

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 non-coding 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. Dead-enylated 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 *dis*-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.