
Preface

Messenger RNA polyadenylation is an important aspect of gene expression in eukaryotes. The process itself is physically and temporally coupled with several other steps in gene expression, ranging from the initiation of transcription to the export of the mature mRNA to the cytoplasm. In the cytoplasm, the poly(A) tail constitutes an essential cis-element that enables the translation of the mRNA, in cooperation with the 5'-cap.

mRNA polyadenylation is an RNA processing event whereby a precursor mRNA is cleaved and a poly(A) tract added to the 3' end of the cleaved RNA. The position along the precursor mRNA where processing occurs contributes to the overall nature of the mature mRNA. Typically, the poly(A) site is situated several tens or hundreds of nucleotides 3' of the translation termination codon that is specific within the protein-coding portion of the mRNA. However, for a majority of genes in eukaryotes, the primary transcript may be processed and polyadenylated at more than one position; the result of this multiplicity is a set of mRNA isoforms, each of which has a different 3'-UTR as well as other mRNA features. This multiplicity of mRNA isoforms gives rise to the possibility that gene expression may be controlled in part by alterations in the 3' end profiles of expressed genes. This possibility has been confirmed in many systems; perhaps most prominent along these lines is the realization that alternative poly(A) site choice is a key determinant of the levels of expression of growth-promoting oncogenes in cancer cells.

Recent years have seen a renewed interest in the process of mRNA polyadenylation in plants. There have been a growing number of reports that link polyadenylation with several important molecular and physiological processes. Moreover, with the advent of genome-scale studies extending back more than 10 years and spanning the “ages” of large-scale EST studies, to microarray-based experiments, and more recently to high-throughput sequencing, it has become apparent that alternative poly(A) site choice may be a common feature of gene expression in plants, and that alterations in 3' end profiles may contribute to the regulation of gene expression in several ways. For these reasons, the field has grown in terms of scope and methodology, and now encompasses a broad spectrum of experimental approaches.

In this volume, the breadth of these studies is encapsulated so that interested readers may appreciate and adapt the tools and outcomes, and also gain a better feel for the current state of the field. The chapters in this volume are grouped into three parts. The first part represents a relatively recent development in the field, namely the use of bioinformatics and computational tools to study polyadenylation. These tools include methods for analyzing and predicting plant polyadenylation signals (Chapters 1 and 2) and for retrieving poly(A) sites from public sequence databases (Chapter 3). Chapter 4 describes computational approaches for analyzing large-scale sequencing data specifically designed for poly(A)-tag sequencing (PAT-seq), while Chapter 5 describes a computational assay that uses PAT-seq to measure poly(A) site choice on a genome-wide scale. Chapter 6 presents a comprehensive approach for studying alternative mRNA processing, including polyadenylation, using data derived from tiling microarrays.

The second part brings into focus the numerous molecular, biochemical, and cellular methods that have been used to characterize polyadenylation in plants. Described in this part are techniques used to characterize polyadenylation complexes and their activities in vitro (Chapters 7 and 8) and basic operation to analyze related proteins (Chapter 9), to study novel aspects of polyadenylation factor structure and function using chemical modification and mass spectroscopy (Chapter 10), for transient expression to study polyadenylation proteins and polyadenylation signals (Chapter 11), and for the determination of mRNA 3' ends using a modification of the so-called 3'-RACE assay (Chapter 12). The third part consists of a compendium of methods used to characterize polyadenylation sites on a genome-wide scale. A high-throughput approach to identifying protein interacting partners, and further to identifying small sequence motifs that may be associated with such interactions, is described in Chapter 13. Chapters 14–16 describe three methods for the generation of sequence tags that query the mRNA-poly(A) junction on a genome-wide scale; these sequence tags are suitable for the so-called next generation sequencing technology. Along with poly(A) tag analyses, we also need to perform RNA-seq to get sequence information beyond poly(A) sites. Thus, a simple, inexpensive protocol for constructing RNA-seq libraries is also included as Chapter 17. Chapter 18 was used to study the relationship between transcription and polyadenylation through RNA polymerase II activities.

It is our hope that this volume will bring together, in one place, the range of methods that are used to study polyadenylation in plants (or other organisms), and in so doing will provide readers convenient ways to employ these tools to advance the understanding of the role of mRNA polyadenylation in plant gene expression.

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