RNA INTERFERENCE TECHNOLOGY

RNA Interference (RNAi) technology has rapidly become one of the key methods used in functional genomics. RNAi is used to block the expression of genes and create phenotypes that can potentially yield clues about the function of these genes. In the postgenomic era, the elucidation of the physiological function of genes has become the rate-limiting step in the quest to develop "gene-based drugs" and RNAi could potentially play a pivotal role in the validation of such novel drugs. In this cutting-edge overview, the basic concepts of RNAi biology are discussed, as well as the current and potential applications. Leading experts from both academia and industry have contributed to this invaluable reference for graduate students, postdocs, and researchers from academia wanting to initiate RNAi research in their own labs, as well as for those working in research and development in biotech and pharmaceutical companies who need to understand this emerging technology.

Krishnarao Appasani is the Founder and Chief Executive Officer of Gene-Expression Systems, a gene discovery company focusing on functional genomics in cancer research.

RNA Interference Technology

FROM BASIC SCIENCE TO DRUG DEVELOPMENT

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Winner of the Nobel Prize in Physiology or Medicine, 1968

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In memory of my parents

For my teachers, family members

and especially my wife Shyamala and sons Raakish and Raghu

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Foreword

Andrew Fire

It has been a privilege to watch the growth of RNA interference technology over the last ten years. Starting with a mixture of curiosity and chagrin, the field has grown into a substantial enterprise which impacts (and utilizes resources from) virtually every field of biomedical research. Research in RNAi derives from a set of apparently unconnected observations: strange pigment patterns in plants, unexpected failures and successes in antisense and overexpression studies, small regulatory RNAs in bacteria. If there is an underlying and recurring scientific lesson, it has been: "Pursue the unexpected." Basic and applied research each advance as a consequence of this pursuit; certainly this has been no better illustrated than in the last ten years of RNAi.

The work of hundreds of researchers in different fields that is reported in this book should provide the reader with both solid information (needed for experimental design and evaluation) and a lively and hopeful scientific story (needed to keep us all going through the long haul of scientific research). Our knowledge of the realm of genetic regulation by small RNAs has grown with remarkable speed. Starting in 1981 with a single known example of a modulatory short RNA (regulating copy number of the ColE1 plasmid), small RNAs are now known to regulate genetic activity at virtually every level: DNA and chromosome structure, transcription, RNA structure and stability, translation, and protein stability. Likewise, our ability to experimentally alter cells using this system has advanced at an unprecedented rate. As recently as 1990, the known examples of experimentally-induced silencing were a few unusual and accidental plant pigmentation patterns; now there are extensive menus of silencing-based methods as part of the "standard" molecular biology toolkit.

Work in this field is by no means finished. We still don't understand all of the modalities of RNA-triggered genetic regulation, why these modalities exist, and how they interact with each other. We don't have a clear picture the full extent of RNA-based regulation. As these questions are further investigated and understood, and as the underlying mechanisms are understood in detail, it will become possible to carry out more and more sophisticated experimental manipulations of genetic function. More questions: How do some organisms encapsulate

RNA triggers to produce a systemic response? How are long term RNAi effects perpetuated? What is the link between RNAi and immunity? What biological effects will come from the selective or global inactivation or augmentation of the RNAi pathway? How can we best use RNAi to discover the most sensitive and critical targets for biological investigation and drug development? Can we cure diseases by specifically triggering the RNAi pathway to attack errant genes? Can we treat other diseases by up- or down-regulating components of the RNAi machinery itself in specific cell types? How will cells and organisms respond in the long term to continuous modulation or use of the RNAi machinery?

We'll all be busy for quite a while in addressing these questions. Based on the first years of the field, one thing that can certainly be expected is a few more surprises.

Stanford, California, USA
August 2, 2004

Foreword

Marshall Nirenberg

RNA interference is a powerful tool that has been used to inhibit gene function either by increasing the destruction of mRNA corresponding to the gene, or in some cases, by inhibiting the transcription of the gene or the translation of mRNA to the corresponding protein. Exploring gene function by the classical approach of generating mutants of a gene often is much more laborious and time consuming then silencing gene function by RNAi using double-stranded RNA or double-stranded oligoribonucleotides about twenty two nucleotide residues in length. This book edited by Krishnarao Appasani is a timely and comprehensive compendium of information on RNAi and will be useful to experts on RNAi as well as investigators in many fields of research who may be interested in using RNAi to explore problems they are studying.

The RNAi field is only six years old. Research on RNAi has been expanding at an extraordinarily rapid rate, yet the field is in its infancy. There is great interest in using RNAi as a means of exploring gene function during embryonic development and in the adult in many organisms. Many aspects of RNAi remain to be explored. For example, the reactions and the molecules required for RNAi targeted destruction of mRNA are incompletely known. Similarly, the mechanisms of RNAi targeted modification of DNA, which regulates, transcription of DNA, as well as RNA targeted inhibition of mRNA translation are only partially known. Also, the functions of most micro RNA genes have not yet been explored. Since RNAi also can be used to regulate gene expression in specific cell types, the possibility that RNAi can be used therapeutically to treat diseases or certain viral infections by targeted gene silencing is an exciting, challenging possibility. However, difficult problems have to be overcome such as the problem of delivery of appropriate double-stranded oligoribonucleotides into cells, the stability, concentration, and toxicity of the oligoribonucleotides, and the length of time the oligoribonucleotides remain in the cells. These are challenging research problems. Nevertheless, the use of oligoribonucleotides as therapeutic agents to silence gene expression has great potential for the future. Libraries of small interfering RNAs (siRNAs) or short hairpin RNAs (shRNA) have been constructed and have been screened in cultured cells. In addition, methods have been devised for high

throughput screening of siRNA or shRNA libraries. RNAi has been used to inhibit replication of viruses in cultured cells such as HIV, hepatitis C virus, and hepatitis B virus. The oncogenic fusion protein p210 in chronic myelogenous leukemia cells promotes cell division in these cells. Both siRNA and a lentivirus vector containing shRNA have been shown to reduce the levels of p210 protein in cell lines and thereby inhibit cell division. In addition, RNAi has been used in intact mice to reduce the function of a mutant gene which results in the movement disorder, spinocerebellar ataxia type one. Treatment of mice by RNAi resulted in improved motor coordination and the cellular changes in the brain characteristic of the disease were no longer visible. RNAi also is being investigated as a therapy for ocular diseases.

It is too early to say how successful RNAi therapy will be. However, it is clear that RNAi is a powerful tool that has revolutionized basic research and that the ability of RNAi to down-regulate almost any gene affords remarkable opportunities to explore the use of duplex oligoribonucleotides as therapeutic agents for many diseases.

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