PREFACE

The major function of the human genome is to encode a vast diversity of RNAs that control essentially every facet of cellular function. The RNAs include well-known RNAs, such as messenger RNAs, transfer RNAs, and ribosomal RNAs, as well as a dizzying set of other RNAs such as long noncoding RNAs, Y-RNAs, microRNAs, small nucleolar RNAs, vault RNAs, piwi-interacting RNAs, and numerous others. A major focus has been mRNAs and uncovering the relationship between their localization and function in normal and disease cells. However, for the large and mysterious class of long noncoding RNAs, studies that reveal their localization and trafficking can provide a first clue about their potential function in cells. For these reasons, methods in localizing RNAs in cells have been important for biomedical researchers. In recent years, these methods have undergone a renaissance with multiple innovations providing researchers with the ability to image RNAs in living cells in real time.

In addition to RNA trafficking, RNA undergoes diverse types of processing events in cells, such as splicing, degradation, and modification. These processes are typically measured by harvesting cells and performing assays such as Northern blotting. However, these approaches fail to reveal the spatial and temporal dynamics of these processes, which may be subjected to regulation. To overcome this, new methods are being developed to image these events in living cells. Methods to image RNA processing are still in their infancy, but are rapidly developing.

This volume is roughly divided into two parts. The first part highlights diverse methods for detecting and imaging different types of endogenous RNA in cells. The second part comprises techniques to study mRNA molecular biology and for using RNA itself as a reporter.

The volume starts with MERFISH (Chapter 1), an impressive technique described by Moffitt and Zhuang. This protocol can be used for visualization of hundreds to thousands of different RNAs in individual cells with single-molecule resolution. In the next chapter, Moon and Park describe an approach that utilizes the MS2-GFP fusion to image single β-actin mRNA dynamics in live cells of mouse brain slices. In Chapter 3, Yoshimura and Ozawa present a protocol that describes fluorescent-protein-based imaging of RNAs utilizing PUM-HD, a domain that can be modified to recognize different 8-base RNA sequences. Jackson et al. (Chapter 4) describe a way to

utilize hairpin-DNA-functionalized gold nanoparticles as an efficient way to label and image mRNAs in live cells. Another application of a fluorescent protein fused to MS2 is described by Peña and Heinlein (Chapter 5). In this chapter, viral RNA is visualized in live plant cells. Chao and colleagues present a powerful technique to track untranslated RNAs and then to report on the first round of translation (Chapter 6). A separate chapter is presented by Coulon and Larson on the topic of fluctuation analysis, which is a way to analyze and interpret multicolor transcriptional time traces (Chapter 7). Ray et al. describe a protocol to use IMAGEtags to image bulk RNA in cells and to study transcription changes in real time (Chapter 8). Finally, Matera and colleagues present a protocol for expression of highly stable circular RNAs and their imaging using the Broccoli fluorescent RNA aptamer.

The second part of the volume describes the methods for studying RNA molecular biology. The first chapter in this series (Chapter 10) is compiled by Grayhack and colleagues and describes RNA-ID, an approach to identify and analyze sequences that regulate gene expression. The group of Lukyanov presents two protocols that use flow cytometry and fluorescence microscopy to report on alternative splicing or nonsense-mediated RNA decay (Chapters 11 and 13, respectively). As another approach to study splicing, Zheng describes IRAS, a fluorescence-based protocol to identify novel alternative splicing regulators (Chapter 12). The final chapter in this volume describes an innovative way to use RNA fluorescence to report on intracellular events. Litke et al. describe a protocol for development of fluorogenic riboswitches to serve as RNA-based biosensors (Chapter 14).

While all methods for imaging RNA and RNA processing are not presented here, we hope that these chapters will be of use to the scientific community and will help to stimulate further development of imaging tools that can provide new insights into the intricacies of RNA biology.

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