Center for Molecular Medicine 542, Stony Brook, New York, USA, and Albert Einstein College of Medicine, Anatomy and Structural Biology and Gruss-Lipper Biophotonics Center, Bronx, New York, USA

Kevin J. Sokoloski
Department of Microbiology, Immunology and Pathology, Colorado State University, Ft. Collins, Colorado, USA

Mhairi Stewart
Zentrum für Molekulare Biologie der Universität Heidelberg (ZMBH), Heidelberg, Germany

Ann-Bin Shyu
Department of Biochemistry and Molecular Biology, The University of Texas Medical School at Houston, Houston, Texas, USA

Sundaresan Tharun
Department of Biochemistry, Uniformed Services University of the Health Sciences (USUHS), Bethesda, Maryland, USA

Sarah Tisdale
Division of Rheumatology, Immunology and Allergy, Brigham and Women's Hospital, Boston, Massachusetts, USA

Chen-Pei D. Tu
Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, Pennsylvania, USA

Sarah Welch
Rutgers University, Department of Cell Biology and Neuroscience, Piscataway, New Jersey, USA

Ambro van Hoof
University of Texas Health Science Center-Houston, Department of Microbiology and Molecular Genetics, Houston, Texas, USA

Elmar Wahle
Institute of Biochemistry and Biotechnology, Martin-Luther-University Halle-Wittenberg, Halle, Germany

J. Timothy Westwood
Department of Cell and Systems Biology and Canadian *Drosophila* Microarray Centre, University of Toronto, Mississauga, Ontario, Canada

Carol J. Wilusz
Department of Microbiology, Immunology and Pathology, Colorado State University, Ft. Collins, Colorado, USA

Marvin Wickens
Department of Biochemistry, University of Wisconsin—Madison, Madison, Wisconsin, USA

Bozidarka L. Zaric
Institut Curie, UMR 7147, Equipe: Recombinaison et Instabilité Génétique, Paris, France

PREFACE

The expression of protein-encoding genes in eukaryotes is regulated at multiple levels, including the initial synthesis of pre-mRNA in the nucleus, the various nuclear processing events that convert pre-mRNA to mRNA, mRNA transport to the cytoplasm, and mRNA translation and, ultimately, degradation in the cytoplasm. All steps are exquisitely controlled to orchestrate the production of protein at the appropriate time and at a suitable level. It follows that a critical step in this orchestration is the proper maintenance of mRNA stability, which is often regulated to influence the amount of encoded protein. Thus, determinants of the rate at which an mRNA is degraded are important regulators of gene expression. As a consequence, the availability of methods and tools to analyze how and when individual mRNAs are either stabilized or turned over is essential to understand this exciting area of biology.

Determinants that dictate the stability of an mRNA include general elements, such as the 5′ methylguanosine cap and the 3′ poly(A) tail, and transcript-specific elements that are recognized by protein factors or noncoding RNAs. Regulation of mRNA stability is largely imparted by the ability of nucleases to access and remove the terminal elements that protect the mRNA. The bulk of mRNA decay appears to proceed through defined pathways that are initiated by deadenylation of the poly(A) tail. Deadenylated mRNA is subsequently subjected to one of two exonucleolytic decay pathways. One pathway continues to degrade the RNA from the 3′ end to generate a capped oligonucleotide that is subsequently hydrolyzed. Alternately, deadenylation can trigger decapping of the mRNA to expose the 5′ end to 5′-to-3′ exonucleolytic decay. Interestingly, recent evidence indicates that these pathways are not necessarily mutually exclusive and can also occur simultaneously. Moreover, mRNAs can be shunted to exonucleolytic decay pathways after initial cleavage by an endonuclease. The rate at which different *trans*-acting factors and *cis*-acting elements coordinate the demise of an mRNA dictates the chemical half-life of the mRNA, which in eukaryotes can range from several minutes to several days. Thus, methods to analyze mRNA levels, mRNA half-lives, mRNA decay pathways, mRNA decay intermediates, and the associated nucleases and their activities are critical for a comprehensive understanding of mRNA turnover.

Significant advances have been made in recent years in ways to analyze both genome-wide mRNA decay and the decay of specific mRNAs in mammalian systems and in numerous model organisms. This volume is

focused on six broad areas of mRNA turnover. The first section relates to methods to study mRNA decapping and consists of four chapters that range from the analysis of decapping activity and the kinetics of decapping to the reconstitution of complexes that promote decapping. The next section includes four chapters that present *in vitro* methods to analyze deadenylation. Methods to reconstitute and test the activities of nucleases that function in mRNA decay are presented in the third section, which addresses how to characterize and detect exoribonucleases and endoribonucleases.

A fundamentally important aspect of mRNA turnover studies involves methods that measure mRNA chemical half-life, and the fourth section consists of six chapters presenting state-of-the-art methods for such measurements in cells of a variety of organisms. Approaches aimed at detecting decay intermediates are detailed in the fifth set of chapters, which describe transcript-specific and genome-wide analyses of mRNA decay products and substrates of nucleases. Last, an exciting new development has been the realization that mRNA decay factors can concentrate in discrete cellular foci. These foci seem to consist of mRNAs undergoing decay and/or mRNAs that are translationally silenced. The final two chapters present approaches to detect and follow the formation of two such foci, processing bodies and stress granules.

