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Switches in gene expression including microRNA and a large number of distinct mRNAs

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In eukaryotic cells, the kinetics of gene expression depend on the interplay of messenger RNAs (mRNAs), proteins, and nonprotein coding RNAs, or, more specifically, microRNAs. Some of microRNAs may target hundreds of mRNAs. To describe this case, the author proposes a kinetic model implying that the microRNA synthesis is suppressed by protein produced via translation of one of the target mRNAs. With physically reasonable model parameters, the model predicts bistability or, in other words, switches in expression of hundreds of genes.

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In cells, the information flows from deoxyribonucleic acid (DNA) to protein through its intermediary ribonucleic acid (RNA) [1]. Specifically, the heritage encoded in DNA is expressed via a templated polymerization called transcription, in which the genes (segments of the DNA sequence) are used as templates to guide the synthesis of RNA by RNA polymerase (RNAP). In turn, RNA or, more specifically, mRNA serves to direct the synthesis of proteins by ribosomes. The gene transcription, performed by RNAP, is often controlled by master regulatory proteins. Due to the feedback between these processes, the kinetics of mRNA and protein formation may be complex even in the simplest genetic networks. In particular, it may exhibit bistability. Practically, this means that with changing a governing parameter one can observe a stepwise transition or, in other words, switch from one regime of gene expression to another regime. Such switches often play a key role in regulation of cellular processes. The corresponding kinetic models are focused on the expression of one or two genes and interplay of mRNA(s) and protein(s) (see, e.g., reviews [2, 3], recent articles [4], and references therein).

The scheme outlined above is fully applicable to prokaryotes whose genomes consist of tightly packed protein-coding sequences. The genomes of the eukaryotic cells contain however relatively rare protein-coding sequences. The rest of the genome includes a lot of transcript units representing nonprotein coding RNA (ncRNA). During the past decade, it has become obvious that such RNAs form the cornerstone of a regulatory network of signalling that operates in concert with the protein network [5 – 9].

One of the most important and interesting subgroup of ncRNAs includes microRNAs (miRNAs) which are 20–22 nucleotides long [5, 10 – 12]. Such RNAs are transcribed as long ncRNA and then generated via a two-step processing pathway including first the formation of a few different ~ 65-nt pre-miRNAs and then conversion of each of them into the corresponding miRNA [10]. The numerous biological functions of miRNAs are based on their abilities to silence target genes [10, 11]. Specifically, a miRNA pairs with a target mRNA and then either prevents translation or results in rapid degradation of the miRNA-mRNA complex.

At present, miRNAs are thought to regulate up to one third of all human genes [13]. The bulk of the data in this field was obtained by analysing the correlations in the miRNA and mRNA expression and also by using computational target predictions (see recent reviews [13, 14]). One of the most interesting concepts drawn from such studies is that some of miRNAs can target hundreds (up to 800 [13]) of mRNAs. The direct experimental identification and validation of miRNA targets are however still rare and represent one of the challenges in miRNA biology [14].

In analogy with mRNAs, the ncRNA/miRNA formation can be controlled by transcription factors (proteins) involved in the regulation of "conventional" genes [11]. The identification and validation of feedbacks in the interplay of ncRNAs, mRNAs, and proteins are now based primarily on the analysis of the correlations in the ncRNA and mRNA expression and computational predictions [15, 16].

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The first kinetic models [17, 18], proposed recently to describe the interaction of miRNA with mRNAs of one or a few tipes, do not predict bistability. The model [19] predicting bistability is focused on interaction of miRNA with mRNA of one type. In this Letter, we introduce a model exhibiting bistability in the interplay of miRA, protein and hundreds of distinct mRNAs. This model clarifies the conditions when this phenomenon is possible and may help to understand the function of the switches in gene expression regulated by miRNAs.

Our model includes synthesis of miRNA ($\mathcal{R}$) and mRNAs of n types (R_i, $1 \le i \le n$)

$$\text{Gene}_* \rightarrow \text{Gene}_* + \mathcal{R}, \tag{1}$$

$$\text{Gene}_i \rightarrow \text{Gene}_i + R_i. \tag{2}$$

The miRNA synthesis is assumed to be regulated by protein (P) produced via translation of one of the mRNAs,

$$R_1 \rightarrow R_1 + P. \tag{3}$$

All these species degrade,

$$\mathcal{R} \rightarrow \emptyset, \;\; R_i \rightarrow \emptyset, \;\; P \rightarrow \emptyset. \tag{4}$$

The interaction between miRNA and mRNAs is considered to occur via their association and degradation,

$$\mathcal{R} + R_i \rightarrow \mathcal{R} \cdot R_i \rightarrow \emptyset. \tag{5}$$

In this generic scheme, the formation of miRNA is represented as a single lumped step (1). If necessary, one can introduce a few intermediate steps (see, e.g., Ref. [18]). For our present goals, the lumped description is sufficient.

