Supplementary Information

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Expression for steady state protein in the presence of miRNA

The concentration of free mRNA increases because of its de novo formation (at rate k_1), due to dissociation of the complex (at a rate $k_4 \times mmi$), and due to the return of undegraded mRNA from the P-body (at a rate $q_1 \times k_9 \times mmi$). The concentration of free mRNA decreases because of its degradation (at a rate $k_7 \times m$) and also when it binds to miRNA and the complex is formed (at a rate $k_3 \times m \times mi$). The net rate of change of the concentration $d(m)/dt$ is given by the difference in the rates of increase and decrease, as represented in (1.1). The differential equations for the other species are also written down based on such a mass balance relating the change in concentration to the rates of the reactions that contribute to an increase or decrease in the concentration of that species.

The differential equations (as given in main text, equations 7-10) are,

$$\frac{d(m)}{dt} = k_1 - k_3 \times m \times mi + k_4 \times mmi - k_7 \times m + q_1 \times k_9 \times mmi \tag{1.1}$$

$$\frac{d(mi)}{dt} = k_2 - k_3 \times m \times mi + k_4 \times mmi - k_8 \times mi + q_2 \times k_9 \times mmi \tag{1.2}$$

$$\frac{d(mmi)}{dt} = k_3 \times m \times mi - k_4 \times mmi - k_9 \times mmi \tag{1.3}$$

$$\frac{d(p)}{dt} = k_5 \times m + k_6 \times mmi - k_{10} \times p \tag{1.4}$$

The above equations were non-dimensionalized using $1/k_7$ as reference time and k_1/k_7 as reference concentration. Parameters κ_i are corresponding dimensionless parameters. The quantities, *m, mi, mmi,* and *p* hereafter represent these scaled concentrations.

The above equations (1.1-1.4) were solved for a steady state, i.e. by equating each differential equation to zero. The steady state expression for *m* is given as,

$$m_{ss-mir} = \frac{2}{1 + \chi + \sqrt{1 + \chi^2 + 2\delta}} \tag{1.5}$$

Where, $\chi = \dfrac{\kappa_3 \kappa_9 (\kappa_2(1-q_1) - 1(1-q_2))}{\kappa_8(\kappa_4 + \kappa_9)}$ and $\delta = \dfrac{\kappa_3 \kappa_9 (\kappa_2(1-q_1) + 1(1-q_2))}{\kappa_8(\kappa_4 + \kappa_9)}$ $\tag{1.6}$

The reaction rate parameters were arranged into three dimensionless numbers. These numbers are given as,

$$a = \kappa_2(1-q_1), \quad b = (1-q_2), \quad c = \frac{\kappa_3 \kappa_9}{\kappa_8(\kappa_4 + \kappa_9)} \tag{1.7}$$

Using Eqn. 1.3, we get steady state expression for *mmi*,

$$mmi_{ss-mir} = \left(\frac{\kappa_3}{\kappa_4 + \kappa_9}\right) \times m_{ss-mir} \times mi_{ss-mir} \tag{1.8}$$

Substituting the above Eqn. 1.8 in Eqn. 1.2, the steady state expression for *mi* is given as,

$$mi_{ss-mir} = \frac{\kappa_2}{\kappa_3 \times m_{ss-mir} - \kappa_4 \left(\dfrac{\kappa_3}{\kappa_4 + \kappa_9}\right) \times m_{ss-mir} + \kappa_8 - q_2\kappa_9\left(\dfrac{\kappa_3}{\kappa_4 + \kappa_9}\right) \times m_{ss-mir}} \tag{1.9}$$

Using Eqn. 1.4 and the above expressions for *mmi* and *mi*, Eqn. 1.8 and 1.9, the expression for steady state protein is given as,

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$$p_{ss-mir} = \frac{\kappa_5 \times m_{ss-mir} + \kappa_6 \left(\dfrac{\kappa_3}{\kappa_4 + \kappa_9}\right) \times m_{ss-mir} \times mi_{ss-mir}}{\kappa_{10}}$$

