Dynamics of the Mixed Feedback Loop Integrated with MicroRNA

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Abstract   The so-called mixed feedback loop (MFL) with microRNA is a small two-gene network where microRNA regulates the translation of mRNA by base pairing with the mRNA. It has been developed to show that the MFL is a representative motif of genetic regulation networks. The present paper is mainly concerned with the issues of dynamics for the mixed feedback loop (MFL) integrated with microRNA. A simple mathematical model of the motif is proposed based on the biochemical interactions. It is shown that, by itself, this motif can serve both as a monostable and a bistable switch depending on a kinetic parameter range. The results emphasize the role of microRNA in the function of genetic modules and the regulation of itself.

Keywords   mixed feedback loop (MFL), microRNA, dynamical Regimes

1   Introduction

Biological cells depend on complex networks of biochemical interaction between different molecules [1, 2, 3]. There has recently been increasing interest in the study of transcription regulation interactions and protein-protein interactions, which in many cases are involved in post-translational regulation. During the last years, it has become evident that another type of interaction plays a prominent role in the regulation of cellular processes, manifested by microRNA molecules that base pair with the mRNA and regulate gene expression post-transcriptionally [2]. It is worth noting that this mode of regulation was found in both pro- and eukaryotes (for review see Storz et al., 2005). Although there are differences in the characteristics of the eukaryotic and prokaryotic regulatory RNAs and in the fine-details of their mechanism of action, both exert their regulatory function mostly by base pairing with the mRNA and influencing translation or mRNA stability [2]. More recently, an analysis of transcriptional networks integrated with post-transcriptional or post-translational has pointed out several motifs of mixed interactions. Especially, an over-represented motif with the mixed feedback loop (MFL) is proposed to show that the role of protein dimerization and the usefulness of modeling mRNA dynamics explicitly [3]. Here, it is intriguing to study the dynamical properties of this type of motifs with microRNA in comparison to other type of motifs.

The main objective of this paper is to investigate dynamics of a specific motif with microRNA. To better understand the possible functions of this basic module, a model of the

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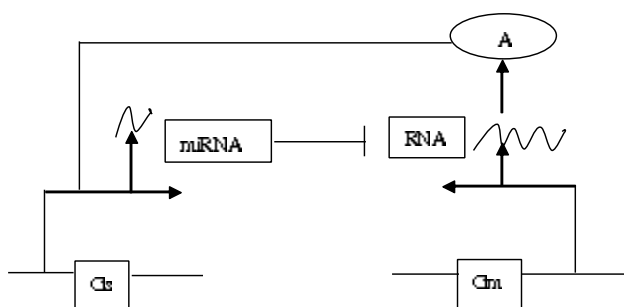*Corresponding author: Jinzhousu@yahoo.com.cn

Figure 1: The proposed model of the MFL motif

mixed feedback loop (MFL) based on the simplest biochemical interactions is proposed, which is a small two-gene network where microRNA regulates the translation of RNA. It is composed of RNA produced from gene G_m and a transcription factor A translated from RNA and microRNA produced from gene G_s, and A regulates the transcription of gene G_s and RNA directly interacts with microRNA. It is shown that, by itself, it can serve both as a monostable and a bistable switch depending on a kinetic parameter range. The results emphasize the role of microRNA in the function of genetic modules and the regulation of itself.

The rest of this paper is organized as follows. Section 2 describes the mathematical model, Section 3 is the main dynamics results of the model. The conclusion is given in Section 4.

2   Mathematical Model

2.1   Model formulation

In this paper, we are mainly interested in analyzing dynamics of a specific motif with microRNA. As previously described, the MFL consists of RNA produced from gene G_m and a transcription factor A translated by RNA and microRNA produced from gene G_s. A regulates the transcription of gene G_s and RNA directly interacts with microRNA. Our aim is to analyze the dynamics of this small genetic module and see what can be achieved in the simplest setting. Therefore, different cellular compartments and separate concentrations for the nucleus and cytoplasm are not considered and biochemical reactions are modeled by simple rate equations. The proposed MFL model is depicted schematically in Fig. 1.

The MFL model consists of four equations that are described and explained below. The concentration of the species G_s, microRNA, RNA and A are denoted by N_g, N_s, N_m and N_p, respectively, and the cell volume is taken as volume unit.

