An Introduction to RNA Interference and Drug Development
RNA interference (RNAi) represents one of the most promising new frontiers in drug discovery. To date, most drugs have focused on areas involving protein function. More recently, the mapping of the human genome has targeted DNA via gene therapies. RNA is the intermediary between DNA and proteins.

**Breakthroughs in understanding RNA’s extensive role in essential cellular behaviors have opened up the potential for a whole new class of drugs based on RNA interference (RNAi).**

Uncovering new understandings in essential cellular function leads to drug discovery breakthroughs. Researchers know that development of potent and safe drugs requires an understanding of relevant biological pathways. With the reservoir of knowledge about disease pathways expanding, there is a growing biochemical foundation upon which new drugs can be intelligently developed.

In the essential pathway that is central to all cells, genes, through an intermediary “messenger” called RNA, produce proteins. Scientists view this pathway as essential, since proteins make up most of a cell’s machinery. The basic steps and core molecular functions inherent to this essential pathway are well understood. Specifically, when a protein is to be made in the cell, the gene’s DNA is copied onto a corresponding piece of single-stranded RNA, known as the messenger RNA, which delivers the protein’s recipe to the cell’s protein-making machinery.

In the past, drug targets have predominantly been focused on the “downstream” part of this essential pathway, namely proteins. Over the years, once armed with an understanding of a specific protein’s relationship to a specific disease family, researchers have exhaustively targeted selected protein receptors and enzymes. These research efforts have produced an array of effective small and large molecule drugs, from drugs that “bind to” or “block” unwanted protein function, such as Cox-2 inhibitors and calcium channel blockers, to drugs that provide a “needed” protein, such as erythropoietin.
While proteins have been targeted extensively for drug discovery, RNAi offers an unexplored area in the essential cellular pathway.

If researchers continue to look only at protein for drug development, they face at least two critical challenges. First, many protein-targeting therapies are plagued by unwanted side effects and low efficacy. Side effects and low efficacy often arise from therapies that target downstream of the gene-to-protein process, as these therapies are made less effective when similar proteins in the body result in undesired action. Second, researchers are bound to see diminishing returns from investing incrementally in one particular arena of drug development, as new drug candidates become increasingly difficult to identify and screen.

In the past decade, following the mapping of the human genome, gene therapy – targeting the DNA itself – has begun to be explored. While offering a powerfully promising and novel approach to therapy development, there are some concerns that there may be some unwanted side effects to “unnatural” intervention into DNA. Many feel that there is good cause to be cautious before widely deploying therapies that basically re-write the essential genetic matter contained in DNA.

Very recently, leveraging a series of important scientific breakthroughs, the possibility of targeting RNA as a tool in drug development has emerged. This revolutionary approach has been heralded by many and offers incredibly broad promise, as drugs can potentially be targeted “upstream” of protein synthesis in a natural way that is available in all cells.

Recent RNA-related discoveries are having immediate practical applications.

Scientists have found that tiny snippets of RNA with two strands instead of the usual one can be used to shut off specific genes. The technique, known as RNA interference (RNAi), is being widely used to discover the functions of genes by turning them “off” and seeing what happens to plants and animals. Harnessing the natural cellular power of RNA offers a novel approach that opens up whole new areas of disease therapy in a way that can be targeted and safe. Shutting off unwanted genetic activity in a targeted and natural manner can be applied to a wide spectrum of breakthrough therapies, from attacking rogue genes in cancer to beating back chronic viruses, such as those that cause hepatitis and AIDS.

How RNAi works

RNA plays a key role in cellular function

It has long been known that RNA’s role extends beyond that of a passive messenger. For example, the ribosome, which makes proteins, is made partly of RNA, and another type of RNA, called transfer RNA, also aids in protein production.

In a process known as RNA interference (RNAi), double-stranded RNA can stop the production of specific proteins, effectively inactivating genes.

