Timing matters

Towards a kinetic model of miRNA-mediated gene silencing

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***Short Abstract* — MicroRNAs are post-transcriptional silencers of gene expression. Though the understanding of molecular mechanism of microRNA regulation has increased over the recent years, little is known about the precise kinetics of the process. We introduce a quantitative model of miRNA regulation and apply our model to the microRNA target identification problem.**

I. Background

MicroRNA (miRNAs) are short, evolutionarily conserved, non-coding RNAs that regulate gene expression at the post-transcriptional level. Biochemical studies suggest that miRNA regulation proceeds in two steps: first, the interaction between the miRNA and its target mRNA results in a rapid inhibition of translation initiation, while subsequent decapping and deadenylation lead to an increase in the mRNA degradation rate [1]. Together, these two processes lead to a decreased level of the protein encoded by the target mRNA. The kinetics and magnitude of miRNA regulation are poorly understood, but they are critical for biological processes regulated by a miRNA whose expression is changing in time, such as pattern formation in development [2], clearance of maternal mRNAs during embryogenesis [3], or more artificial settings such as experiments aimed at identifying genes regulated by a miRNA. Such experiments measure changes in mRNA [4] or protein [5] levels induced by transiently perturbing miRNA expression, and then analyze these changes under steady-state assumptions, leading to results that are difficult to interpret. For instance, we have previously shown that properties such as the structural accessibility of miRNA binding sites, the extent of complementarity to the miRNA seed, the AU content of the mRNA and the location of the site within the 3'UTR all influence the efficiency of miRNA-induced mRNA degradation. While the same properties describe miRNA binding sites under evolutionary selective pressure, they surprisingly fail to characterize sites leading to protein down-regulation in a miRNA over-expression experiment [6].

II. Results

Here, we hypothesize that this apparent discrepancy may be due to the kinetic aspects of miRNA regulation. We first show that the changes inprotein levels resulting from perturbing the expression of a miRNA cannot be analyzed under steady-state assumption. We then introduce an ordinary differential equation model of miRNA action that summarizes themolecular mechanism of miRNA-mediated gene regulation. The model is parameterized by 16 reaction rates, some of which we estimated from published biophysics experimental data [7], and which we are currently attempting to estimate precisely by performing time-series measurements of mRNA and protein levels of a luciferase reporter following the induction of a cognate miRNA. Initial exploration of the behavior of the model indicates that the estimated rates are consistent with the timing of a miRNA perturbation experiment. It also predicts that the protein levels of genes regulated by the perturbed miRNA should be more affected than the mRNA levels. Sensitivity analysis further shows that the rates characterizing different reactions may control very diverse aspects of the miRNA regulation kinetics and magnitude. Finally, it suggests that two gene-dependent parameters – the miRNA-target mRNA binding rate and the half-life of the encoded protein – may explain why the properties that characterize functional miRNA binding site fail to describe sites leading to protein down-regulation.

III. Conclusion

We show that the kinetics of miRNA-mediated gene silencing must be taken into account when designing and analyzing experiments aiming at identifying miRNA targets, and propose an explanation for why properties that describe functional miRNA binding sites appear not to characterize sites that cause protein down-regulation.

References

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