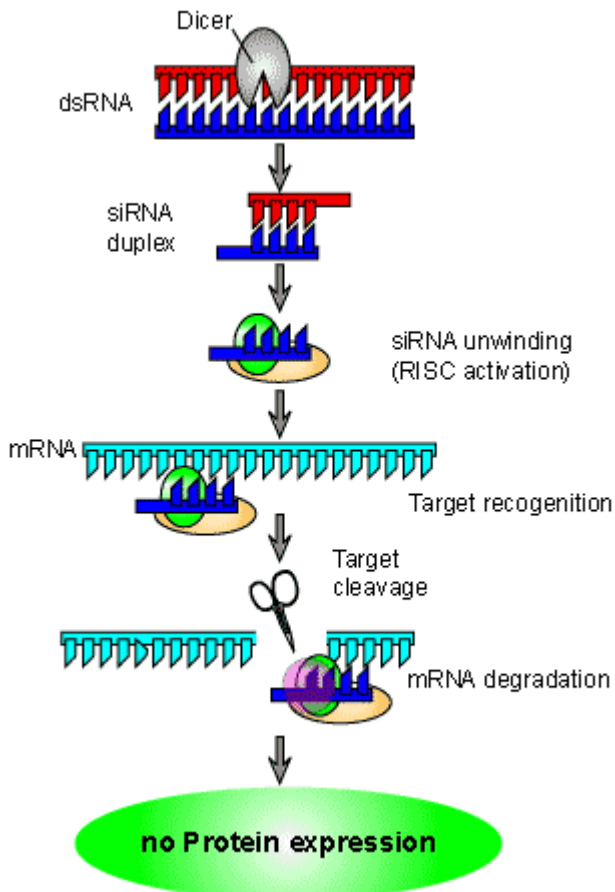


si RNA

RNA interference refers to the inhibition of gene expression by small double-stranded RNA molecules. This phenomenon was initially demonstrated in *C. elegans*, in which the injection of dsRNA molecules inhibited complementary gene expression. Though the use of siRNA has become a widely used tool for down-regulating gene expression, the existence of a naturally occurring pathway in eukaryotes has been well described. The origin of endogenous siRNA (or miRNA) may be transposons, viruses, repetitive sequences and genes. The process of producing effective endogenous siRNA is regulated by three enzymes. RNA-dependent RNA polymerases convert single-stranded RNA into double-stranded RNA. Alternatively, DNA-dependent RNA polymerases produce dsRNA by transcribing inverted DNA repeats. The resulting large RNA molecules are subject to digestion by ribonuclease III (Dicer) to produce short double-stranded siRNA molecules. Argonaute proteins are then required to bind siRNA molecules to form a complex known as RISC (RNA-induced silencing complex). RISCs may then promote epigenetic silencing through RNA-directed DNA methylation or by target RNA cleavage. Though protein translation may be knocked down considerably, siRNA does not normally eliminate the expression of a gene target completely.



The use of RNA interference for artificially manipulating gene expression was initially limited by the activation of cellular antiviral mechanisms. Exposure of cells to sequences longer than 30 nucleotides induces interferon gene expression resulting in non-specific RNA degradation and reduced protein synthesis. However, this problem was circumvented by designing 19 to 22 nucleotide siRNA sequences. Methods for siRNA delivery into cells include liposome-based addition of purified ribonucleotides to the media or transfection of plasmid vectors designed to express siRNA molecules. Plasmid vectors rely on the use of two RNA Polymerase III promoters (U6 and H1) to drive transcription of the siRNA molecule. The target sequence (19 to 29 nucleotides) is placed in a sense and antisense orientation with a small spacer group in between (short hairpin RNA or shRNA). Once transcribed, a hairpin structure is formed that can be recognized and cleaved by Dicer. Alternatively, RNA duplexes may be transcribed without hairpin structures and directly processed by the RISC. Currently, there are a variety of plasmid and viral vectors that utilize similar concepts to produce siRNA or shRNA molecules.

Following the discovery of siRNA, several studies attempted to identify the optimal characteristics required for siRNA design. Some of the requirements include using sequences shorter than 30 nucleotides to avoid PKR activation, sequence stability at the 5' end of the antisense strand relative to the 3' terminus and inserting a TT overhang. Based on studies like these, a number of algorithms have been developed by academic and industrial labs to predict the most effective target sequences for a given gene. Though most of these programs are not perfect, the likelihood of obtaining a predicted sequence is superior to designing sequences without consideration of the recommended features. Synthesis and testing of multiple sequences may be required. The design of siRNA experiments may contain some potential pitfalls, thus the design should be done to include appropriate controls and measurable endpoints. A negative control should consist of a non-complementary sequence with thermodynamically similar properties as the effective

siRNA sequence. When transfecting a plasmid vector to introduce siRNA or shRNA, the ratio of lipid to nucleic acid should be equal and the control vector should contain a sequence that is transcribed and processed intracellularly. Validation of the siRNA effect should also be done by measuring RNA and protein expression. Using the lowest possible concentration of siRNA may help avoid unwanted side effects. Off-target effects can be dose-dependent. Before you start your study, run experiments with different siRNA concentrations to determine the level of knockdown you need to see a change in phenotype.

Useful websites

Design of siRNA

http://www.ambion.com/techlib/tb/tb_506.html

<http://www.promega.com/siRNADesigner/>

<http://i.cs.hku.hk/~sirna/software/sirna.php>

<http://www.sirnazizard.com/>

Transfection

http://www.iscid.org/encyclopedia/siRNA_Transfection

<http://www.ambion.com/techlib/tn/121/9.html>

<http://www.scientistsolutions.com/t641-sirna+transfection.html>

Tips

<http://www.rnaiweb.com/>

<http://www.si-rna.com/>

<http://www.rockefeller.edu/labheads/tuschl/sirna.html>

<http://sirna.sbc.su.se/>

http://www.protocol-online.org/prot/Research_Tools/Online_Tools/SiRNA_Design/

<http://web.mit.edu/sirna/>

<http://www.in-pharmatechnologist.com/Materials-Formulation/Delivery-technology-could-speed-up-siRNA-development>

<http://biowww.net/browse-65.html>

http://cshprotocols.cshlp.org/cgi/collection/rna_interference_rnai_siRNA

<http://biotech.about.com/od/glossary/g/siRNA.htm>

Good luck

Dr Julie Williams