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### Review Series Introduction

Small molecules and antibodies have revolutionized the treatment of malignant diseases and appear promising for the treatment of many others. Nonetheless, there are many candidate therapeutic targets that are not amenable to attack by the current generation of targeted therapies, and in a small but growing number of patients, resistance to initially successful treatments evolves. This Review Series on the medicinal promise of posttranscriptional gene silencing with small interfering RNA and other molecules capable of inducing RNA interference (RNAi) is motivated by the hypothesis that effectors of RNAi can be developed into effective drugs for treating malignancies as well as many other types of disease. As this Review Series points out, there is still much to do, but many in the field now hope that the time has finally arrived when “antisense” therapies will finally come of age and fulfill their promise as the magic bullets of the 21st century.

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# On future's doorstep: RNA interference and the pharmacopeia of tomorrow

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**Small molecules and antibodies have revolutionized the treatment of malignant diseases and appear promising for the treatment of many others. Nonetheless, there are many candidate therapeutic targets that are not amenable to attack by the current generation of targeted therapies, and in a small but growing number of patients, resistance to initially successful treatments evolves. This Review Series on the medicinal promise of posttranscriptional gene silencing with small interfering RNA and other molecules capable of inducing RNA interference (RNAi) is motivated by the hypothesis that effectors of RNAi can be developed into effective drugs for treating malignancies as well as many other types of disease. As this Review Series points out, there is still much to do, but many in the field now hope that the time has finally arrived when “antisense” therapies will finally come of age and fulfill their promise as the magic bullets of the 21st century.**

Although the advent of specifically targeted antibodies and small molecules has made an extraordinary difference to the lives of patients with various maladies, many of them suffering from malignant diseases, most of the individuals that physicians are called upon to treat have yet to experience the miracle of targeted therapies. Further, for those that have responded to the various agents available, resistance to once effective remedies is becoming an increasingly important problem (1–3). The importance of the work described in this Review Series on posttranscriptional gene silencing with small interfering RNA (siRNA) derives from the promise of siRNA to simultaneously promote the goal of “targeted, less toxic” therapies and expand the universe of patients who might benefit from them.

Numerous gene silencing strategies have evolved over the years, and these have been primarily directed either to the genes themselves (4–6) or to the mRNAs they encode (7, 8). Although some exceptionally clever techniques for direct gene targeting have been developed (6, 9), the perceived ease with which mRNA can be accessed has resulted in most therapeutic efforts being directed toward this approach (10, 11). A number of modalities are available for mRNA targeting, and of these, the “antisense” strategies have been the most widely applied. These antisense strategies are all based on delivering into cells a nucleic acid strand, either DNA or RNA, that is reverse complementary to the mRNA encoding the protein that one would like to extinguish. By processes still unknown, the antisense nucleic acid (ASNA) strand and the mRNA target come into proximity and then hybridize if the strands are physically accessible to each other. Stable mRNA-ASNA duplexes can interfere with splicing of heteronuclear RNA into mature mRNA (12, 13); can block translation of mature mRNA (14, 15); or can lead to the destruction of the mRNA, either by endogenous nucleases, such as RNase H (16, 17), that are recruited into the mRNA-ASNA duplex or by intrinsic enzymatic activity engineered into the ASNA sequence, as is the case with ribozymes (18, 19) and DNazymes (20, 21).

