
Preface

Native small regulatory RNAs of about 22 nt in eukaryotes have emerged as evolutionarily conserved molecules that can repress translation or degrade the RNA transcripts of target genes, depending on the degree of complementarity. They are called microRNAs (miRNAs). Consequently, miRNAs fine-tune protein synthesis, manifesting numerous biological characteristics as a result of the control and coordination of large sets of genes. miRNAs are noncoding regulatory RNAs important for development and cell homeostasis, including exonic and intronic miRNAs. Exonic miRNAs, such as *lin-4* and *let-7*, are transcribed by RNA polymerases II (Pol-II) or III (Pol-III) as a large oligonucleotide precursor, and further processed by Drosha and Dicer RNases to form mature miRNAs.

Unlike the express processes of exonic miRNAs, intron-derived miRNAs (Id-miRNAs) are generated during intron processing of messenger RNAs (mRNAs). In mammals, both kinds of miRNAs are endogenous single-stranded molecules that mediate their activities through partial complementarity with the target genes, whereas short interfering RNAs (siRNAs) are mostly exogenous double-stranded molecules acted upon with complete complementarity against the target genes. Conceivably, siRNAs are synthetic molecules consisting of two perfectly matched miRNAs; one sense and one antisense, exerting miRNA-like activity.

miRNAs, small single-stranded hairpin RNAs capable of interfering with intracellular mRNAs that contain partial complementarity, are useful for the design of new therapies against cancer polymorphism and viral mutation. This characteristic is different from siRNA because a rigid complete complementarity is required for siRNA-induced RNAi gene silencing. miRNA was originally discovered in *Caenorhabditis elegans* as native RNA fragments that modulate a wide range of genetic regulatory pathways during animal development. Recently, findings of intron-derived miRNA in *C. elegans*, mouse, and human have led to a novel therapeutic strategy, using the miRNA generated by polymerase II (Pol-II) RNA transcription and splicing. The advantages of using miRNA over siRNA are that they are (1) long-acting, (2) stable in vivo, (3) highly RNA promoter-compatible, (4) multiple targeting, and (5) of no overt toxicity. This type of gene therapy is highly target-selective and suppresses sequence-specific genes, including mutants and polymorphisms. After comparing miRNA with numerous small regulatory RNAs involving sequence-specific gene silencing, it is clear that most are miRNA-like molecules.

Even though siRNA is not native in mammalian and human cells, their native counterparts in lower animals are processed by similar mechanisms, such as Dicer and RISC (Science 2002; 297:2056–2060). miRNA represents a broad spectrum of cellular self-defense tools against transgenes and viral genes that provide a unique vehicle for gene therapy.

The ability to efficiently, stably produce and deliver sufficient amounts of miRNA into the proper target cell without overt toxicity requires fine tuning of the technology before it can be tried clinically. The pharmacokinetics, cellular safety, and functional stability of miRNA expression in animals needs to be examined to ascertain that the artificial miRNAs are stable, effective, and non-toxic in vivo. In eukaryotic cells, the Pol-II-based transcription process is highly regulated and can be adjusted through diverse RNA promoters and transcription factors, thus, the Pol-II-mediated miRNA generation system provides efficient and safe application in gene therapy.

Until the early 1990s, the dogma was that DNA, the blueprint, was transcribed to mRNA, which was then translated into polypeptide or protein, resulting in a specific function reflecting a specific trait. On the other hand, the Human Genome Project completed the process of determining, sequencing, and mapping about 30,000 protein-coding genes among the 3 billion nucleotide base pairs of the human genome; however, the conventional genes only contribute about 3% of the human genome. For many years, the small noncoding RNAs were considered unwanted debris and discarded. These molecules probably were removed during molecular sizing procedures of RNAs; however, increasing lines of evidence suggest that the noncoding portions (i.e., intron) of gene transcripts play an important role in the regulatory pathways of global function in cells and organisms. With the advent of various miRNA genes, it became possible to interpret physiological variations that reflect individual differences, including weights, heights, and responses to various drugs. The functional role of miRNAs meant that suddenly one DNA may consist of multiple genes with pluripotent functions; some for translation, and others for regulation of the quantity of timely protein synthesis. This new discovery of miRNA genes, which has been facilitated by the rapid accumulation of computerized sequence data for human miRNAs, means that biomedical researchers can now manipulate specific mRNA expression using miRNAs in their research plans.

In view of the high conservation of the miRNAs in modulation of gene expression, the main objective of *MicroRNA Protocols* is to provide diverse, novel, and useful descriptions of miRNAs in several species, including plants, worms, flies, fish, chicks, mice, and humans. These include some useful adaptations and applications that could be relevant to the wider research community who are already familiar with the identification of miRNAs. For example, a

variety of different adaptations are described that have been employed to develop miRNAs as a potential drug design.

miRNA has opened a new avenue for our understanding of gene expression and will become one of the most widely applied techniques in biomedical research, playing a major role in the molecular investigation of disease pathogenesis. Determination of the applicable miRNAs at the molecular level is already beginning to inform the design of new therapeutic strategies. It is our hope that *MicroRNA Protocols* will stimulate the reader to explore diverse ways to understand the mechanisms by which miRNAs facilitate the molecular aspects of biomedical research.

Shao-Yao Ying