Scientists can copy double-stranded RNA (dsRNA) from the specific gene they wish to shut off. When appropriately introduced into the cell, the base pairs of dsRNA and targeted messenger RNA (mRNA) match exactly, meaning the dsRNA will have the highest possible specificity as a therapy, corresponding to high potency and low side effects. The matching of the base pairs results in the degradation of the specifically targeted mRNA, effectively stopping the activity of the targeted gene and the production of its proteins by eliminating their intermediary.

RNAi is naturally observed in all species including mammals

Scientists believe that the underlying mechanism of RNA interference evolved from a natural early defense mechanism against viruses. While RNAi was first observed in plants over a decade ago, it has taken recent breakthroughs to provide the understanding needed to translate this initial discovery into the potential for the development of human therapies. Unlike the cells of simpler organisms, such as plants and insects, mammalian cells react to the presence of dsRNA with a more sophisticated response, which is a natural defense mechanism against dsRNA from viruses. In the event that they detect long strands of dsRNA, mammalian cells have an extra level of self-defense that triggers cellular suicide with the simultaneous release of interferon to warn neighboring cells of a viral invasion. However, researchers have now shown that RNA interference is observed in mammalian cells when the dsRNA is introduced into mammalian cells in ‘short pieces’ that are approximately 21-25 base pairs long.

These shorter pieces of dsRNA are called small interfering RNA (siRNA), and specific siRNA have been shown to deactivate specific genes. Researchers have observed this targeted gene deactivation across a wide array of genes and species, including a variety of plants and animals.
RNAi enables targeted mRNA degradation

The key actor in the RNAi process is a 21-25 base pair RNA strand, called siRNA. Through a natural cellular process, a specific siRNA degrades a targeted mRNA, thereby deactivating an undesirable gene expression.

1. siRNA is targeted to a specific gene. This can occur through a natural process or be designed through chemical synthesis.
2. Within cells, siRNA unwinds and is incorporated into RISC, a stable protein-RNA complex.
3. siRNA is directed to a targeted mRNA that is known to be involved in a disease pathway.
4. This mRNA is cleaved and undergoes degradation, thereby interrupting the protein synthesis of the targeted gene.
**Biotechnology can leverage the natural process of RNAi for therapeutic benefit**

Within cells, siRNAs are incorporated into an RNA-induced silencing complex (RISC), forming a stable protein-RNA complex that can recognize and destroy target mRNAs. siRNAs of the characteristic 21-25 base pair length readily form a naturally stable RISC complex. The RISC complex activates with the addition of ATP, resulting in the unwinding of the siRNA that then targets mRNA with its base pairs providing it with built-in specificity for a unique substrate.

RNAi is distinguished from antisense technologies, an earlier attempt to harness the innate therapeutic power of DNA or RNA. Whereas antisense technologies focused on interrupting the translation of mRNA to protein by introducing single strands of RNA targeted to bind with the mRNA, RNAi mimics a natural process by using double stranded siRNAs. Because it leverages a natural process and acts predictably through the RISC complex, RNAi is highly specific, in contrast to antisense technology which has proven hard to target and unstable in the body. Antisense therapies seek to “block” a key cellular biochemical process, as compared with RNAi which “directs” the cell’s own biology to detect and shut off rogue genetic activity and corresponding protein synthesis.

**Key milestones in the evolution of the new field of RNA Interference (RNAi)**

- First scientific observation in plants of what is known today as RNAi 1990
- dsRNA shown to be capable of gene silencing in worms 1998
- Discovery of RNA-induced silencing complex (RISC) 2000*
- siRNA of 21-25 base pair length shown to induce RNAi in mammals 2001*
- RNAi shown to reduce the activity of viruses, such as HIV and Hepatitis C 2002*

*Contributions made by scientists affiliated with Alnylam Pharmaceuticals, Inc.

**RNAi-based therapies offer a number of inherent and fundamental benefits:**

1. high specificity – with the built-in accuracy of Watson-Crick base pairings, RNAi-based therapies can target only specific gene activity in contrast to small molecules which intervene in an array of biologically important processes beyond the relevant disease pathway;

2. good safety profile potential – RNAi-based therapies mimic a natural process and use complexes and compounds that exist in the body today, thereby avoiding the toxicity that can result from introducing unfamiliar elements and molecules into the body;

3. more rapid early-stage drug development – RNAi-based therapy development relies predominantly on documented gene sequence data and leveraging a natural process.

