Target-Specific and Global Effectors in Gene Regulation by MicroRNA

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ABSTRACT MicroRNAs are responsible for post-transcriptional gene silencing as part of critical cellular pathways and intercellular coordination, for example during embryonic development. Yet, the basic mechanism by which this silencing is accomplished is still not understood. For example, it is not known to what extent and through what process does the suppression of protein accumulation accompany a reduction in mRNA level. Here we present a simple quantitative modeling approach to microRNA mediated silencing. We show how differential responses of the mRNA- and protein levels may be tuned by target-specific parameters and how global effectors may alter this behavior for some—but not all—miRNA targets in the cell.

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MicroRNAs (miRNAs) are endogenous small RNA molecules that regulate genes post-transcriptionally through specific basepairing with messenger RNAs. In recent years, miRNAs in flies, fish, worms, and humans have been shown to be involved in pathways of development, programmed cell death, and cancer. In all known cases, microRNAs silence a target gene or, more often, a set of target genes. (For reviews, see, e.g., (1,2).)

While evidence for the functional roles of miRNA keeps accumulating, the mechanism by which gene silencing is achieved has remained elusive (3,4). Early reports suggested that unlike the reduced protein level, the level of polysomes is not affected by microRNAs, as long as the miRNA-mRNA basepairing is imperfect. This is in contrast with the RNAi pathway, in which small interfering RNA (siRNA) molecules bind mRNA targets through perfect basepairing, directly promoting cleavage (5). However, recent findings suggest that this idea may be oversimplified (6–8). For example, a recent set of reports presents contradictory results even for a specific miRNA (9–11).

Although the detailed mechanism is as yet unknown, evidence point in favor of a two-step model, where binding of miRNA to the mRNA promotes a secondary process (e.g., ribosome runoff or deadenylation) which ultimately leads to mRNA accumulation in its processed state, perhaps in specific cellular structures such as processing bodies or stress granules (12,13). The existence of the second step suggests that some parts of this mechanism (affecting the transition from bound to processed state) are controlled by cellular components in a global fashion, obeying the same dynamical rules for all miRNA regulated targets. Here we use a modeling approach to compare target-specific versus global contributions to different observables, focusing on the differential effects on mRNA and protein levels.

We assume that the dynamical variables are three different mRNA concentrations: free mRNA denoted by m; bound miRNA-mRNA, denoted by m^*; processed mRNA, denoted by m^{**}; and the free miRNA concentration, given by s. Each of these states has a fixed interaction with other pieces of cellular machinery (such as ribosomes, degradation enzymes, etc.) and hence can be characterized by a set of reaction parameters (governing protein production rates, decay, etc.) from that state. Binding (unbinding) of a free mRNA to a miRNA occurs with rate κ_+ (κ_-); η_+ (η_-) are the transition rate to (from) the processed state; and three λ-rates define degradation of the mRNA at its different states. This formulation leads directly to the mass-action equations

$$\begin{aligned}
\frac{dm}{dt} &= \alpha_{\mathrm{m}} + (\kappa_- m^* - \kappa_+ sm) - \lambda_{\mathrm{m}} m \\
\frac{dm^*}{dt} &= (\kappa_+ sm - \kappa_- m^*) + (\eta_- m^{**} - \eta_+ m^*) - \lambda^*_{\mathrm{m}} m^* \\
\frac{dm^{**}}{dt} &= (\eta_+ m^* - \eta_- m^{**}) - \lambda^{**}_{\mathrm{m}} m^{**} \\
\frac{ds}{dt} &= \alpha_{\mathrm{s}} - \lambda_{\mathrm{s}} s + (\kappa_- m^* - \kappa_+ sm) + (1-q)\lambda^{**}_{\mathrm{m}} m^{**} \\
&\quad + \lambda^*_{\mathrm{m}} m^*. \qquad (1)
\end{aligned}$$

Here the α-terms account for synthesis of the RNA species. For simplicity, we assume here that proteins are produced (at equal rates) in both the free and bound states, but not at all in the processed one. Of particular importance is the parameter q which accounts for the probability for a miRNA to be co-degraded with the mRNA in the processed state. In the limit $\eta_- = 0$, this model can be also applicable for silencing by siRNA through direct cleavage of the message.

Key to our analysis is the assumption that the binding and degradation rates, $\kappa_\pm$ and λ, are specific to the mRNA-miRNA pair, whereas the transition rates are global, the same for all complexes in a given cell. Underlying this assumption is accumulating evidence suggesting that the transition to the processed

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state is a multistep process, which involves many cellular components (3,4). The availability of these components, and thus the rates they infer, are likely to be condition-dependent.