(1.10)

Simplifying the above expression (Eqn. 1.10), using Eqn. 1.5, 1.8 and 1.9, the expression for protein in terms of reaction rate parameters is given as,

$$P_{ss-mir} = \frac{2\kappa_5 + \dfrac{2\kappa_2\kappa_3\kappa_6}{\kappa_8(\kappa_4 + \kappa_9) + \dfrac{2\kappa_3\kappa_9(1-q_2)}{1+\gamma+\chi}}}{\kappa_{10}(1+\gamma+\chi)}$$

(1.11)

Where, $\gamma = \sqrt{1 + \dfrac{\kappa_3^2\kappa_9^2(\kappa_2 - 1 - q_1\kappa_2 + q_2)^2}{\kappa_8^2(\kappa_4 + \kappa_9)^2} + \dfrac{2\kappa_3\kappa_9(\kappa_2 + 1 - q_1\kappa_2 - q_2)}{\kappa_8(\kappa_4 + \kappa_9)}}$

Parameters γ and χ can be expressed in terms of these numbers as,

$$\gamma = \sqrt{1 + c^2(a-b)^2 + 2c(a+b)} \text{ and } \chi = c(a-b)$$

(1.12)

Rearranging the terms and simplifying the expression (1.11), the steady state protein level can be expressed as

$$P_{ss-mir} = \frac{2\kappa_5}{\kappa_{10}(1+\gamma+\chi)} + \frac{2\,c\,\kappa_2\kappa_6}{\kappa_9\kappa_{10}(2bc+1+\gamma+\chi)}$$

(1.13)

Steady state protein level in the absence of miRNA is given as

$$P_{ss} = \frac{\kappa_5}{\kappa_{10}}$$

(1.14)

The ratio (r) of P_{ss-mir} to P_{ss} is,

$$r = \frac{P_{ss-mir}}{P_{ss}} = \frac{2}{1+\gamma+\chi} + \frac{2c \times \kappa_2\kappa_6}{\kappa_9\kappa_5(2bc+1+\gamma+\chi)}$$

(1.15)

Defining the fourth dimensionless number $d = \dfrac{\kappa_2\kappa_6}{\kappa_9\kappa_5}$

The ratio (r) of P_{ss-mir} to P_{ss} is given as,

$$r = \frac{P_{ss-mir}}{P_{ss}} = \frac{2}{1+\gamma+\chi} + \frac{2cd}{2bc+1+\gamma+\chi}$$

(1.16)

Expression for the ratio (r) in case of intronic miRNA
In case of intronic miRNA, the miRNA is synthesized from the same precursor mRNA. This is represented as equal reaction rate constants of mRNA and miRNA synthesis.
$k_1 = k_2$.
In terms of dimensionless parameters, $\kappa_1 = \kappa_2 = 1$

(1.17)

The expression for ratio (r) of P_{ss-mir} to P_{ss} remains unchanged as equation (1.16) but the dimensionless numbers can be simplified as,

$$a = (1-q_1), b = (1-q_2), \ c = \frac{\kappa_3\kappa_9}{\kappa_8(\kappa_4 + \kappa_9)}, d = \frac{\kappa_6}{\kappa_9\kappa_5}$$

(1.18)

Condition of translational activation and equal total mRNA[1]

Translational activation indicates that $r > 1$. The other condition of equal total mRNA both in the presence and absence of miRNA can be written as,

$$m_{ss} = m_{ss-mir} + mmi_{ss-mir}^i \qquad (1.19)$$

Protein ratio is,

$$r = \frac{P_{ss-mir}}{P_{ss}} = \frac{\kappa_5 \times m_{ss-mir} + \kappa_6 \times mmi_{ss-mir}}{\kappa_5 \times m_{ss}} \qquad (1.20)$$

Substituting from Eqn. 1.19 in 1.20

$$r = \frac{P_{ss-mir}}{P_{ss}} = \frac{\kappa_5 \times m_{ss-mir} + \kappa_6 \times mmi_{ss-mir}}{\kappa_5 \times m_{ss-mir} + \kappa_5 \times mmi_{ss-mir}} \qquad (1.21)$$

From the above equation it is clear that to satisfy the condition of translational activation with same total mRNA, κ_6 must be greater than κ_5.

