Preface

Besides being the physical link between DNA and proteins, RNA plays several other key roles, including RNA catalysis and gene regulation mediated mainly by non-coding small RNAs. This regulation can occur at some of the most important levels of genome function, such as chromatin structure, chromosome segregation, transcription, RNA processing, RNA stability, and translation.

The discovery of catalytic RNAs has opened up a wealth of opportunities to allow investigators to regulate gene expression post-transcriptionally using ribozymes and derivates. In addition to ribozymes, a new RNA-based strategy for gene expression in mammalian cells has recently been described. This strategy known as RNA interference (RNAi) has been shown to function in mammalian cells after introduction of small interfering RNAs (siRNAs) or expression of short hairpin RNAs (shRNAs) by RNA polymerase III promoters. Although much is known about the mechanisms of RNAi, there are a number of challenges that applications of this gene-silencing technology need to overcome, including the activation of innate immunity, off-target effects, and in vivo delivery. The present book on RNA is intended to cover some the most important functions of RNA, particularly small RNAs, and to facilitate the use of RNA agents in human therapies.

Since most, if not all, biological functions are mediated by structure on some level, and it is clear that natural RNA modifications play a vital role in such a structure, **Chapter 1** highlights the importance of this process in gene regulation. And **Chapter 2** describes a detailed method for analyzing naturally occurring RNA modifications. It should be noted that modified nucleotides occur in almost all classes of endogenous RNAs, and there are about 100 different base modifications known that may carry out several functions. Recent studies with chemically modified siRNAs have provided convincing evidence that 2′-ribose modifications can evade the recognition of siRNAs by immune receptors**. Chapter 3** elegantly describes the current strategies, allowing the separation of immune activation from gene silencing. Regarding siRNA chemical modifications, the major challenge is to identify the modification that eliminates siRNA unwanted effects without interfering with gene silencing.

Although much has been accomplished regarding RNA delivery to mammalian cells, obstacles regarding the in vivo delivery of siRNAs remain. Also, technologies that mediate targeted delivery of siRNAs are needed to improve their therapeutic efficacy and safety. **Chapters 4–7** describe the most current methods for delivery, including the use of innovative nanotechnologies for the delivery of oligonucleotides and siRNAs. Also, a large number of targeting strategies using antibodies, peptide, and RNA aptamers are described in **Chapter 7**.

To design an effective siRNA sequence, one must consider the base composition of the chosen site and whether the site will be accessible. **Chapter 8** describes a method for the identification of effective siRNA sequences. Also, **Chapters 9–11** offer valuable protocols for the design, the delivery, and assessments of shRNA activity on tumor growth and viral infection.

A recently established transfected cell array technology has opened new experimental dimensions in the field of functional genomics. This technology allows for the transfection

of several thousands of different siRNAs in microarray format. Thus, the silencing of several genes in a spatially separated manner can be monitored. **Chapters 12** and **13** describe this new technology, which is expected to facilitate functional genomics and drug target validation.

As indicated above, eukaryotes produce several types of small RNAs that function in diverse pathways. These small RNAs are conventionally grouped on their origin into two classes: microRNAs (miRNAs) and siRNAs. MiRNAs are usually generated from the dsRNA region of the hairpin-shaped precursors, whereas siRNAs are derived from long double-stranded RNAs. **Chapter 14** highlights the biogenesis of miRNAs and shows that intron-derived miRNA can induce RNAi not only in vitro but also in adult mice. Given that several effector proteins are shared by siRNA and miRNA pathways, there is a potential danger of inhibiting the miRNA biogenesis by exogenously or endogenously expressed siRNAs. **Chapter 15** underlies the risk of this danger.

Currently, different methodologies are used to profile miRNA expression. These include Northern blotting with radiolabeled probes, cloning approaches, quantitative PCR-based amplification, and microarray-based expression profiling. A collection of four chapters describes these recent technologies (**Chapters 16–19**).