Differential effects of miRNA on protein- and mRNA-levels may be controlled by specific and global effectors

Let us first focus on the case when miRNAs are extremely abundant in the cell. Here, there are no free mRNAs and one readily finds that the ratio of bound mRNA in the unprocessed to processed states is just $\theta \equiv (\lambda_m^{**}+\eta_-)/\eta_+$. Given this ratio, the fold of reduction in mRNA and protein level is given, respectively, by

$$w_M = \lambda_m / \left(\frac{\theta\lambda_m^{*}+\lambda_m^{**}}{\theta+1}\right), \quad w_P = w_M\left(\frac{\theta}{1+\theta}\right). \qquad (2)$$

The term in brackets in w_M is a weighted degradation rate, and the one in w_P is a renormalized translation rate. In these expressions, the only contribution of global parameters comes in via θ.

Very different effects on mRNA and protein levels are expected for those targets with small θ, namely those that spend significant time in the processed state. This requires that cellular conditions would set $\eta_- \leq \eta_+$. Still, even under such conditions, distinct effects on mRNA and protein levels would only occur for those targets for which λ_m^{**} is at most comparable with η_+.

In contrast, for targets characterized by a large θ, the levels of protein and mRNA are equally repressed ($w_M \approx w_P$). For those targets which are efficiently degraded in the processed state, $\lambda_m^{**} \gg \eta_+$, the degradation rate of mRNA is effectively replaced by the global parameter η_+. Conversely, if a large value of θ is only the result of the ratio between global parameters, then $w_M \approx \lambda_m/\lambda_m^{*}$, which is not expected to be large.

It is therefore possible that the same miRNA would strongly affect the mRNA level of one target but not on that of another; similarly, the same miRNA-target pair may exhibit different behavior under different cellular conditions (Fig. 1 *A*). This enables the cell to accomplish disparate goals. For example, preventing inadvertent fluctuations from producing protein in cells that are to be permanently silenced would necessitate removing the mRNA; keeping a gene off in a state that would allow rapid switching on would best be accomplished by keeping mRNA high and obviating the need for new transcription.

Our prediction can easily be tested on a global scale, by comparing the effect of endogenously introduced miRNA on the level of mRNA (using DNA microarray) and on the level of proteins (e.g., using protein microarray) under different conditions. Under stressful conditions, processing bodies accumulate, and one expects an increased η_+. One would then be able to identify target which show ''inconsistent'' behavior, such as the black target in Fig. 1 *A*.

Many target mRNAs have multiple binding sites for a specific miRNA. Within our model, this number should affect the miRNA binding rates (κ) but not the transition rates (η); the latter are probably dominated by transport, not by reactions. From Eq. 2 we therefore find that the number of binding sites can only affect the strength of repression if the rate of mRNA degradation at the processed state λ_n^{**} is influenced by the number n of bound miRNA. Now global parameters appear through a set of values $\theta_n \equiv (\lambda_n^{**}+\eta_-)/\eta_+$. A suggestive interpretation of the results is through an effective parameter θ_{eff}, which changes, as the miRNA concentration increases, from one θ_n to the next. This can have some interesting consequences, as we have seen, e.g., that θ is what controls the ratio of protein to mRNA suppression (Fig. 1 *B*). In general, since θ_{eff} determines the effect of global parameters on the behavior of a given target, this behavior may change with the miRNA level.

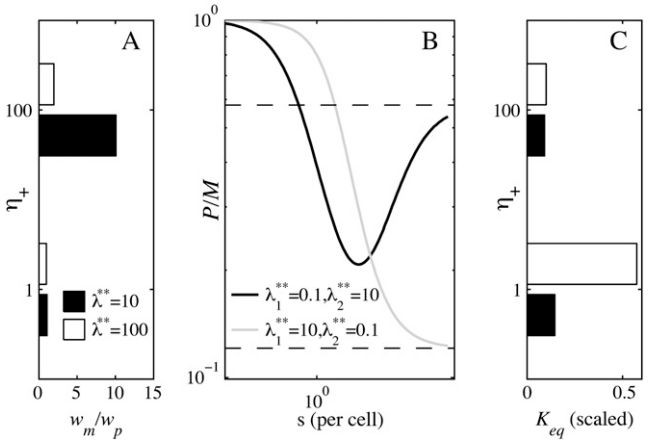

FIGURE 1 Global and target-specific effectors can interplay to alter the response of a target gene to miRNA. (*A*) The ratio between fold-change in mRNA level, w_M, and fold-change in protein level, w_M, for two targets (*black* and *white*) of different degradation rates. As the cellular conditions change to make the processing rate η_+ more efficient, a target with small λ_m^{**} has its mRNA level unaltered while protein level is strongly repressed, whereas a target with large λ_m^{**} experiences similar repression in both mRNA and protein level. (*B*) For a target with two binding sites, the ratio proteins/mRNA may differ as the abundance of miRNA changes. Dashed lines are the ratios w_M/w_P using λ_m^{**} = 0.1 (*bottom*) or λ_m^{**} = 10 (*top*). (*C*) The equilibrium constant characterizing the level of miRNA at the onset of repression can be changed significantly by global factors for a target with highly unstable in the processed state but not for a target which is relatively stable (large and small λ_m^{**}, respectively). To ease the presentation K_{eq} of the second target is scaled-down by a factor 10. Unless noted otherwise, we set (arbitrarily, and thus with no specifying units) $\lambda = \lambda^{*} = \lambda^{**}/10$, $\eta_- = \eta_+/8 = 1$, and $\kappa_+ = \kappa_-/10 = 10$.