Limiting cases of dimensionless numbers

1. $c = 0$, $k_9 \neq 0$

   The value of c can be zero when k_3 is zero and/or k_8 or k_4 have very large value. Under this condition using equations (1.12 and 1.16), the ratio r reduced to 1 indicating equal steady state level of protein even in the presence of miRNA.

2. $c = 0$, $k_9 = 0$

   Under this condition, d is undefined. Therefore the expression for protein steady state value in the presence of miRNA was obtained by using (1.11), which reduces to

   $$P_{ss-mir}\big|_{\kappa_9=0} = \frac{\kappa_5}{\kappa_{10}} + \frac{\kappa_2 \kappa_3 \kappa_6}{\kappa_8 \kappa_4 \kappa_{10}} \qquad (1.22)$$

   Using 1.14, the ratio can be calculated as,

   $$r\big|_{\kappa_9=0} = 1 + \frac{\kappa_2 \kappa_3 \kappa_6}{\kappa_8 \kappa_4 \kappa_5} \qquad (1.23)$$

   The above equation indicated that under the condition of $k_9 = 0$, contrary to the normal inhibitory action of miRNA, the protein level even in the presence of miRNA can be equal or greater than that in the absence of miRNA.

3. $a = 0$

   The third case would be valid at $k_2 \sim 0$ or $q_1 \sim 1$ which means very low miRNA synthesis or near complete return of mRNA. The expression for the ratio is given as

   $$r\big|_{a=0} = 1 + \frac{dc}{1+c} \qquad (1.24)$$

   Under this condition the protein level in the presence of miRNA would be more than that in the absence of miRNA but it would reach to a certain maximum value ($1+d$).

4. $b = 0$

   The ratio of protein steady state is given as,

   $$r\big|_{b=0} = \left(\frac{1}{1+ac}\right)(1+dc) \qquad (1.25)$$

   The above condition would be valid when $q_2 \sim 1$, implying complete return of miRNA. In this case steady state protein level in the presence of miRNA can be lower, equal or higher than that in the absence of miRNA depending upon the values of k_2 and c.

Supplementary Table

Reaction	Rate equation	Parameter Value
mRNA synthesis $\varphi \rightarrow m$	k_1	$k_1 = 0.02$ (molecule/sec)
miRNA synthesis $\varphi \rightarrow mi$	k_2	$k_2 = 0.1$ (molecule/sec)
mRNA-miRNA binding $m + mi \rightarrow mmi$	$k_3 \times m \times mi$	$k_3 = 1$ (molecule^{-1}sec^{-1})
complex dissociation $mmi \rightarrow m + mi$	$k_4 \times mmi$	$k_4 = 0.02(\text{sec}^{-1})$
Translation by mRNA $\varphi \rightarrow p$	$k_5 \times m$	$k_5 = 0.01$ (sec^{-1})
Translation by the complex $\varphi \rightarrow p$	$k_6 \times mmi$	$k_6 = 0.001$ (sec^{-1})
mRNA degradation $m \rightarrow \varphi$	$k_7 \times m$	$k_7 = 0.002$ (sec^{-1})
miRNA degradation $mi \rightarrow \varphi$	$k_8 \times mi$	$k_8 = 0.0025$ (sec^{-1})
Complex degradation $mmi \rightarrow \varphi$	$k_9 \times mmi$	$k_9 = 0.002$ (sec^{-1})
Protein degradation $p \rightarrow \varphi$	$k_{10} \times p$	$k_{10} = 0.001$ (sec^{-1})
mRNA returning to cytoplasm $mmi \rightarrow m$	$q_1 \times k_9 \times mmi$	$q_1 = 0.2$
miRNA returning to cytoplasm $mmi \rightarrow mi$	$q_2 \times k_9 \times mmi$	$q_2 = 0.4$