In our treatment, P is considered to suppress the miRNA synthesis. Specifically, the miRNA formation is assumed to run provided that m regulatory sites of Gene_* are free of P. The P association with and dissociation from the gene are considered to be rapid so that these steps are at equilibrium. In this case, the mean-field kinetic equations for the numbers of R_i, P, and $\mathcal{R}$ copies in a cell are as follows

$$\frac{dN_i}{dt} = w_i - r_i N_* N_i - k_i N_i, \tag{6}$$

$$\frac{dN_P}{dt} = \kappa_P N_1 - k_P N_P, \tag{7}$$

$$\frac{dN_*}{dt} = w_* \left(\frac{K_P}{K_P + N_P}\right)^m - \sum_i r_i N_* N_i - \kappa N_*, \tag{8}$$

where w_i is the rates of the R_i synthesis, κ_P is the rate constant of the P synthesis, w_* is the rate of the $\mathcal{R}$ synthesis in the absence of suppression by P, $[K_P/(K_P + N_P)]^m$ is the probability that all the regulatory sites are free of P (this probability corresponds to the P association-dissociation equilibrium; K_P is the corresponding constant), k_i, κ and k_P are the rate constants of the R_i, $\mathcal{R}$ and P degradation, and r_i are the rate constants of steps (5).

Analysing Eqs. (6)–(8), we consider that the cell is under steady-state conditions. In this case, equation (6) yields

$$N_i = w_i/(k_i + r_i N_*). \tag{9}$$

Substituting this expression into Eq. (8) results in

$$w_* \left(\frac{K_P}{K_P + N_P}\right)^m - \sum_i \frac{r_i w_i N_*}{k_i + r_i N_*} - \kappa N_* = 0. \tag{10}$$

To express in this equation N_P via N_*, we rewrite Eq. (7) as

$$N_P = (\kappa_P/k_P) N_1 \tag{11}$$

and use expression (9) for N_1,

$$N_1 = w_1/(k_1 + r_1 N_*). \tag{12}$$

Substituting (12) into (11) yields

$$N_P = \kappa_P w_1/[k_P(k_1 + r_1 N_*)]. \tag{13}$$

Substituting this expression into Eq. (10), we obtain

$$w_* \left(\frac{K_P}{K_P + \kappa_P w_1/[k_P(k_1 + r_1 N_*)]}\right)^m - \\ - \sum_i \frac{r_i w_i N_*}{k_i + r_i N_*} - \kappa N_* = 0. \tag{14}$$

Equation (14) for N_* can easily be solved numerically and then one can calculate all the other variables. Looking at Eq. (14), one can notice that it always has at least one solution. If $m \ge 2$, equation (14) may have three solutions. As usual in such cases, the lower and upper solutions are stable and the intermediary solution is unstable. Thus, with appropriate parameters, equation (14) predicts bistability.

Our analysis of Eq. (14) in a wide range of model parameters (the total number of parameters is $3n + 6$) indicates that the bistability is possible for physically reasonable parameters even if n is high, e.g., 100 as we use below. To explicitly illustrate the type of constraints we have here, it is instructive to outline the principles behind the choice of "reasonable parameters". First of all, we should note that the direct measurements of kinetic parameters for miRNAs are now lacking. Nevertheless, the estimates of the scale of the parameter for miRNAs are feasible, because the mechanisms of formation and degradation of miRNAs are similar to those of mRNAs. For this reason, we can employ for miRNAs the same range of parameters as for mRNAs.

The rate constants of mRNA and protein degradation are often in the range from 0.1 to $1\,\mathrm{min}^{-1}$ (see, e.g., the review by Kaern et al. [2]). To be specific, we employ $k_1 = \kappa = k_P = 0.1\,\mathrm{min}^{-1}$. The rate constants k_i with $i > 1$ are selected at random in the range from 0.1 to $1\,\mathrm{min}^{-1}$.

