

Small RNA Activation of Gene Expression

Small RNA-mediated gene regulation through gene silencing was observed when exogenous double-stranded RNAs (dsRNAs) were introduced into *C.elegans*¹. This exogenously introduced RNA is known as a small interfering RNA (siRNA). An endogenous gene silencing process was also discovered in *C. elegans* whereby *lin-4* encodes a small RNA molecule known as a microRNA (miRNA) that is complementary to the 3' untranslated region of the *lin-14* messenger RNA (mRNA)². Collectively these two processes of gene silencing are known as RNA interference (RNAi).

The mechanism of RNAi is mediated through RNA-protein interactions. Precursor RNAs are processed by an RNase, Dicer, into mature 19 to 21-nucleotide small dsRNAs with 2-base pair overhangs at the 3' ends. For siRNA, the small RNA duplexes are assembled into an RNA-protein complex called the RNA-induced silencing complex (RISC), which contains the protein Argonaute 2 (AGO2). RISC facilitates both the interaction between the target mRNA and the complementary strand of the small RNA duplex and the cleavage of the mRNA³. In the case of miRNAs, AGO2 facilitates degradation of the target mRNA through a number of mechanisms including deadenylation and endonuclease cleavage⁴. The silencing of gene expression by small RNAs has made this an attractive therapeutic technique to treat diseases resulting from gene overexpression⁵.

Antisense RNA (agRNA) is another potential therapeutic reagent that has emerged from the study of RNAi that combines siRNA with antisense RNA. agRNAs are small duplex RNAs that are complementary to DNA. agRNAs silence genes by binding to complementary DNA sequences at the transcription start site when the two strands of DNA are separated and thus prevent transcription⁶. Corey, *et al.* demonstrated that agRNAs targeting the transcriptional start site of the progesterone receptor (PR) can potently silence the expression of PR⁷. Surprisingly, when the target site for agRNAs within the PR promoter was moved enhanced gene expression was actually observed⁸. Similarly, enhanced gene expression was also observed by Li, *et al.*, when attempting to silence the E-cadherin, p21 and VEGF genes using an agRNA approach⁹. This observed process of RNA activation, RNAa, was sequence specific, independent of the interferon response and had slower kinetics than RNAi^{8,9}. **This leads to the hypothesis that small RNA-mediated gene regulation is not limited to gene silencing, but also includes enhanced gene expression through transcriptional activation affecting promoter activity.**

To elucidate the mechanism of RNAa, Li, *et al.* examined possible roles for pre-miRNA, Dicer and AGO2¹⁰. They verified that RNAa can, in fact, occur through miRNAs and requires both Dicer and AGO2. Corey, *et al.* used both chromatin and RNA immunoprecipitation assays to examine the interactions that occur at the promoter between RNA, DNA and proteins¹¹. They observed changes in protein, RNA and DNA interactions at the PR promoter that affected promoter activity to cause enhanced gene expression.

The ability of small RNA duplexes to enhance gene expression makes them attractive potential therapeutics for treating diseases such as cancer. The possibility of RNAa as a therapeutic agent to enhance p21 expression has shown promise in bladder cancer¹². However, the exact mechanism of RNAa still needs to be completely elucidated.

References

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