The following three equations model the transcriptional regulation of gene N_s, and the

interaction of RNA and microRNA.

$$\begin{cases} \dfrac{dN_g}{dt} = \theta[N_g : N_p] - \beta N_g N_p, \\[2mm] \dfrac{dN_s}{dt} = \rho_f[N_g : N_p] + \rho_b N_g - d_s N_s - \alpha N_s N_m, \\[2mm] \dfrac{dN_m}{dt} = g_m - d_m N_m - \alpha N_s N_m. \end{cases} \qquad (1)$$

where it is assumed that gene G_s exists under two forms, with A bound to its promoter with probability $[N_g : N_p]$ and without A with probability N_g. Since $[N_g : N_p] + N_g = 1$, the first equation of (1) is sufficient to describe the transition between the two forms. Specifically, A proteins bind to the promoter at a rate β, and when bound they are released at a rate θ. The regulation of transcription of gene G_s by protein A is described by the second equation. When A is bound to the G_s promoter, the transcription is initiated at a rate ρ_f, and otherwise, it is initiated at a rate ρ_b. Thus, $\rho_f > \rho_b$ corresponds to transcriptional activation by A and $\rho_f < \rho_b$ to transcriptional repression. Since regulation of gene G_m is not considered it is simply assumed in the third equation that RNA is produced at a given basal rate g_m. The second crucial interaction of the MFL, the direct interaction between microRNA and RNA is taken into account by assuming that they associate at a rate α. In addition, a first-order degradations for RNA at a rate d_m and microRNA at a rate d_s have also been assumed in the third equation, respectively. As given, the description is strictly valid for a single copy gene.

The production of the A protein is described by the following equation that complete our description of the MFL module:

$$\frac{dN_p}{dt} = g_p N_m - d_p N_p + \theta[N_g : N_p] - \beta N_g N_p. \qquad (2)$$

where it is assumed that A proteins are produced from the transcripts of G_m at a rate g_p, and degraded at a rate d_p simultaneously. The last two terms of Eq. (2) come from the (small) contribution to the concentration of A in solution of the binding(unbinding) of A to (from) the G_s promoter.

As described in Eq. (1), the complexation N_x between RNA and microRNA proceeds at a rate α, For simplicity, we suppose that the complex N_x does not dissociate back into their original components. Since the complex N_x does not feed back on the dynamics of the other species, its concentration does not need to be monitored and the complex N_x is not explicitly considered in the following.

2.2 Values of kinetic parameters

As is known, even in this simple model, ten kinetic constants should be specified. It is useful to consider the possible range of their values both to assess the biological relevance of the different dynamical regimes and to orient the model analysis [3].

Half-lives of RNA range from a few minutes to several hours and are peaked around 20 min in yeast [7]. Therefore, $d_s = d_m = 0.05 min^{-1}$ can be taken as a typical value.

For the transcription factor-gene promoter interaction, typical values appear to be a critical concentration $\frac{\theta}{\beta} = A_0$ in the nanomolar range, a bound state lifetime of several

minutes and activated transcription rates per minute. Therefore, we assume $\frac{\theta}{\beta} = 40$, that θ is of the same order as d_m [3]. ρ_f and ρ_b range from $5min^{-1}$ to $500min^{-1}$, since the production rate of RNA range from $0.1min^{-1}$ to $10min^{-1}$ [6] and the production rate of microRNA is 50 times faster than that of RNA [2].

Protein half-lives vary from a few minutes to several days. The hour appears as a typical value [3]. We choose $d_P = 0.01min^{-1}$ and $g_p = 3min^{-1}$. RNA is produced at a given basal rate g_m and the rate value range from 0.1 molecule min^{-1} to 10 molecule min^{-1}. The rate constants for binding of microRNA to RNA was taken as $\alpha = 10min^{-1}$.

It is convenient to introduce dimensionless variables in Eqs. (1) and (2) to decrease as far as possible the number of independent parameters. For this reason, we first normalize the microRNA concentration by the concentration that gives a production of RNA equal to microRNA. Thus, we define the dimensionless concentration $G = N_g, P = N_p, S = \frac{N_s}{g_m}$, and define $M = \frac{N_m}{g_m}$. With the four substitutions, Eqs. (1) and (2) can be rewritten as

$$
\begin{cases}
\dfrac{dG}{dt} = \theta(1 - G - G\dfrac{P}{A_0}), \\
\dfrac{dS}{dt} = \rho_0(1 - G) + \rho_1 G - d_s S - \gamma MS, \\
\dfrac{dM}{dt} = 1 - d_m M - \gamma MS, \\
\dfrac{dP}{dt} = \delta M - d_p P + \theta(1 - G - G\dfrac{P}{A_0}).
\end{cases}
\tag{3}
$$

where we have defined the following parameters $\rho_0 = \frac{\rho_f}{g_m}$, $\rho_1 = \frac{\rho_b}{g_m}$, $\gamma = \alpha g_m$ and $\delta = g_p g_m$. The model still depends on eight parameters. The influence of two key parameters ρ_0 and ρ_1, which measure the strengths of the two possible states of microRNA production (with or without A bound to gene G_s as compared to that of RNA is particularly examined in the following.

