The aim of gene therapeutics is to add a gene product, usually a protein that is deficient or that when supplied in excess can prevent, stabilize, or reverse a human disease state. However, the ability to turn off or downregulate genes can be equally important in nucleic acid-based drug development. For example, a viral infection and illness results from the expression of exogenous genes encoded by the viral genome. Cancer can result from the inappropriate expression of normal genes, or the expression of a gene that has acquired a gain-of-function mutation, either of which can alter the cell cycle and promote uncontrolled cellular proliferation.

In the next four issues of *Human Gene Therapy*, we plan to present a series of reviews covering topics related to therapeutic applications of small RNAs. The review series will focus on microRNAs and RNA interference (RNAi) although other RNA-based therapies including those based on aptamers will also be covered. The biomedical potential of these small RNA technologies as well as their limitations will be discussed. Because the diversity of RNA-based therapeutics has exploded during the past 5 years, the final review will discuss some of the business and financial implications of this growing technology.

The idea for having a series dedicated to therapeutics based on microRNA/RNAi evolved from questions asked by some of my gene therapy colleagues, such as “What is the difference between RNAi and microRNA-mediated gene knockdown?” and “Will RNAi really work when past attempts to knock down gene expression have not been overly successful?” Of course, there is no easy or definitive answer to either of these questions. Nonetheless, to try to answer these questions, we need to begin with a broad definition. MicroRNAs and/or RNAi molecules are short, double-stranded RNAs (length, about 21 base pairs) that can partially or fully silence genes at the posttranscriptional level. This occurs when an mRNA contains the small RNA cognate target sequence. The reason why RNAi has the potential to be more robust and hence more successful than past strategies designed for gene knockdown is because the endogenous microRNA/RNAi machinery is present in all mammalian cells. Although we in the biomedical research arena like to define and split processes, it is important to note that there are distinct commonalities and differences between what are commonly referred to as microRNA and RNAi pathways. The dividing lines are not always distinct but in general, RNAi results in a target sitespecific cleavage event, whereas microRNA knockdown results from translational mRNA repression and/or enhanced mRNA turnover. The RNAi cleavage pathway is activated when there is a complete complementary match between the small RNA and its mRNA target, whereas microRNA-mediated repression occurs when there is a partial mismatch between these two RNA sequences. Most notably in lower organisms, these RNAs can sometimes result in transcriptional repression by causing chromatin/DNA modifications at the corresponding gene. In mammals, this level of regulation is still debatable.

The endogenous microRNA gene sequences within a mammalian genome represent at least 500 or so different RNA species that regulate one-third of our genes. This process adds another layer to control complex genetic regulatory networks involved in development, cell cycling, immunity, and metabolic pathways that are just beginning to be realized. Of course, how these microRNAs are controlled is another area of great interest. Nonetheless, numerous studies are beginning to establish how the dysregulation of these circuits can be important in the pathogenesis of serious human diseases including neurologic impairment, cancer, immune dysfunction, and infection to name a few.

While the process known as RNAi was first described from an inadvertent result in plant transgenesis studies, the elucidation of the process was established in *Caenorhabditis elegans* by Fire, Mello, and coworkers in the late 1990s (Fire et al., 1998), resulting in their sharing the 2006 Nobel Prize in Physiology or Medicine. At the time of the discovery, there was great debate as to whether or not RNAi occurred in mammalian systems, based on concerns about how such double-stranded RNAs might induce interferon responses. Nonetheless, Tuschl and colleagues (Elbashir et al., 2001) showed that RNAi was functional in mammalian cells, opening a whole new approach to treat human disease.

RNAi-based therapeutics can be divided into two categories. The first is the delivery of the mature siRNA molecules as double-stranded naked RNA or RNA complexed with a carrier. The RNA nucleotides can be modified to reduce toxicity and/or prolong activity when delivered into nuclelease-containing body fluids. The activity of the siRNA will be limited because of their finite half-life once inside the cell. The second approach is to use RNA as a transcriptional template to make short hairpin RNAs (shRNAs) that are then processed into mature siRNAs. These approaches will be limited by the vector but generally can result in more robust and sustained RNAi activity.
In the last several years, there has been an explosion in the number of efforts to use these small RNAs in preclinical models of human disease. There are at least half a dozen approved clinical trials, with many more coming down the pipeline. These include both vector-expressed shRNAs and siRNA-based approaches. It is likely that both approaches will have their place in clinical medicine, with the specific type of therapy dictated by the disease process. As the results of these clinical trials move forward, it will be important to ascertain whether the efficacy is due to specificity of gene knockdown, and not to a variety of possible nonspecific responses. It is important to move forward while at the same time maintaining the highest level of integrity and scrutiny to avoid preventable mishaps.

During the last decade, the important role that small RNAs play in biological homeostasis has become more apparent, but with new technologies, including large-scale sequencing platforms, we have just started to scratch the surface. Not only is the elucidation of the endogenous microRNA/RNAi pathways leading to important new conceptual insights about gene regulation and function, it is becoming clearer that many other flavors of small RNAs exist in our cells. The mechanisms by which these are generated, as well as their role in gene regulation, are not yet known. This is clearly an exciting time for RNA biologists as well as the physician scientists who wish to exploit this technology for the treatment of human disease. One thing is clear: small RNAs as a therapeutic platform are here to stay.

REFERENCES