Global and specific signals determine equilibrium constants for miRNA that act catalytically

The limit $q = 0$ is the case where miRNAs act as enzymes to catalyze the suppression of free mRNA. This has typically been shown to be the case for siRNA in the RNAi pathway (14). In this case, the free-mRNA concentration at a given miRNA concentration s is $m = \alpha_m/\lambda_m[1 + w_M(s/K_{eq})]/[1 + (s/K_{eq})]$, with

$$K_{\mathrm{eq}} = \frac{\theta(\kappa_- + \lambda_{\mathrm{m}}^*) + \lambda_{\mathrm{m}}^{**}}{\kappa_+} \frac{\lambda_{\mathrm{m}}}{\theta\lambda_{\mathrm{m}}^* + \lambda_{\mathrm{m}}^{**}}, \quad (3)$$

which sets the value of miRNA concentration at the onset of repression. Again, target-specific rates are compared with global ones (through θ) to determine the value of K_{eq} (Fig. 1 *B*). Thus, the responsiveness of a given target to a miRNA can be changed under different cellular conditions.

Unlike what occurs for transcriptional regulation, here the equilibrium constant and the strength of repression (w_{M}) are not independent. In both cases, however, this factor is independent of the transcription rate of the target mRNA, and specifically of the number of copies of its genes. This is in contrast to observations made in Doench and Sharp (15), and to the stoichiometric mode, as discussed below.

In a noncatalytic mode of action, target synthesis rate is another specific signal that determines miRNA efficacy

The more general case $q > 0$ allows for the possibility that cleavage of an mRNA molecule in the processed state is accompanied by turnover of the bound miRNA. This scenario is motivated by the fact that the processed state may be thought of as a localization to a cytoplasmic body, which is enriched in ribonucleatic agents. Moreover, the $q = 0$ steady-state limit is only valid if all the relevant timescales (such as, e.g., escape from the cytoplasmic body) are shorter than the biologically relevant time. It is instructive to solve first the equations for nonfree mRNA in Eq. 1, yielding

$$\begin{aligned} 0 &= \alpha_{\mathrm{m}} - \lambda_{\mathrm{m}} m - (\lambda_{\mathrm{m}}/K_{\mathrm{eq}}) sm \\ 0 &= \alpha_{\mu} - \lambda_{\mathrm{s}} s - Q(\lambda_{\mathrm{m}}/K_{\mathrm{eq}}) sm, \end{aligned} \quad (4)$$

where $Q = q\lambda_{\mathrm{m}}^{**}/(\lambda_{\mathrm{m}}^{**} + \theta\lambda_{\mathrm{m}})$. All global parameters appear here through K_{eq} and Q. This form of the model reveals an interesting symmetry between free mRNA and free miRNA steady-state pools. A model of this form has been introduced for a class of bacterial small RNA that may cleave along with their mRNA target (16,17).

The steady-state mRNA concentration is given now by $m = [(Q\alpha_{\mathrm{m}} - \alpha_{\mathrm{s}} - \varepsilon) + \sqrt{(Q\alpha_{\mathrm{m}} - \alpha_{\mathrm{s}} + \varepsilon)^2 + 4\alpha_{\mathrm{m}}\varepsilon}]/(2Q\lambda_{\mathrm{m}})$, with $\varepsilon = \lambda_{\mathrm{s}}/K_{\mathrm{eq}}$. Expecting ε to be small compared with other rates, silencing is accomplished when the miRNA synthesis rate α_{s} exceeds $Q\alpha_{\mathrm{m}} + \varepsilon$, reflecting a competition between the miRNA synthesis and the rescaled mRNA transcription (16,17). Thus, the synthesis rate of miRNA determines the repression strength, whereas the steady-state concentration of free miRNA determines the sharpness of the transition. In contrast to the catalytic case, here the fold of repression mediated by a given miRNA transcription rate depends strongly on the target mRNA amount, as suggested by Doench and Sharp (15).

In some organisms (e.g., plants and nematodes), RNA interference is accompanied by amplification of the siRNA population (18). This amplification is the result of synthesis of siRNAs initiated by binding of an siRNA to its target. Within our model, siRNA amplification can be modeled by having $q < 0$. The significance of miRNA amplification is most transparent when $|Q| > \varepsilon/\alpha_{\mathrm{m}}$. Now, the repression is finite even for small α_{s}, indeed it is finite in the limit $\alpha_{\mathrm{s}} \to 0^+$, where it takes the value $\varepsilon/(|Q|\alpha)$.

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