All chapters begin with a brief overview of a specific area of mRNA turnover and progress to a detailed description of relevant methods. This volume is part of a three-volume series. The first companion volume (Vol. 448) focuses on RNA turnover in bacteria, archaea, and organelles, and the last volume (Vol. 450) covers the analysis of specialized and quality control RNA decay in eukaryotes. Cumulatively, this series presents all the latest approaches and methods to assess how RNA decay contributes to gene regulation in many commonly used organisms. The series aims to provide a valuable arsenal of tools with which one can study the exciting and rapidly expanding arena of mRNA turnover as it pertains not only to basic research but also to therapeutics.

METHODS IN ENZYMOLOGY

VOLUME I. Preparation and Assay of Enzymes
Edited by SIDNEY P. COLOWICK AND NATHAN O. KAPLAN

VOLUME II. Preparation and Assay of Enzymes
Edited by SIDNEY P. COLOWICK AND NATHAN O. KAPLAN

VOLUME III. Preparation and Assay of Substrates
Edited by SIDNEY P. COLOWICK AND NATHAN O. KAPLAN

VOLUME IV. Special Techniques for the Enzymologist
Edited by SIDNEY P. COLOWICK AND NATHAN O. KAPLAN

VOLUME V. Preparation and Assay of Enzymes
Edited by SIDNEY P. COLOWICK AND NATHAN O. KAPLAN

VOLUME VI. Preparation and Assay of Enzymes *(Continued)*
Preparation and Assay of Substrates
Special Techniques
Edited by SIDNEY P. COLOWICK AND NATHAN O. KAPLAN

VOLUME VII. Cumulative Subject Index
Edited by SIDNEY P. COLOWICK AND NATHAN O. KAPLAN

VOLUME VIII. Complex Carbohydrates
Edited by ELIZABETH F. NEUFELD AND VICTOR GINSBURG

VOLUME IX. Carbohydrate Metabolism
Edited by WILLIS A. WOOD

VOLUME X. Oxidation and Phosphorylation
Edited by RONALD W. ESTABROOK AND MAYNARD E. PULLMAN

VOLUME XI. Enzyme Structure
Edited by C. H. W. HIRS

VOLUME XII. Nucleic Acids (Parts A and B)
Edited by LAWRENCE GROSSMAN AND KIVIE MOLDAVE

VOLUME XIII. Citric Acid Cycle
Edited by J. M. LOWENSTEIN

VOLUME XIV. Lipids
Edited by J. M. LOWENSTEIN

VOLUME XV. Steroids and Terpenoids
Edited by RAYMOND B. CLAYTON

VOLUME XVI. Fast Reactions
Edited by KENNETH KUSTIN

VOLUME XVII. Metabolism of Amino Acids and Amines (Parts A and B)
Edited by HERBERT TABOR AND CELIA WHITE TABOR

VOLUME XVIII. Vitamins and Coenzymes (Parts A, B, and C)
Edited by DONALD B. MCCORMICK AND LEMUEL D. WRIGHT

VOLUME XIX. Proteolytic Enzymes
Edited by GERTRUDE E. PERLMANN AND LASZLO LORAND

VOLUME XX. Nucleic Acids and Protein Synthesis (Part C)
Edited by KIVIE MOLDAVE AND LAWRENCE GROSSMAN

VOLUME XXI. Nucleic Acids (Part D)
Edited by LAWRENCE GROSSMAN AND KIVIE MOLDAVE

VOLUME XXII. Enzyme Purification and Related Techniques
Edited by WILLIAM B. JAKOBY

VOLUME XXIII. Photosynthesis (Part A)
Edited by ANTHONY SAN PIETRO

VOLUME XXIV. Photosynthesis and Nitrogen Fixation (Part B)
Edited by ANTHONY SAN PIETRO

VOLUME XXV. Enzyme Structure (Part B)
Edited by C. H. W. HIRS AND SERGE N. TIMASHEFF

VOLUME XXVI. Enzyme Structure (Part C)
Edited by C. H. W. HIRS AND SERGE N. TIMASHEFF

VOLUME XXVII. Enzyme Structure (Part D)
Edited by C. H. W. HIRS AND SERGE N. TIMASHEFF

VOLUME XXVIII. Complex Carbohydrates (Part B)
Edited by VICTOR GINSBURG

VOLUME XXIX. Nucleic Acids and Protein Synthesis (Part E)
Edited by LAWRENCE GROSSMAN AND KIVIE MOLDAVE

VOLUME XXX. Nucleic Acids and Protein Synthesis (Part F)
Edited by KIVIE MOLDAVE AND LAWRENCE GROSSMAN

VOLUME XXXI. Biomembranes (Part A)
Edited by SIDNEY FLEISCHER AND LESTER PACKER

VOLUME XXXII. Biomembranes (Part B)
Edited by SIDNEY FLEISCHER AND LESTER PACKER

VOLUME XXXIII. Cumulative Subject Index Volumes I-XXX
Edited by MARTHA G. DENNIS AND EDWARD A. DENNIS