The table below characterizes some of the inherent differences between RNAi and conventional small molecule drug evolution, looking at different key milestones in the discovery and development process:

<table>
<thead>
<tr>
<th>Target ID [discovery]</th>
<th>Validation, translating hits to leads [discovery]</th>
<th>Drug-ability [discovery]</th>
<th>Advancement through drug development [development]</th>
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<tbody>
<tr>
<td>RNAi</td>
<td>Use genome to find other potential undesirable targets</td>
<td>No known barriers</td>
<td>Higher probability of success in safety and efficacy</td>
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<td>Conventional Small Molecule Drugs</td>
<td>Range of tools in trial-and-error method: - Libraries - Screening - Structural Biology</td>
<td>Requires exhaustive enzyme panel experiments; medicinal chemistry optimization</td>
<td>Primarily limited to enzymes and receptors only</td>
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RNA's roles in disease and their application to the development of therapies

Many diseases are fundamentally gene-based, and these are the diseases for which targeting RNA via RNAi offers the most immediate therapeutic promise. There are three broad categories of diseases that are gene-based. Viral infection includes diseases caused by a “foreign invader” into the body. Cancer includes diseases that are manifestations of mutations. Inflammation includes diseases resulting from the over-expression of specific genes.

Since RNAi is a ubiquitous, naturally occurring cellular process, the potential for RNAi extends to other unmet patient needs across a broad range of diseases.

<table>
<thead>
<tr>
<th>Disease Category</th>
<th>Representative Disease</th>
<th>Potential Differentiation of RNAi</th>
<th>RNAi Therapeutic Approach</th>
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<tbody>
<tr>
<td>Viral Infection</td>
<td>Hepatitis C</td>
<td>Specific to virus yields reduced side effects and better response rates vs., for example, interferon</td>
<td>Destroys the viral RNA genome</td>
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<td></td>
<td>Gene Target: Hepatitis C RNA</td>
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<tr>
<td>Cancer</td>
<td>Types of lung and pancreatic cancers</td>
<td>Specific to attacking only mutated oncogene vs. small molecule kinase inhibitors that affect other targets</td>
<td>Wipes out expression of mutated protein that causes cancer cells to proliferate</td>
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<tr>
<td></td>
<td>Gene Target: RAS mutation</td>
<td></td>
<td></td>
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<tr>
<td>Inflammation</td>
<td>Rheumatoid Arthritis</td>
<td>More potent and works upstream vs. protein receptor blockers</td>
<td>Shuts off protein synthesis</td>
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<td></td>
<td>Gene Target: TNF-alpha protein in joints</td>
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## Glossary of relevant terms

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
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<tr>
<td><strong>Protein</strong></td>
<td>A primary component of cells, containing linked amino acids whose order is specified by a gene. Proteins build up most of the structures in cells and act as “little machines” that work together inside of cells to accomplish most cellular functions.</td>
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<td><strong>Receptor</strong></td>
<td>A protein, usually on the cellular surface, that interacts with binding substances, including drugs, to produce a biological response.</td>
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<td><strong>RISC</strong></td>
<td>RNA-induced silencing complex (RISC), forming a naturally stable protein-siRNA complex that can recognize and destroy target mRNAs.</td>
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<td><strong>RNA</strong></td>
<td>Ribo-nucleic acid. A naturally-occurring molecule key to essential cellular function which, like DNA, is made up of a string of bases.</td>
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<td><strong>siRNA</strong></td>
<td>Small interfering RNA. “Short pieces” of dsRNA, approximately 21-25 base pairs long, which are central to RNAi.</td>
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<td><strong>Small molecule</strong></td>
<td>A “small” chemical, often in the form of a drug, which can be absorbed through the gut. Contrasted with “large” protein therapeutics which must be administered by injection to bypass the gut.</td>
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Select References

General Reviews of RNAi


Select references to key milestones in RNAi