3   Dynamical Regimes

We provide here several dynamical regimes of the MFL with microRNA in different parameter regimes, and summarize their characteristics. It have observed that depending on the values of ρ_0 and ρ_1, the MFL can be monostable, and exhibit bistability.

3.1   Monostable steady states

The simplest case occurs when the production rate of microRNA is higher or lower than the production rate of RNA, irrespective of the state of the G_s promoter. That is when both ρ_f and ρ_b are either higher or lower than g_m, the MFL has a single stable state to which it relaxes starting from any initial conditions.

When both microRNA production rates are higher than the production rate of RNA (i.e.$\rho_0 > 1$ and $\rho_1 > 1$) and the RNAs are quickly paired with microRNA and are unable to translate proteins which interact with G_s promoter. The concentration RNA of unpaired with microRNA is, therefore, low and results from a simple balance between production and complexation. The high concentration of uncomplexed microRNA is the effective

result from transcription at the free promoter rate, complexation, and degradation.

$$N_s \cong \frac{\rho_b - g_m}{d_s}, \qquad N_m \cong \frac{g_m}{\alpha N_s}. \tag{4}$$

An equally simple but opposite result holds when both microRNA production rates are lower than the production rate of RNA (i.e., $\rho_0 < 1$ and $\rho_1 < 1$), Then, the concentration of uncomplexed RNA is high, the G_s promoter is occupied by proteins A which is translated by uncomplexed RNA and a low concentration microRNA results from a balance between complexation and production.

$$N_s \cong \frac{\rho_f}{\alpha N_m}, \qquad N_m \cong \frac{g_m - \rho_f}{d_m}. \tag{5}$$

The dynamics of the MFL with microRNA is richer when the production RNA is intermediate between the two possible production rates of microRNA. We consider the case when $\rho_b > g_m > \rho_f$ (i.e., $\rho_0 < 1 < \rho_1$) in turn.

3.2   Transcriptional repression and bistability

When protein A is a transcriptional repressor, then two stable steady states can coexist. Let us first suppose that no A is bound to the G_s promoter. Then the production rate of microRNA is larger than the production rate of RNA, and all produced RNA are so quickly paired with microRNA not to translate protein A. This stably prevents the binding of A proteins to the G_s promoter and maintain a steady state with low RNA and high microRNA concentrations approximately equal to

$$N_s^1 \cong \frac{\rho_b - g_m}{d_s}, \qquad N_m^1 \cong \frac{g_m}{\alpha N_s^1}. \tag{6}$$

The second opposite possibility is that A is sufficiently abundant to repress the transcription of gene G_s. Then, since the production rate of RNA has been supposed to be higher than the production rate of microRNA in the repressed state, microRNAs are quickly paired but unpaired RNA are present and translate protein A to maintain the repression of the gene G_s transcription. This gives rise to a second stable state with high RNA and low microRNA concentrations approximately equal to

$$N_s^2 \cong \frac{\rho_f}{\alpha N_m^2}, \qquad N_m^2 \cong \frac{g_m - \rho_f}{d_m}. \tag{7}$$

We provide here a more detailed analysis on the second case of the MFL as follows. It can be observed that the free gene, microRNA and A protein concentrations are given in a steady state as a function of the concentration of RNA.

$$\begin{cases} G = \dfrac{A_0}{A_0 + M\frac{\delta}{d_p}}, \\[3mm] S = \dfrac{\rho_1 A_0 + \rho_0 M\frac{\delta}{d_p}}{(A_0 + M\frac{\delta}{d_p})(d_s + \gamma M)}, \\[3mm] P = M\dfrac{\delta}{d_p}. \end{cases} \tag{8}$$

Thus, the concentration of RNA itself satisfies the following equation:

$$\frac{\rho_1 A_0 + \rho_0 M \frac{\delta}{d_p}}{(A_0 + M \frac{\delta}{d_p})(d_s + \gamma M)} \gamma + d_m M = 1. \tag{9}$$

This leads to

$$\frac{(\rho_1 a + \rho_0 M)M}{(a + M)(b + M)} + d_m M = 1, \tag{10}$$

where $a = \frac{d_p A_0}{\delta}, b = \frac{d_s}{\gamma}$.

In order to simplify above analysis, it is useful to note that b is a small parameter (approximately equal to 3×10^{-3} with respect to the previous estimations when g_m is set properly). For $b = 0$, we can obtain a solution $M_2 \cong \frac{a(\rho_1 - 1)}{1 - \rho_0}$. For sufficient small b, two other steady states are possible. A steady state with a small concentration of M, $M_1 \cong \frac{b}{\rho_1 - 1}$ exists when $\rho_1 > 1$. Inversely a steady state with a large concentration of M, $M_3 \cong \frac{1 - \rho_0}{d_m}$ exists when $\rho_0 < 1$.