VOLUME XXXIV. Affinity Techniques (Enzyme Purification: Part B)
Edited by WILLIAM B. JAKOBY AND MEIR WILCHEK

VOLUME XXXV. Lipids (Part B)
Edited by JOHN M. LOWENSTEIN

VOLUME XXXVI. Hormone Action (Part A: Steroid Hormones)
Edited by BERT W. O'MALLEY AND JOEL G. HARDMAN

VOLUME XXXVII. Hormone Action (Part B: Peptide Hormones)
Edited by BERT W. O'MALLEY AND JOEL G. HARDMAN

VOLUME XXXVIII. Hormone Action (Part C: Cyclic Nucleotides)
Edited by JOEL G. HARDMAN AND BERT W. O'MALLEY

VOLUME XXXIX. Hormone Action (Part D: Isolated Cells, Tissues, and Organ Systems)
Edited by JOEL G. HARDMAN AND BERT W. O'MALLEY

VOLUME XL. Hormone Action (Part E: Nuclear Structure and Function)
Edited by BERT W. O'MALLEY AND JOEL G. HARDMAN

VOLUME XLI. Carbohydrate Metabolism (Part B)
Edited by W. A. WOOD

VOLUME XLII. Carbohydrate Metabolism (Part C)
Edited by W. A. WOOD

VOLUME XLIII. Antibiotics
Edited by JOHN H. HASH

VOLUME XLIV. Immobilized Enzymes
Edited by KLAUS MOSBACH

VOLUME XLV. Proteolytic Enzymes (Part B)
Edited by LASZLO LORAND

VOLUME XLVI. Affinity Labeling
Edited by WILLIAM B. JAKOBY AND MEIR WILCHEK

VOLUME XLVII. Enzyme Structure (Part E)
Edited by C. H. W. HIRS AND SERGE N. TIMASHEFF

VOLUME XLVIII. Enzyme Structure (Part F)
Edited by C. H. W. HIRS AND SERGE N. TIMASHEFF

VOLUME XLIX. Enzyme Structure (Part G)
Edited by C. H. W. HIRS AND SERGE N. TIMASHEFF

VOLUME L. Complex Carbohydrates (Part C)
Edited by VICTOR GINSBURG

VOLUME LI. Purine and Pyrimidine Nucleotide Metabolism
Edited by PATRICIA A. HOFFEE AND MARY ELLEN JONES

VOLUME LII. Biomembranes (Part C: Biological Oxidations)
Edited by SIDNEY FLEISCHER AND LESTER PACKER

VOLUME LIII. Biomembranes (Part D: Biological Oxidations)
Edited by SIDNEY FLEISCHER AND LESTER PACKER

VOLUME LIV. Biomembranes (Part E: Biological Oxidations)
Edited by SIDNEY FLEISCHER AND LESTER PACKER

VOLUME LV. Biomembranes (Part F: Bioenergetics)
Edited by SIDNEY FLEISCHER AND LESTER PACKER

VOLUME LVI. Biomembranes (Part G: Bioenergetics)
Edited by SIDNEY FLEISCHER AND LESTER PACKER

VOLUME LVII. Bioluminescence and Chemiluminescence
Edited by MARLENE A. DELUCA

VOLUME LVIII. Cell Culture
Edited by WILLIAM B. JAKOBY AND IRA PASTAN

VOLUME LIX. Nucleic Acids and Protein Synthesis (Part G)
Edited by KIVIE MOLDAVE AND LAWRENCE GROSSMAN

VOLUME LX. Nucleic Acids and Protein Synthesis (Part H)
Edited by KIVIE MOLDAVE AND LAWRENCE GROSSMAN

VOLUME 61. Enzyme Structure (Part H)
Edited by C. H. W. HIRS AND SERGE N. TIMASHEFF

VOLUME 62. Vitamins and Coenzymes (Part D)
Edited by DONALD B. MCCORMICK AND LEMUEL D. WRIGHT

VOLUME 63. Enzyme Kinetics and Mechanism (Part A: Initial Rate and Inhibitor Methods)
Edited by DANIEL L. PURICH

VOLUME 64. Enzyme Kinetics and Mechanism
(Part B: Isotopic Probes and Complex Enzyme Systems)
Edited by DANIEL L. PURICH

VOLUME 65. Nucleic Acids (Part I)
Edited by LAWRENCE GROSSMAN AND KIVIE MOLDAVE

VOLUME 66. Vitamins and Coenzymes (Part E)
Edited by DONALD B. MCCORMICK AND LEMUEL D. WRIGHT

VOLUME 67. Vitamins and Coenzymes (Part F)
Edited by DONALD B. MCCORMICK AND LEMUEL D. WRIGHT

VOLUME 68. Recombinant DNA
Edited by RAY WU

VOLUME 69. Photosynthesis and Nitrogen Fixation (Part C)
Edited by ANTHONY SAN PIETRO

VOLUME 70. Immunochemical Techniques (Part A)
Edited by HELEN VAN VUNAKIS AND JOHN J. LANGONE