Analysis of mammalian gene function using small interfering RNAs

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RNA interference (RNAi) represents an evolutionary conserved cellular defense mechanism for controlling the expression of alien genes in protists, filamentous fungi, plants, animals and humans. RNAi is triggered by double-stranded RNA (dsRNA) and causes sequence-specific degradation of homologous mRNAs. The mediators of target RNA cleavage are duplexes of 21-nt small interfering RNAs (siRNAs) produced by Dicer RNAse III cleavage of long dsRNAs or RNA hairpins. Duplexes of 21-nt siRNAs with symmetric 2-nt 3' overhangs or RNA hairpins with a single 2-nt 3'-overhang are efficient triggers of RNAi in mammalian cells. siRNAs and hairpin RNAs of less than 30 base pairs do not trigger sequence-unspecific effects in mammalian cells (interferon response) and produce "knockdown" cells with characteristic "knockdown" phenotypes.

We are characterizing the human ribonucleoprotein complexes involved in RNAi and showed the participation of the human Argonaute family members, eIF2C1 and eIF2C2, in the formation of the target RNA-cleaving RNA-induced silencing complex (RISC). Active RISC contains a single-stranded antisense RNA that guides the target RNA cleavage. eIF2C2 has also been shown to be associated with microRNAs that are expressed from conserved genes that encode short hairpin RNAs. We have identified over one hundred mammalian microRNAs, and recently also recorded the miRNA profile of D. melanogaster to better understand the phylogenetic conservation of this class of genes and their respective targets. We are now developing reagents to dissect the specific roles of the various human Argonaute proteins and explore the links that point towards a possible role in mediating sequence-specific chromatin changes.

To make siRNAs as more reliable or more efficient reagents for gene silencing in target validation but also as therapeutic reagents, we have examined the variation of gene silencing as a function of positional variation and also compared different organisms and cell lines. We find cell-type dependent global effects and cell-type independent positional effects. RNase-protecting phosphorothioate and 2'-fluoro pyrimidine RNA backbone modifications of siRNAs did not significantly affect silencing efficiency, although cytotoxic effects were observed when every second phosphate of a siRNA duplex was replaced by phosphorothioate.

Synthetic RNA hairpin loops were subsequently evaluated for silencing as a function of stem-length and loop composition. As long as the 5' end of the guide strand coincided with the 5' end of the hairpin RNA, 19 to 29 base pair hairpins effectively silenced lamin A/C, but when the hairpin started with the 5' end of the sense strand, only 21 to 29 base pair hairpins were highly active. We hope that these studies enable us to make a more efficient transition from synthetic siRNAs to siRNA expression vectors for gene silencing in cases were synthetic siRNAs are more difficult to deliver than siRNAs expressed from viral vectors.