RNA interference: When it is regulated by the NIH Guidelines?

What is RNA interference (RNAi)?

RNA interference (RNAi) is a method of suppressing gene expression in cells by introducing a double stranded RNA molecule that is complementary to a portion of your target gene. This double stranded RNA gets taken up by a complex designed to fight RNA based viruses. Using the introduced RNA as a targeting template, the complex binds to any RNA that is complementary to that template and degrades it, in this case the mRNA of your target gene. In this way, expression of a target gene is reduced or eliminated.

What are the most commonly used methods of RNAi?

Depending on the organism worked with, there are different methods of RNAi that can be used. Focusing on mammalian cell culture and mammalian animal models, the primary methods include: short interfering RNA (siRNA), short hairpin RNA (shRNA), microRNA (miRNA). In organisms, like *C. elegans* and *Drosophila,* long double stranded RNA dsRNA may be used also.

siRNA: When using siRNA the researcher will select a 20-22 nucleotide sequence that is unique to the target gene. A RNA olgionucleotide of this sequence and its complement are synthesized, mixed and allowed to anneal to form an RNA duplex. This duplex is then transfected into cells or introduced into animals. The duplex is then taken up by the RNAi machinery as outlined above. The resulting suppression of the target gene's mRNA is transient (3-7 days) as the amount of siRNA within the cells is reduced by degradation and cell division.

shRNA: To achieve a longer lasting gene suppression, the shRNA method was developed. In this method, a DNA cassette is made that contains a 19-29 nucleotide target sequence, a loop domain, and then a 19-22 nucleotides sequence complementary to the target sequence. When transcribed, the RNA twists into the short hairpin structure which brings the complementary target RNA sequences together to form an RNA duplex. This duplex is then processed and taken up by RNAi machinery as above. These shRNA DNA cassettes are placed into plasmid vectors and either directly administered to cells or animals or used to make viral vectors which then introduce the construct by infection. Depending on the method of introduction, the resulting shRNA gene suppression can be transient or persistent.

miRNA: miRNA are naturally occurring genes that code for an RNA that adopts a short hairpin structure. This hairpin is then processed by the RNAi machinery into a mature miRNA of 21 nucleotides which suppresses gene expression as described above. Many miRNAs are complementary to more than one gene's mRNA and thus can suppress the expression of several gene products at once. As with shRNA, the miRNA gene is placed into plasmid vectors and either directly administered to cells or animals or used to make viral vectors which then introduce the construct by infection. Depending on the method of introduction, the resulting shRNA gene suppression can be transient or persistent.

dsRNA: Many organisms, most notably *C. elegans* and *Drosophila*, have the capacity to take up dsRNA segments of 200-400 nucleotides in length and then process them into 21 nucleotide pieces which are taken up by the RNAi complex as above. These dsRNA are generally expressed from a plasmid though there are kits for *in vitro* generation of dsRNA. Whole *C. elegans* and *Drosophila* cell culture can passively absorb dsRNA. Targeted microinjection of dsRNA is also used. Gene suppression is transient but long lived. Some RNAi mediated suppression in *C. elegans* persists into first generation offspring.

How is RNAi regulated under the NIH Guidelines for Research Involving Recombinant DNA Molecules?

In section I-B of the *NIH Guidelines,* recombinant DNA molecules are defined as either: *"(i) molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or (ii) molecules that result from the replication of those described in (i) above."* It further goes on to say that, *"If the synthetic DNA segment is not expressed in vivo as a biologically active polynucleotide or polypeptide product, it is exempt from the NIH Guidelines."*

siRNA duplexes are synthesized and not replicated within recipient cells. Thus, it is not considered recombinant DNA and is exempt from the NIH Guidelines. In contrast, plasmids containing shRNA , miRNA and dsRNA cassettes are replicated within *E. coli* during their creation and amplification protocols. Thus, they are rDNA and regulated by the *NIH Guidelines.* A risk assessment that evaluates the nature of the insert, the intended recipient, and the transmission method used would be needed to determine the exact regulations that apply to any given experiment.

For assistance regarding the biosafety requirements for working with these agents, please contact Vanderbilt Biosafety at biosafety@vumc.org.