MATHEMATICAL MODELING AND ANALYSIS EXPLAINS ABBRENT POSITIVE EFFECTS OF MICRO-RNA ON TARGET MRNA

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Abstract

Proteins are formed using messenger RNA (mRNA) as templates. Micro-RNA (miRNA) are small RNA molecules that bind to target mRNA strands. One miRNA can bind to several mRNA and each mRNA can be bound by several miRNA. The net result of this binding is a reduction in the concentration of the protein formed. Hence under most experimentally-studied conditions, depletion in the miRNA results in an increase in the target protein concentration, and an increase in miRNA formation results in a reduction in target protein concentration. However, in several experimental studies it has been demonstrated that some miRNA-target pairs show seemingly aberrant results: the target protein levels are positively correlated with miRNA levels. Through a mathematical model of the reactions governing miRNA and mRNA formation, interaction, and catalytic protein production, we seek to provide testable hypotheses for the mechanism of such aberrant effects. We show that when modeled as a one-miRNA-one-target interaction, a set of dimensionless numbers are sufficient to establish the conditions when such aberrant effects may be seen. We also show that when modeled as a two-miRNA-one-target interaction, such parameter constraints are not required to observe aberrant effects, and that the apparent positive regulation of targets by miRNA could be an artifact resulting from competition for mRNA. We discuss how these results can be generalized to competing catalyst poisons.

Keywords
microRNA, messenger RNA, protein expression model, dimensionless numbers, competition, post-transcriptional regulation, catalyst poison.

Introduction

Gene expression is highly regulated in order to accurately control the spatiotemporal concentrations of proteins in cells. Small RNAs such as microRNAs (miRNA) have been identified as important regulators of their target proteins. miRNA act by binding to complementary or partially complementary sites on target messenger RNA (mRNA). About 60% of expressed mRNA have binding sites for one or more miRNAs (van Rooij, 2011).

The predominant action of miRNA on its targets is destabilization of target transcripts (Guo et al. 2010), through multiple mechanisms including accelerated degradation of mRNA. miRNA activity has been termed as catalytic as one miRNA has a nonstoichiometric effect on target strands due to selective degradation of the target mRNA strand from the complex (Baccarini et al. 2011).

The role of miRNA in regulation of gene expression has been thought to be repressive in action, with decreased target transcript concentrations resulting from miRNA activity. Recent reports (Vasudevan, 2012) demonstrate an unexpected positive role of miRNA. Overexpression of miRNA in such cases leads to increased expression of its target, and, conversely, inhibition of miRNA causes decrease in translational levels of its target.

Since biological regulation often involves complex regulation, it is possible that these unexpected effects are due to such feedbacks. For instance a target of an miRNA may regulates another target negatively. Such an incoherent regulation may lead to the observed unexpected effects when one regulator (miRNA) is perturbed. We explore the possibility of observing such effects using only the known facts about miRNA effects. We show that there exists a operating range defined by four dimensionless numbers where such effects are to be expected. These include some infrequently observed interactions, such as when miRNA binding stabilizes the transcript even though it decreases the translation efficiency. We show that even when individual effects are intuitive, (apparent) unexpected positive effects may result from the multiple competing nonlinear effects of miRNA on common targets. We discuss the implications of this work on the study of competing catalyst poisons.

A model for regulation by miRNA

We develop a model that includes miRNA and target expression, miRNA-mRNA binding, and the effect of such binding on degradation and translation rates (Figure 1). All the reactions are assumed to follow mass-action kinetics. mRNA formation is assumed to be unregulated (zero-order) and the catalyst (mRNA) effect is assumed to be first-order. ODEs are formulated and solved either analytically using Mathematica; or integrated using Matlab and the result compared to the analytical solution.

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from the effect of interaction and competition. We also show that this apparent positive effect is not observed when the miRNA interact noncompetitively with their common target (Nyayanit and Gadgil, 2015).

Results

We first consider the interaction of one miRNA with one mRNA, by setting the initial concentration and formation rate of the second miRNA to zero. This leads to a system of four differential equations with 12 reaction rate parameters. We express the ratio (r) of the steady state protein concentration scaled to the unregulated or no-miRNA concentration (r=p/p_{rel}) in terms of the parameters. We reformulate the equations in terms of four dimensionless numbers, and show that r can be expressed in terms of these four dimensionless numbers (Gokhale and Gadgil, 2012). Figure 2 is an operating diagram for the relative protein ratio, where positive values of the log-scaled ratio indicate regions where the unexpected effect will be observed.

![Figure 1. Reaction model for regulation by miRNA. Rate constants are in italics and species in bold. Unfilled arrows depict catalytic formation of protein from bound and free mRNA, dashed arrows represent nonstoichiometric degradation/return.](image)

![Figure 2. An operating diagram showing regions (in green) where unexpected effects are predicted, as a function of four dimensionless numbers (a-d).](image)

We next explore whether such positive effects of individual miRNA are required. We use parameters such that the effect of a single miRNA on its target is negative when there is no competition from another miRNA. We identify conditions (Fig 3) for a 2miRNA–1mRNA system where increase in one miRNA concentration has a positive effect on the concentrations of the target, thus establishing that such an observation of an unexpected effect may not always imply any inherent positive effect of the miRNA on the target, but rather could be an observation resulting

![Figure 3: Simulations showing unexpected miRNA effects (top four lines) where protein concentration increases with increase in miRNA formation rate, even when the individual effect of the miRNA is negative.](image)

Discussion

The ‘beauty’ of dimensionless numbers has been appreciated (Aris, 1997) and employed by reaction engineers and mathematical biologists (Stahl, 1961) for many decades. Here, we present an example where dimensionless numbers are used to construct an operating diagram for miRNA regulation, specifying the regimes where expected (product protein concentration is inversely related to the formation rate of the catalyst poison miRNA) and unexpected effects can be observed. We further show that even in the ‘red zone’ parameter range, where individual miRNA repress target proteins, unexpected effects are possible due to the effect of competition by other miRNA with different binding and poisoning capabilities. We are in the process of extending this to a general study of catalytic process kinetics in the presence of multiple poisons. We thus demonstrate the use of chemical reaction engineering principles to analyze and shed light on seemingly unintuitive intracellular processes.

References


Nyayanit, D., and Gadgil, C. J. (2015). Mathematical modelling of combinatorial regulation suggests that apparent positive regulation of targets by miRNA could be an artifact resulting from competition for mRNA. RNA, 21, 307

