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## Preface

The transfer of hereditary information from genes to proteins is one of the essential processes in all living organisms on our planet. Some genes are expressed without modulation throughout the life of a cell, while many others require various degrees of control to precisely balance cellular metabolism with environmental conditions. For many years, researchers attributed this regulatory function to protein molecules, which can direct gene expression at multiple levels, in response to various input signals, and with different degrees of selectivity. Even when the control of gene expression was achieved via direct interactions between proteins and mRNAs, the active role was routinely assigned to proteins, while RNAs were considered merely as recipient molecules. The discovery of RNA interference and multiple bacterial regulatory RNAs caused a shift from the perception of proteins as the predominant regulators of gene expression to the acknowledgement of the importance of RNAs in many regulatory circuits. Such a viewpoint received strong support several years ago after the discovery of riboswitches and related RNA sensors – mRNA regions capable of alternating their conformations in response to the presence of cellular metabolites and other physical or chemical cues. These classes of RNA pass on cellular and environmental information directly to transcription or translation machinery without the assistance of proteins.

The riboswitches are commonly defined as evolutionarily conserved mRNA regions capable of specific binding to metabolite molecules, and, as a result, adopting a particular RNA conformation that modulates gene expression. This definition restricts riboswitches to mRNAs responding to small organic molecules, and this volume is primarily focused on the techniques used for the identification and characterization of such RNAs. However, the meaning of the term riboswitch can be broadened to incorporate classes of RNA that undergo conformational transitions in response to other stimuli in order to control the expression of genes. Therefore, several contributions to the book are devoted to the mRNAs which adopt complexly folded conformations and directly sense environmental signals or recognize molecules other than metabolites. Among the many RNA-based regulatory systems, there is a special place for mRNAs which sense the regulatory signal delivered by a specific RNA. These RNAs participate in diverse regulatory mechanisms, overviewed in the book, and often require *in vivo* techniques, described in two chapters, for the elucidation of their function.

This volume includes comprehensive and up-to-date coverage of various methods used to study riboswitches and other RNAs involved in gene expression control. Although some protocols utilize the intrinsic properties of metabolite molecules and can be applied exclusively to elucidate the function of metabolite-binding RNAs, a majority of the methods originate from rigorously tested procedures previously used for the characterization of various RNA molecules and RNA–ligand complexes.

Several chapters in this book describe classical and emerging biochemical techniques, such as chemical and enzymatic RNA synthesis, RNA structure probing, and footprinting. The latter two techniques, powerful and fast methods of gaining preliminary structural information, have become routine procedures in a number of laboratories. Nevertheless,

many other researchers hesitate to utilize these techniques to the fullest extent due to difficulties in the reproducibility and interpretation of the results. The expert contributions to this volume will guide one through all the obstacles in these techniques and will ultimately convert troublesome applications into facile and readily reproducible protocols.

Modern biophysical methods also represent a major part of the current volume. Several chapters include the description of cutting-edge technologies used to study riboswitch structures and their folding. These contributions cover a wide range of protocols from X-ray and NMR experiments to single-molecule fluorescent studies and isothermal titration calorimetry. Biophysical techniques are complemented by SELEX and related protocols, which allow for a transition from *in vitro* to *in vivo* experiments. *In vivo* methods, however, are not limited by the elucidation of the function of engineered riboswitches. Microbiologists, cell biologists and geneticists can appreciate the chapters focusing on various aspects of the identification and characterization of riboswitches and regulatory RNAs in living cells. One chapter is devoted to probably the most intriguing and demanding aspects of riboswitch research: computerized searches for riboswitches in genomic sequences, which could be helpful in the prediction of the alternative secondary and tertiary riboswitch structures, and in the identification of the candidate molecules that partner with riboswitches.

Research success in any given field is critically dependent upon advances in experimental methodologies. This volume summarizes and illustrates key methods that depict the remarkable recent progress in the riboswitch field and other areas of RNA biology. The wide scope of this book is a feature that will undoubtedly appeal to researchers with different backgrounds, working in various areas of modern biology ranging from evolutionary biology to pharmacology and biotechnology. Moreover, the protocols presented in the book can be successfully utilized by researchers with different levels of expertise, both beginners, who wish only to try the “RNA kitchen”, and experts, who would like to expand their RNA methodology.

I thank the authors who contributed to this volume and whose work will hopefully help readers find new stimulating approaches suited for application in their experiments.

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