MicroRNAs generate gene expression thresholds with ultrasensitive transitions

Shankar Mukherji[1,*], Margaret S. Ebert[2,3,*], Grace X. Zheng[2,4], John S. Tsang[5], Phillip A. Sharp[2,3], and Alexander van Oudenaarden[2,6,#]

***Short Abstract* — MicroRNAs are an abundant class of post-transcriptional regulator whose repressive effects are thought to have broad reach in terms of number of targets but modest effect for any given target. In this study we performed a single-cell analysis of the effects of microRNA-mediated repression. We observe that microRNAs generate gene expression thresholds by which target protein production is robustly silenced below a given transcriptional activity and is activated in an ultrasensitive fashion as the transcriptional activity is increased. The threshold can be tuned by modulating the microRNA concentration and the number of microRNA binding sites in the target 3'-UTR.**

***Keywords* — microRNA, gene expression, post-transcriptional regulation**

I. Purpose

MicroRNAs (miRNAs) are short, highly conserved non-coding RNA molecules that repress gene expression in a sequence-dependent manner. Each miRNA is predicted to target hundreds of genes [1-4], and a majority of protein-coding genes are predicted to be miRNA targets [4,5]. Bulk measurements on populations of cells have indicated that, although pervasive, repression due to miRNAs is on average quite modest (~2-fold) [2,3,6]. Information on the magnitude of repression in single cells, however, has been lacking. Here we perform single-cell measurements using quantitative fluorescence microscopy and flow cytometry to monitor a target gene's protein expression in the presence and absence of regulation by miRNA. We find that while the average level of repression is modest and in agreement with previous population-based measurements, the repression among individual cells varies dramatically. In particular, we show that regulation by miRNAs establishes a threshold level of target mRNA below which protein production is highly repressed. Beyond this threshold, there is a regime in which expression responds ultrasensitively to target mRNA input until reaching high enough mRNA levels to almost escape repression by miRNA. We constructed a mathematical model, similar to models used to describe small RNA (sRNA) reguation in bacteria [7] and protein-protein titration effects [8,9], describing repression of target gene expression by both non-catalytic and catalytic activity of miRNA. The model predicted, and experiments confirmed, that the ultrasensitive regime could be shifted to higher target mRNA levels by transfecting additional miRNA or by increasing the number of miRNA binding sites in the 3' UTR of the target mRNA. The ultrasensitive transition is not observed when the miRNA targets a perfectly complementary site that can undergo catalytic cleavage. These results demonstrate that even a single species of miRNA can act both as a switch to effectively silence gene expression and as a fine-tuner of gene expression.

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[1]Harvard-MIT Division of Health Sciences and Technology, E-mail: mukherji@mit.edu, [2]MIT Department of Biology, [3]Koch Institute for Integrative Cancer Resarch, [4]MIT Computational and Systems Biology Graduate Program, [5]MIT Department of Physics, [6]Harvard Biophysics Graduate Program, [*]These authors equally contributed, [#]To whom correspondence should be addressed: avano@mit.edu

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