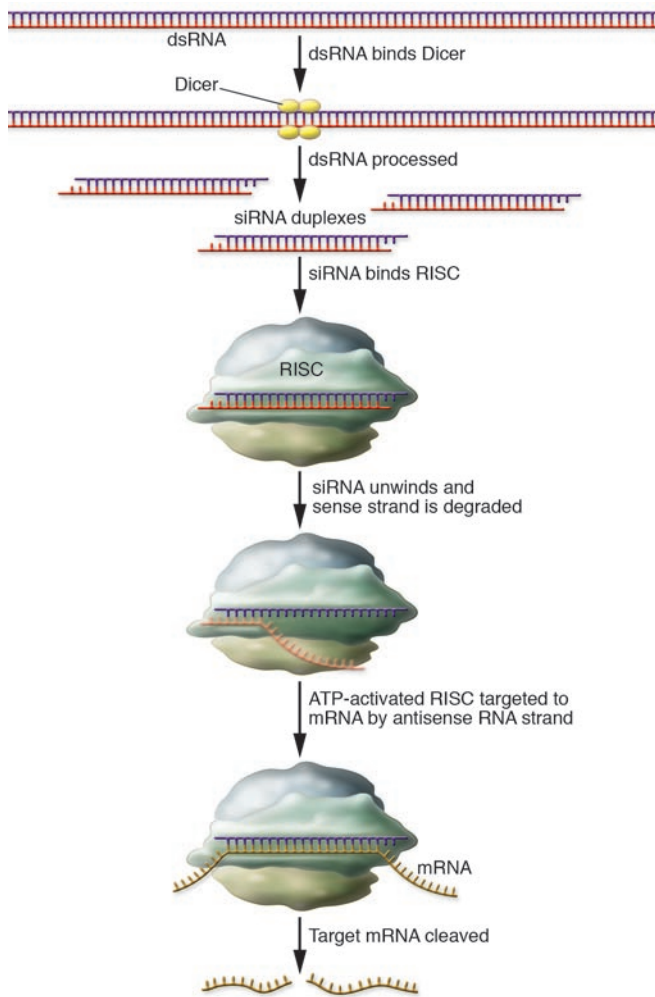
In the last several years, RNA interference (RNAi) (22, 23) has emerged as an exciting potential alternative to the more classical antisense approaches (11, 24, 25). Indeed, it is so robust and has had such a profound effect on the way science is now conducted that two of the major contributors to the field were recently awarded a Nobel Prize in Physiology or Medicine for their contributions (26–28). In brief, RNAi is the process by which double-stranded RNA (dsRNA) targets mRNA for destruction in a sequence-dependent manner. The mechanism of RNAi involves processing of dsRNA into approximately 21- to 23-bp fragments that hybridize with the target mRNA and initiate its destruction (Figure 1); this mechanism has been intensively studied. An enzyme called Dicer processes dsRNA into the short fragments (29–32). These small cleavage products are then incorporated into a larger, multi-protein RNA-induced silencing complex (RISC), which simultaneously scans the complementary mRNA sequence for homology to the small, now unwound, RNA fragment and then promotes the destruction of the mRNA through an enzymatic activity integral to the complex (23, 33–35). RNAi is in fact a natural process, and this is perhaps best exemplified by the discovery of naturally encoded structural hairpin RNA molecules that are called microRNAs (miRNAs), which are now known to play extremely important roles in regulating gene expression at the posttranscriptional level. Most human miRNA loci are located within intronic regions and are transcribed by RNA polymerase II. The primary transcripts are cleaved by the nuclear ribonuclease Drosha (36, 37) to release approximately 70-nt pre-miRNAs, which are subsequently processed by the RNAi machinery to generate mature, approximately 22-nt, miRNAs that are increasingly being shown to play a critical role in normal development and malignant cell transformation (38–41).

Although many hope, with good reason, that RNAi will be the “true grail” of targeted therapy, there remain many obstacles that must be overcome before this becomes reality. Indeed, these are quite well known because they are virtually identical to the roadblocks that have plagued the other gene silencing approaches. These issues include the stability of the molecules in plasma and intracellularly (42, 43), the ability of these molecules to hybridize with their mRNA target and promote its destruction (44, 45), and the ability to deliver these molecules into target cells (42, 46, 47).

**Nonstandard abbreviations used:** ASNA, antisense nucleic acid; dsRNA, double-stranded RNA; miRNA, microRNA; RISC, RNA-induced silencing complex; RNAi, RNA interference.

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**Figure 1**

The RNAi pathway. The enzyme Dicer processes dsRNA molecules into shorter dsRNA fragments, typically 21–23 bp in length, termed siRNA. The siRNA duplexes are incorporated into the RISC, where the sense strand is degraded, leaving the antisense “guide” strand free to hybridize with its complementary sequence in the mRNA. This initiates mRNA strand cleavage by an enzyme native to RISC.

entirely clear. As a general rule, oligodeoxynucleotides are taken up primarily through a combination of adsorptive and fluid-phase endocytosis (54). The mechanism of uptake of dsRNA is likely to be similar and has recently been characterized in liver where it depends on lipoprotein receptors and the mammalian homolog of the *C. elegans* transmembrane protein Sid1 (55, 56). After internalization, confocal and electron microscopy studies have indicated that the bulk of the oligonucleotides enter the endosome/lysosome compartment, in which most of the material becomes either trapped or degraded. Biological inactivity is the predictable result of these events. In this Review series, Sagir Akhtar and Ibrahim Benter describe the delivery problem and outline possibilities for solving it (57). Sometimes, however, it is necessary to get off the road entirely and just drive around the obstructions that are blocking one’s path. If it is not possible to get siRNA molecules into cells, then the strategy of expressing short hairpin, miRNA-like molecules from viral vectors, which can then enter the silencing pathway, might be an approach that could solve the delivery and stability problems simultaneously. This is a classic gene-therapy approach, and its challenges and possibilities are outlined in the Review by Mark Kay and Dirk Grimm (58).

The power of gene silencing with nucleic acid molecules is undisputed in the laboratory, and RNA-based medicines are now working their way into the clinic (59–61). Although some theoretical concerns have already been raised concerning the safety of certain RNAi-mediated therapies (62, 63), most workers in this field remain optimistic that the problems described above are solvable and that the dawn of nucleic acid therapeutics, though often promised and now seriously delayed, is finally upon us.

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It is also worth noting that unintended silencing with siRNA degradation products (off-target silencing), is also being increasingly recognized as a potential problem, not only as a laboratory artifact but as a source of unwanted medicinal side effects (48–52).

Of these core issues, stability might seemingly be the most easily tackled, and in the first article of this Review series (53), David Corey discusses how chemically modifying the nucleic acid strand remains an important and active area of research into methods to improve siRNA stability and activity. How to deliver these molecules as a therapeutic remains a major, and largely unsolved, issue, except perhaps for the liver, which seems more amenable to uptake of siRNA molecules than other organs for reasons that are not

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