Obviously, Eq. (10) is equivalent to the following one:

$$d_m M^3 + (a d_m + b d_m + \rho_0 - 1)M^2 + (a b d_m + a\rho_1 - a - b)M - ab = 0. \tag{11}$$

Note that the MFL with microRNA may have multiple (i .e three) fixed points only if Eq. (11) has three different positive roots. It is easy to see that if the following conditions are satisfied:

$$\begin{cases} \rho_0 < 1 - (a d_m + b d_m) \\ \rho_1 > 1 + (\frac{b}{a} - b d_m) \end{cases} \tag{12}$$

which implies that $\rho_0 < 1$ and $\rho_1 > 1$.

Eq. (3) is solved by numerical integration starting from suitable initial conditions directly. Two concerns are discussed below:

The high S state.

We show that the state with a high concentration of S is stable. In this case, M and P quickly reach their quasiequilibrium concentration. Let $s = S \frac{\gamma d_p}{\delta}$, M and P reach on a fast time scale their quasiequilibrium concentration:

$$M \cong \frac{d_p}{\delta s}, \qquad p \cong \frac{1}{s}. \tag{13}$$

Therefore, the dynamics of MFL with microRNA reduces to the following two equations:

$$\begin{cases} \frac{dG}{dt} = \theta(1 - G), \\ \frac{ds}{dt} = \frac{\gamma d_p}{\delta}[\rho_0(1 - G) + \rho_1 G] - d_s s - \frac{\gamma d_p}{\delta}. \end{cases} \tag{14}$$

It clearly show that the high S fixed point is stable and the concentrations tend toward those of the high S fixed points;

$$G \cong 1, \quad s = \frac{\frac{\gamma d_p}{\delta}(\rho_1 - 1)}{d_s}, \quad M \cong 0, \quad P \cong 0. \tag{15}$$

This steady state exists only if $\rho_1 > 1$, and the production of microRNA is high enough to pair with RNA and to prevent RNA traslating the transcriptional repressor protein A.

The high M state.

The high M state can be analyzed in a very similar way based on the fact that the microRNA and RNA quickly reach quasiequilibrium states. However, both of them cannot be in quasiequilibrium at the same time. For instance, when $M \gg S$, only S reaches its quasiequilibrium. When M and P concentration are high at all, and we set $p = \frac{d_p P}{A_0}$, $m = \frac{d_m \gamma M}{\rho_0}$, G and S reach on a fast time scale their quasiequilibrium state:

$$G \cong \frac{d_p}{p}, \qquad S \cong \frac{d_m}{m}. \tag{16}$$

Therefore, the dynamics of MFL with microRNA reduces to the following two equations:

$$\begin{cases} \dfrac{dm}{dt} = \dfrac{d_m \gamma}{\rho_0} - d_m m - d_m \gamma, \\ \dfrac{dp}{dt} = \eta m - d_p p. \end{cases} \tag{17}$$

Let $\eta = \frac{d_p \rho_0 \delta}{d_m A_0 \gamma}$, It can be from Eq. (17) seen that the high M fixed point is stable and, as found above, satisfies

$$G \cong 0, \quad S \cong 0, \quad m \cong \frac{\gamma(1 - \rho_0)}{\rho_0}, \quad p \cong \frac{\eta m}{d_p}. \tag{18}$$

This steady state is possible only if $\rho_0 < 1$, that is when the S production rate is not high enough to prevent translation by M and to prevent the repression by P.

4 Conclusions

In this paper, we have investigated the issues of dynamics for the mixed feedback loop (MFL) integrated with microRNA. A simple model of such motif is proposed based on the biochemical interactions. The analysis shows that, by itself, this motif can serve both as a monostable and a bistable switch depending on a kinetic parameter range. The results emphasize the role of microRNA in the function of genetic modules and the regulation of itself.
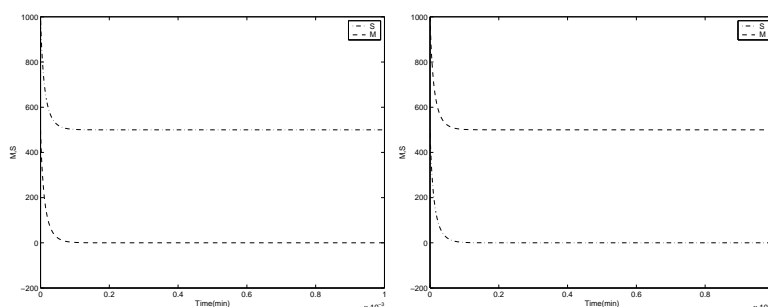
Acknowledges

Figure 2: Time evolution of the concentrations of *M* and *S* respectively. Parameter are the same as that in Sec. 2. i.e., ρ_f=5 min^{-1}, ρ_b=30 min^{-1} and g_m=10 mol min^{-1}. The left one : high *S*; The right one: high *M*.

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