Catch and Release: Interactions of the Viral RNA Silencing Suppressor (p19) with Human RISC Complexes

Renata A. Rawlings

Short Abstract — **In response to viral infection, eukaryotes can activate the RNA interference (RNAi) pathway initiated by the RNase III enzyme Dicer that cleaves stretches of exposed viral genomes into small interfering RNAs (siRNAs). As a countermeasure, many viruses have evolved viral-derived suppressors of RNAi (VSRs) that tightly bind siRNAs to thwart RNAi-mediated degradation. Using fluorescence quenching and electrophoretic mobility shift assays, we probe siRNA binding by the dimeric VSR (p19). We find that the siRNA:p19 interaction is reversible and efficiently competes with recombinant human Dicer to inhibit formation of RNAi-related assembly complexes. Based on these results, the possible modes of Dicer:p19 competition were examined computationally, revealing evidence of a ternary complex between siRNA, p19, and human Dicer. The effects of such a complex and the observed "catch and release" of siRNAs by p19 was monitored by a time-dependent RNAi model that incorporates the effect of p19 on the mRNA levels of viral-derived gene therapy vectors. From our experimentation and quantitative modeling we can postulate fundamental principles for the optimization of p19 in conjunction with RNAi-based techniques and therapeutics, and anticipate these results to be relevant for the further development of p19 as an RNAi suppressor to assist viral-derived gene therapy vectors.**

Keywords — **RNA Interference, Gene Therapy, VSR , p19.**

I. Purpose

Although RNAi is an innate immune response, it has become most extensively used as a tool for functional genomics [1], gene therapy [2-3], vaccine production [4] and therapeutics [5]. For such applications, viruses offer a natural model system for regulating RNAi efficiency and identifying points of further development. The VSR p19 has been shown to aid in the over-expression of gene therapy vector proteins [6], yet questions remain about the precise mechanism of RNAi suppression. We find quantitative evidence of a ternary complex between siRNA, p19, and human Dicer. Additionally, we model the effect of p19 on the mRNA levels of viral-derived gene therapy vectors and find p19 to be tunable and able to increase either the amount of mRNA production or the length of the viral replication phase. Our model predicts p19 to be time invariant and able to reinitiate viral replication after steady-state suppression has been reached. Taken together we can postulate optimization strategies for the use of p19 in conjunction with RNAi-based techniques.

II. Results

From our model we can predict the following principles for optimal use of p19 in tuning RNAi regulation.

A. *Proximity*

p19 expressed close to the source of infection maximizes the benefit of siRNA shuttling through its ternary interaction with Dicer.

B. *Increased concentration*

Higher p19 concentrations produce increased viral mRNA production, longer rising phases and much slower suppression, increasing the observed duration of infection.

C. *Timing of Incubation*

The time incubated before harvesting cells can significantly affect yield. Optimal incubation times scale linearly with the effective concentration of p19 in the cell.

D. *Timing of Introduction*

Addition of p19 before full suppression of infection increases the rising phase of mRNA production and the time needed to reach peak mRNA levels. Late introduction of p19 is observed to reinitiate mRNA accumulation even after equilibrium has been reached.

III. Conclusion

We see that p19 can significantly increase the yield of a viral-derived gene therapy vector, under optimal conditions, and exhibits time invariant reinitiation of viral replication.

References

[1] Belles X. "Beyond Drosophila: RNAi in vivo and functional genomics in insects." *Annu Rev Entomol* **55**,111-128.

[2] Barichievy S, *et al*. (2009) "RNA interference-based gene expression strategies aimed at sustained therapeutic inhibition of HIV." *Curr Top Med Chem* **9** (12),1065-1078.

[3] Schnettler E*, et al.* (2009) "The NS3 protein of rice hoja blanca virus complements the RNAi suppressor function of HIV-1 Tat." *EMBO Rep* **10** (3), 258-263.

[4] Zheng N*, et al.* (2009) "Boosted expression of the SARS-CoV nucleocapsid protein in tobacco and its immunogenicity in mice." *Vaccine* **27** (36), 5001-5007.

[5] Tiemann K & Rossi JJ. (2009) RNAi-based therapeutics-current status, challenges and prospects." *EMBO Mol Med* **1** (3), 142-151.