In the 1980s, the discovery that certain RNAs can perform catalysis has led to the development of a new class of therapeutic RNAs called *trans*-cleaving ribozymes. Such ribozymes bind mRNA through base-pairing interactions and subsequently cleave the bound target mRNA in vivo. During recent years, much progress has been made toward assessing the potential therapeutic utility of ribozymes, with the hammerhead, hairpin, ribonuclease P, and *Tetrahymena* ribozymes being the main focus of this translational research. By using the *Tetrahymena* group I intron-based *trans*-splicing ribozyme, in **Chapter 20,** Kim and colleagues describe the conversion of human telomerase reverse transcriptase-encoding RNA to therapeutic transgene herpes simplex virus thymidine kinase, and **Chapters 21 and 22** cover the recent therapeutic use of hairpin and ribonuclease P ribozymes.

In addition to RNA catalyst, small structured single-stranded RNAs or DNAs, also known as aptamers, are attractive tools in therapy. They are in vitro-evolved RNA structures that bind with high affinity to a given ligand. The high-affinity binders are usually selected from a random pool of nucleotides by a process called systemic evolution of ligands by exponential enrichment (SELEX). **Chapters 23 and 24** describe the use of cancer cells to select cell-specific RNA aptamers and the selection of aptamers binding to human S100 calcium-binding protein B, respectively. **Chapter 25** describes the design of 2′-modified small RNAs able to function as TLR-7/8 antagonists. These novel agents could be used to treat TLR-induced diseases such as SLE.

Cancer immunotherapy offers an attractive therapeutic addition, delivering treatment of high specificity, low toxicity, and prolonged activity. In the case of vaccines, several mechanisms operating under physiologic conditions to modulate immunity contribute to immunologic unresponsiveness seen in most patients. Critical to the initiation of immunity, dendritic cells (DC) are key participants in immune regulation. Therefore, modulation of DC-using RNAi is especially important in overcoming immune tolerance to tumor cells. In this respect, **Chapter 26** describes the modulation of DC function by dual siRNAs targeting indoleamine 2,3-dioxygenase (IDO) and simultaneously activating RIG1 protein. IDO is a key immunomodulatory enzyme that promotes peripheral immune tolerance by inhibiting T-cell activation and proliferation through tryptophan catabolism.

One approach to stimulate effective cytotoxic T-cell (CTL) responses in cancer patients would be to rebuild this process in vitro, that is, to isolate DCs from the patient and load them with tumor antigens in such a manner that the antigens will be processed and presented to T cells. This approach has been accomplished by incubating DCs with peptides and proteins or by transfecting the cells with DNA constructs. Transfecting DCs with mRNA-encoding antigens is yet another method to load DCs with antigens. Messenger RNAs can be isolated directly from tumor cells or synthesized in vitro from DNA constructs. **Chapter 27** describes an elegant protocol for the generation of immunogenic DC transfected with mRNAs.

The generation of therapeutic T-cell populations for adoptive immunotherapy of cancer requires extensive ex vivo cell processing, including the isolation or the creation of antigen-specific T cells and their subsequent propagation to clinically relevant numbers. However, novel antibody-based immunotherapeutic strategies that exploit chimeric immune receptors (CIR), expressed on the surface of transduced human peripheral blood mononuclear cells (PBMC), to redirect potent non-MHC-dependent cytotoxicity to tumor cells expressing a tumor-associated antigen or self-antigens (e.g., CD19, CD20, CD33) has been described. Of note, this strategy overcomes the problem of MHC restriction. **Chapter 28** describes the manufacturing of T cells expressing CIRs. These genetically modified T cells expressed the chimeric receptor and killed cancer cells. These preclinical data confirm the feasibility of this approach to manufacturing T-cell products.

Based on several observations, vaccine strategies will be unsuccessful unless they are coupled with a means of counteracting the suppressive influence caused by certain immune cells and tumor cells. **Chapter 29** describes the recent advances in breaking self-tolerance to tumor cells. Notably, bone marrow transplantation (BMT) is currently used in the treatment of a variety of diseases such as leukemia. However, significant complications still limit the efficacy of this treatment, including the occurrence of graft-versus-host disease (GvHD) and infections. **Chapter 30** highlights the challenges facing this treatment and describes the possibilities of overcoming GvHD as well as genetic manipulations of hematopoietic $CD34^+$ stem/progenitor cells using RNAi.

Topics covered in this volume will be of interest to researchers, clinicians, teachers, and biotech companies interested in RNA-based therapies. It is my hope that the readers will benefit from this collection of excellent chapters dealing with RNA as tools and therapies.

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