Research Tool with Therapeutic Potential

RNA interference (RNAi) refers to sequence-specific downregulation of gene expression via small RNAs, often by the use of small interfering RNAs (siRNA) 21–23 bases in length. A widespread, highly conserved, endogenous mechanism for gene regulation, RNAi may have evolved as a defense mechanism against viruses.

Anti-Dogma

Kevin V. Morris, Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, CA, is looking at how siRNAs can regulate gene expression. Projects in his laboratory include mechanistic determination of siRNA-mediated transcriptional gene silencing (TGS) in human cells, and posttranscriptional and transcriptional gene silencing of human immunodeficiency virus (HIV)-1. “Our laboratory is interested in turning off genes with RNA at the promoter level,” he says. “It may not be RNAi,” he notes, referring to evidence that synthetic double-stranded RNAs, once inside a cell, have been shown to turn off targeted promoters via the antisense strand. “This goes against the dogma that promoters are not transcribed. They are transcribed, at low levels, and the transcript is the target of the antisense. So you can look at RNAi as protected antisense. Antisense RNA can direct chromatin remodeling and provide a means for epigenetic modification of promoter regions in addition to other epigenetic phenomenon like histone deacetylation and DNA methylation. Therefore, in this context, RNA can not only regulate the expression of genes via a promoter with sequence homology, but can also regulate genes up or downstream via chromatin structural changes.

“You can look at RNAi as protected antisense.”

Figure courtesy of Kevin V. Morris, The Scripps Research Institute, La Jolla, CA.

Mechanistic insights into RNA directed transcriptional gene silencing in human cells. Small RNAs, approximately 21–28 bp in length can modulate transcriptional gene silencing when designed to target RNA polymerase II (RNAPII) promoters (Reference 1). The siRNAs or antisense RNAs (approximately 21 bp) are (1) transfected into the target cells where they (2) possibly interact with argonautes 1 and 2 (Ago-1 and Ago-2, respectively) (References 2 and 3). Next, the siRNAs are shuttled into the nucleus (3), possibly via interactions with Ago-1 or when the cell undergoes cellular division. The antisense strand of the siRNA (4) then interacts with a low-copy RNAPII expressed transcript that spans the siRNA targeted RNAPII promoter. The interaction of the antisense strand of the siRNA may then interact or recruit a transcriptional silencing complex (TSC) possibly containing DNA methyltransferase 3A (Reference 4), the histone methyltransferase EZH2 (Reference 5), Ago-1 and TAR binding protein (TRBP) (Reference 3). The subsequent recruitment of the TSC by the antisense RNA targeted to the low-copy promoter associated RNA results in the local chromatin being epigenetically modified and silencing of the genes expression via histone methyl-marks that are repressive, such as H3K9me2 or H3K27me3. Sustained RNA targeting or particular promoters may be more susceptible to RNA-directed DNA methylation which can result in a more permanent epigenetic silencing event.

Morris wondered if RNAi was a vestigial mechanism or if it would be operational at some level in human cells. He and colleagues have found endogenous RNAs transcribed from introns that regulate genes downstream. “So what’s cool is you could control a deregulated gene. Many groups have shown that you can turn genes off. Could you turn genes on? It seems so.” He notes that working with human cells is more difficult than using model systems; although

he is interested in mechanisms, the current tools are limiting. However, he expects RNAi will prove to be the predominant mechanism in gene regulation. “I’m willing to bet the effector molecules are there. They will be in low copy number and hard to detect,” he concludes.

Plants First

Robert Martienssen, Professor, Cold Spring Harbor Laboratory,

Image courtesy of Rob Martienssen, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Arabidopsis mutant in RNAi that looks like a pitcher plant, called "hairy," an allele of argonaute.

