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RNA Interference, Editing, and Modification

Methods and Protocols

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Dedication

This book is dedicated to Eric and Katherine Christian, who provided continual support and joy.

Preface

Two of the more fascinating biological phenomena that have been discovered in recent years are RNA editing and RNA interference. Each of these processes has been found in a cross-section of biological systems, including mammals, viruses, plants, and a range of model organisms (*C. elegans*, *Drosophila*, and various lower eukaryotes). RNA editing, which results in an RNA product different from that predicted by the genome, occurs through a variety of mechanisms. Alterations can occur at either the base level, in which one base is changed to another (substitutional editing/base modification), or via the addition and/or deletion of nucleotides relative to the original template (insertion/deletion editing). RNA interference (RNAi) involves the specific degradation of targeted mRNAs. Although RNA interference, editing, and modification use different enzymes and mechanisms, the targets of each of these reactions are often specified by RNA molecules. Indeed, the discovery of guide RNAs (gRNAs) that direct nucleotide insertion and deletion in trypanosome mitochondria set the precedent for subsequent discoveries of the small nuclear RNAs (snoRNAs) that target pseudouridylylation and methylation of stable RNAs and the small double-stranded RNA fragments (siRNAs) that mediate RNAi. Other small RNAs are known to mediate translational regulation during development (small temporal RNAs [stRNAs]) and mRNA stability (microRNAs [miRNAs]), and the recent identification of more than a hundred small "noncoding" RNAs has led to the realization that they may represent only the proverbial "tip of the iceberg." With the current availability of a large number of complete genomes, this area is one of the fastest growing areas in gene discovery efforts. RNA interference has also proven to be a powerful reverse-genetics tool, and has been used, for example, in the identification of trans-acting factors involved in RNA editing in trypanosomes. More recently there have been some intriguing hints of a possible biological connection between RNA interference and editing, based on both genetic studies in worms and colocalization studies in flies.

RNA Interference, Editing, and Modification is written primarily for those working directly in the fields of gene silencing, RNA interference, editing, and modification, as well as bioinformaticists trying to identify genomic regions that encode RNAs that are not translated into proteins and geneticists and others wanting to use RNA interference as a means of knocking out expression of individual genes and examining associated phenotypes. *RNA*

Interference, Editing, and Modification is split into two parts. Part I describes methods used in transient and stable gene silencing in worms, flies, trypanosomes, mammals, and plants, with an emphasis on parameters that must be considered for each system. Part II includes assays and methods used in studying RNA editing mechanisms in a wide range of organisms, including both systems that involve the conversion of one base to another and insertion/deletion editing. Each topic begins with a brief overview covering both historical background and scientific significance to provide a broader context for readers new to the respective areas. In addition, there are four chapters that focus on methods for the identification and characterization of small RNAs, many of which are involved in RNA interference or modification.

The overall aim of *RNA Interference, Editing, and Modification* is to present, as clearly as possible, methods that represent the current "state-of-the art" in the fields of RNA interference, editing, and modification. The level of detail provided is such that prior experience with the technique should not be required to replicate the methods described. Since the underlying biological mechanisms usually differ somewhat between species, each section will include multiple protocols representative of the major experimental systems in a given field. It is hoped that by presenting methods developed for a range of organisms, this book may lead to the modification or adaptation of assays and approaches for use in other biological systems.

Finally, I would like to thank the authors for their contributions, which were uniformly excellent, and series editor John Walker for his editorial skills and advice. Their thoroughness and commitment made this book possible.

Jonatha M. Gott

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