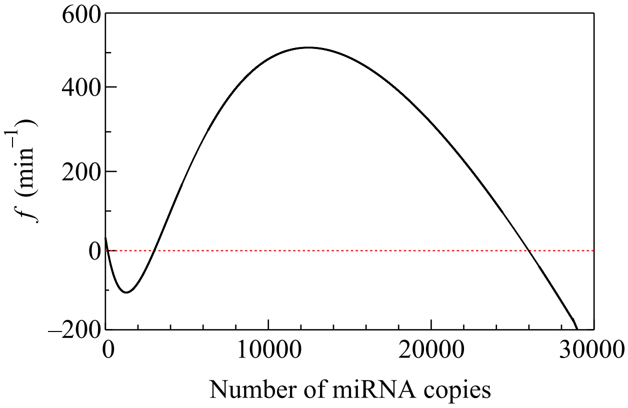
The population of each mRNA may be in the range from a few copies to a few thousands of copies [20]. For high n, the bistability is possible provided that in the absence of miRNA the average population of mRNA, $\langle N_0 \rangle \simeq \langle W_i \rangle / \langle k_i \rangle$, is not too large, because otherwise miRNA will not be able to appreciably influence mRNAs. For this reason, we use $w_1 = 10\,\mathrm{min}^{-1}$ and distribute w_i with $i > 1$ at random in the range from 0 to $20\,\mathrm{min}^{-1}$. In this case, we have $\langle N_0 \rangle \simeq 20$. In contrast, the miRNA population in the absence of mRNAs should be high in order to be able to influence many mRNAs, and accordingly we employ $w_* = 5 \cdot 10^3\,\mathrm{min}^{-1}$. In this case, $w_*/\kappa = 5 \cdot 10^4$.

The P population may be in the range from a few copies to many thousands of copies [20]. To obtain bistability, the parameters controlling this population can be chosen quite arbitrarily. In particular, we use $\kappa_P = 1$ and $w_1 = 10\,\mathrm{min}^{-1}$. In this case, in the absence of miRNA, the P population is $N_P = \kappa_P w_1 / k_P k_1 = 10^3$.

To observe bistability, the variation of N_P from low values up to the maximum value, $N_P = \kappa_P w_1 / k_P k_1 = 10^3$, should result in appreciable change of the rate of the miRNA synthesis. This is possible if K_P is lower than 10^3. To be specific, we employ $m = 4$ and $K_P = 400$.

In addition, we take into account that miRNA finds complimentary fragments of a mRNA chain for association. This means that for a given miRNA the distribution of the association rate constants is not expected to be wide, and accordingly we fix these rate constants as $r_i = r$. To obtain bistability, it is desirable to have high value of r. It cannot however be higher than that predicted by the theory of diffusion-limited reactions. In particular, our estimates [18] indicate that r should be lower than $3 \cdot 10^{-3}\,\mathrm{min}^{-1}$. For this reason, we use $r = 10^{-4}\,\mathrm{min}^{-1}$.

With the specification above, equation (14) predicts bistability as shown in Figure. Two stable solutions correspond to high and low expression of miRNA, respectively. In the former case, the population of $\mathcal{R}$ is $N_* = 2.6 \cdot 10^4$. In the latter case, one has $N_* = 140$.



Left-hand part of Eq. (14) as a function of N_*. In this case, equation (14) has three solutions. The lower and upper solutions are stable and the intermediary solution is unstable. (For the parameters, see the text)

In the example above, the rate constants k_i and w_i were distributed in relatively wide ranges. If these constants are fixed as $k_i = 0.1\,\mathrm{min}^{-1}$ and $w_i = 10\,\mathrm{min}^{-1}$ (in this case, $\langle N_0 \rangle \simeq 100$) and the other parameters are kept the same, the bistability is predicted as well (not shown).

In combitation, the two examples presented above indicate that the conditions for observation of bistability are not too severe. Physically, the explanation of bistability is straightforward: (i) If the miRNA population is high, it suppresses the mRNA population via step (5) and accordingly suppresses the rate of protein formation as well. The latter results in low protein concentration. Thus, this regime is stable because the protein concentration is not sufficient to suppress the miRNA synthesis. (ii) If the miRNA population is low, it is not able suppresses the mRNA population. In this case, the mRNA and protein populations are relatively high. Due to high protein concentration, the miRNA synthesis is suppressed, and accordingly the miRNA population cannot be appreciably increased. For this reason, the latter regime is stable as well.

In conclusion, we may repeat that one of the most interesting concepts in the miRNA biophysics is that some of miRNAs can target hundreds of mRNAs [13, 14]. We have proposed a kinetic model describing this case. The specifics of our model is that the miRNA synthesis can be suppressed by protein which is produced via translation of one of the mRNAs. With physically reasonable model parameters, the model predicts bistability. In other words, the model predicts switches in gene expression including microNRA and hundreds of mRNAs.

Validating the choice of the appropriate parameters, we have shown the conditions desirable for observation of this phenomenon. The understanding of these conditions is of intrinsic interest and also may have practical implications, because the biological functions of miRNAs have been tracked out in a wide variety of cellular processes. For example, thousands of mammalian mRNAs are highly expressed at developmental stages before miRNAs expression and their levels tend to fall as the miRNAs that target them begin to accumulate [21]. Abnormal levels of miRNA expression were observed in many types of human cancer [22]. Our model represents the first attempt of interpretation of such global miRNA-related changes in gene expression in terms of chemical kinetics.

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