Cold Spring Harbor, NY, is also interested in epigenetic mechanisms of gene regulation and the interaction of RNAi with the genome, using *Arabidopsis* and maize (corn) as models. He notes that in plants, epigenetics has been shown to underlie many interesting phenomena (e.g., paramutation, or the way alleles can influence each other when present in the same organism). He predicts the same thing may be happening in mammals. "This has major implications for disease and how we think of evolution in plants, which are sensitive to their environment. Flowering time, which is key for survival, depends upon temperature and day length," he says. Research in plants and other model organisms, he notes, lead the way to discoveries in other systems. For example, transposable elements, first described in maize, have been found in other organisms, including humans. Non-coding RNAs have been implicated in mammals in X chromosome inactivation and imprinting. "Does RNAi play a role?" asks Martienssen. "It's not as easy to address as you think," he says.

The advent of high-throughput sequencing has affected how people look at RNA populations, making it easier to study smaller groups of cells and obscure organisms to see if RNAi explains certain observations. "Sequencing centers democratize the process and will change the way people think about science," Martienssen says. He notes that RNAis are especially abundant in plants, but had been overlooked in gel electrophoresis studies in the past because, due to their small size, they ran off the ends of the gels people were using. Nevertheless, more advanced technologies should have an impact, for example, the use of microarrays in chromatin profiling studies, to identify epigenome-wide transcripts from heterochromatin.

Approaching the Clinic

John J. Rossi, Professor, Division of Molecular Biology, and Dean, Graduate School of Biological Sciences, Beckman Research Institute, City of Hope, Duarte, CA, points out that the use of RNAi for therapeutics would eliminate a lot of the up-front development required with small molecule drugs. Both he and Morris point out that a major pit-

fall, which is how to get small RNAs into cells, remains to be addressed, whether one is interested in research into mechanisms or in designing therapeutic interventions. Rossi predicts that delivery schemes will be a big area of future research. Some strategies that are being developed include antibody-conjugated small RNAs targeted for receptor-mediated uptake, aptamer-mediated uptake, inclusion in liposomes, tagging with cholesterol, and "pseudoselective uptake" via targeted receptors present on a cell type of interest (e.g., transferrin receptors expressed on tumor cells). Rossi's group is interested in therapeutic applications and is using lentiviral vectors to deliver RNAi targeting HIV into hematopoietic stem cells and T cells, with clinical studies to begin soon.

Therefore, Rossi believes that delivery is less of a problem than it was a few years ago. "All the approaches [to delivery] are valid and have their specific niche. This is pointing in the direction to revolutionize diseases intractable to therapeutic intervention." One problem that targeted RNAi may solve is how to get systemically injected therapeutics to cross the blood brain barrier. Just published work shows that it is possible to use

Image courtesy of John J. Rossi, Beckman Research Institute, City of Hope, Duarte, CA.

RNAi is multifaceted. dsRNA, double-stranded RNA; siRNA, small interfering RNA; miRNA, microRNA; RISC, RNA-induced silencing complex; AAAA, poly-adenosine tail.

a neurotropic rabies viral glycoprotein to target RNAi to neuronal cells. The ultimate goal will be to treat brain diseases.

“All approaches are valid and have their specific niche.”

Both Martienssen and Rossi point out that there are huge areas of the genome that are regulated at the level of chromatin. Rossi acknowledges that the discovery of the regulatory role of RNA in one system has led to searching in others. Although in the past the smallness and low copy number of regulatory RNAs may have been an impediment, the field is more tractable now. He also credits high throughput sequence technology, what he terms deep sequencing or massive parallel sequencing, with furthering the field.

Unanswered Questions

Morris notes that there are many questions that remain to be answered, for example, are there small RNAs that are cell-type specific and could be used to differentiate specific cells types (e.g., stem cells)? And, if specific genes can be turned on or off with RNAi, how long-lasting would the effect be? Another far-reaching issue, particularly for drug development, is what the off-target effects may be, and how they can be avoided.

References (cited in the Figure Caption)

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Note: This version corrects errors that appear in print.