Saturation and competition drive siRNA and microRNA activity: It's more than the binding sites

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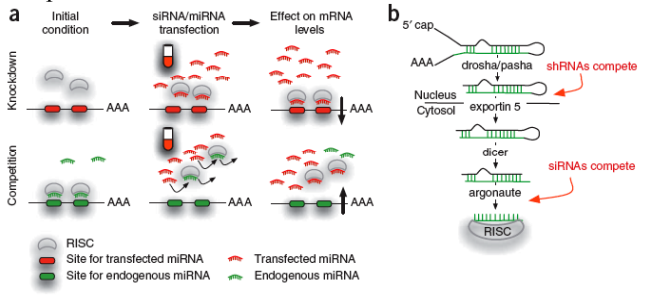
Abstract

Post-transcriptional regulation by microRNAs and siRNAs will depend on systems-level properties, as well as characteristics of individual binding sites in target mRNA molecules. *Saturation* of protein machinery in the RNAi pathway may affect both siRNA and microRNA targeting. By analyzing hundreds of experiments, we show that targets of *endogenous* miRNAs are expressed at significantly higher levels after transfection. *Competition* between different mRNAs for the microRNAs or siRNAs could, according to basic chemical kinetics, affect the targeting quantitatively causing crosstalk between different mRNAs. We show that the number of transcripts with predicted sites for a microRNA/siRNA inversely correlates with the amount of down regulation.

Keywords **— microRNA, siRNA, systems biology, bioinformatics.**

Saturation: unexpected *up*regulation of genes after RNAi

Transfection of small RNAs (such as small interfering RNAs (siRNAs) and microRNAs (miRNAs)) into cells typically lowers expression of many genes. Unexpectedly, increased expression of genes also occurs. We investigated whether this upregulation results from a saturation effect—that is, competition among the transfected small RNAs and the endogenous pool of miRNAs for the intracellular machinery that processes small RNAs.



To test this hypothesis, we analyzed genome-wide transcript responses from 151 published transfection experiments in seven different human cell types. We show that targets of endogenous miRNAs are expressed at significantly higher levels after transfection, consistent with impaired effectiveness of endogenous miRNA repression. (1) This effect exhibited concentration and temporal dependence. Notably, the profile of endogenous miRNAs can be largely inferred by correlating miRNA sites with gene expression changes after transfections. .

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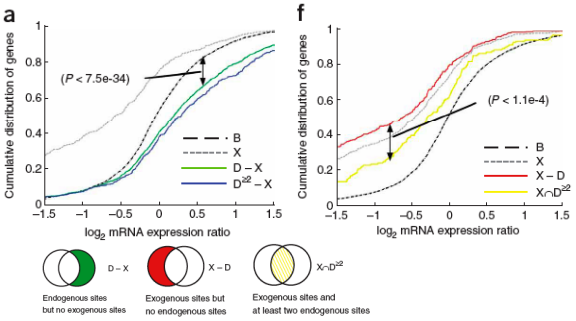
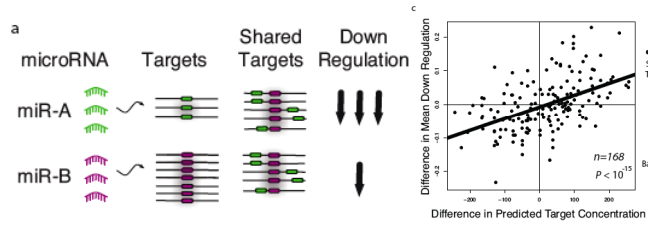


Target abundance dilutes microRNA and siRNA activity

Simple chemical kinetics predicts that the level of microRNA regulation will depend upon concentration of mRNA transcripts with target sites in the cell; that is, target abundance acts as a rate-limiting step in degrading target transcripts. To test this we analyze 143 microRNA and siRNA transfection experiments and show that down-regulation by miRNAs and siRNAs depends on total target mRNA abundance. Comparing pairs of miRNAs with high and low target abundance, we show that similar sites can result in very different amount of regulation as a result of differential target abundance.(2)



Conclusions

The paradigm of microRNA and siRNA targeting should shift away from the simple discretization of "target" or "not a target" and towards a more quantitative framework. The competition and saturation effects have practical implications for miRNA target prediction, the design of siRNA and shorthairpin RNA(shRNA) genomic screens and siRNA therapeutics

References

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