Table S1: Parameter values obtained from model by Shimoni et al[2], except for k_2, k_6, q_1 and q_2, values for which are discussed in main text.
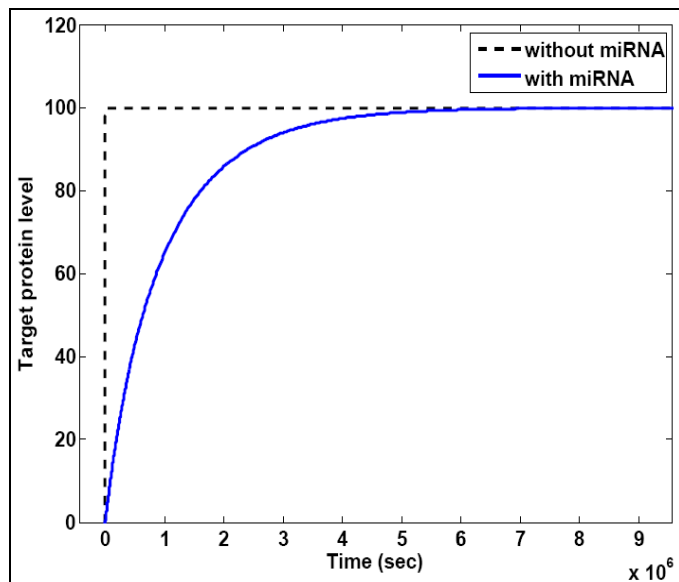
Supplementary Figures

Figure S1 - Effect on transient of target protein. Time course profile of target protein ($k_9 = 0$, $k_6 = 0$; Initial condition- All components at zero level).
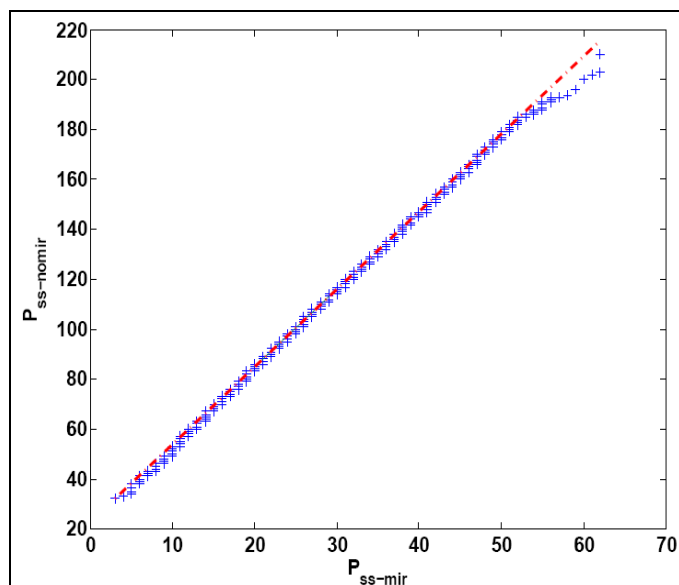

Figure S2 – QQplot of steady state protein distribution in the presence of miRNA vs. that in the absence of miRNA.

Reference:

1.   R. D. Mortensen, M. Serra, J. A. Steitz and S. Vasudevan, *Proceedings of the National Academy of Sciences*, **108**, 8281.

2.   Y. Shimoni, G. Friedlander, G. Hetzroni, G. Niv, S. Altuvia, O. Biham and H. Margalit, *Mol Syst Biol*, 2007, **3**, 138.