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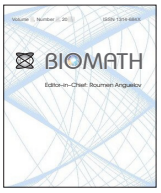

Dynamical Analysis of the MicroRNA-Mediated Protein Translation Process

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***Abstract*—Mathematical modeling of kinetic processes with different time scales allows a reduction of the governing equations using quasi-steady-state approximations (QSSA). A QSSA theorem is applied to a modified mathematical model of the microRNA-mediated protein translation process. By an appropriate normalized procedure the system of seven nonlinear ordinary differential equations is rewritten in a form suitable for model reduction. In accordance with the terminology of the QSSA theorem, it is established that two of the protein concentrations are "fast varying", such that the corresponding kinetic equations form an attached system. The other four concentrations are "slow varying", and form a degenerate system. Another variable appears to be a constant. Analytical relationships between the steady-state values of the fast varying concentrations and the slow varying ones, are derived and interpreted as restrictions on the regulatory role of microRNAs on the protein translation process.**

***Keywords*-nonlinear dynamics; microRNA; protein translation; QSSA theorem**

I. Introduction

MicroRNAs are short (an average of 20–22) RiboNucleic Acid (RNA) molecules that regulate the function of eukaryotic messenger RNAs (mRNAs) and thereby play an important role in development, cancer, stress responses, and viral infections. Recently, remarkable progress was made in the understanding of microRNA biogenesis, its functions and mechanisms of action. MicroRNAs affect gene expression by specific inhibition of target mRNAs. The exact mechanism of this inhibition is still a matter of debate. In the past few years, several mechanisms have been reported, some of which are contradictory [1], [2], [3]. They include in particular inhibition of translation initiation (acting at the level of cap-40S or 40S-AUG-60S association steps), inhibition of translation elongation or premature termination of translation. In order to verify some of these hypotheses, two simple mathematical models of protein translation are proposed as systems of ordinary differential equations in [4]. By their analysis the authors in [4] demonstrated that it is impossible to distinguish alternative biological hypotheses using the steady state data on the rate of protein synthesis. In [5], however, it is shown that dynamical data allow to discriminate some of the mechanisms of microRNA action. The authors in [5] demonstrated this fact using the same models as those in [4] for the sake of comparison but they applied different methods. As a result of their investigation, they formulated a hypothesis that the effect of microRNA action is measurable and observable only if it affects the dominant system (generalization of the limiting step notion for complex networks) of the protein translation machinery. Following the last investigation here we consider one of the models proposed in [4] (so called eIF4F/subunit joining model) as our aim is to show that considerations of time hierarchy in genetic interactions allows us to reduce the number of differential equations of the above-mentioned model and thereby to determine the driving reactions and control parameters in the microRNA repression mechanism. For this purpose we will use the method of the Quasi-Steady-State Approximation (QSSA) theorem proved in the basic paper [6].

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II. QSSA THEOREM

In general the mathematical modeling of biochemical reactions with different time scales leads to hierarchical system of the form:

$$\epsilon \frac{d\vec{x}}{dt} = \vec{f}(\vec{x}, \vec{y}) \quad (1)$$

$$\frac{d\vec{y}}{dt} = \vec{g}(\vec{x}, \vec{y}) \quad (2)$$

where $\vec{x} \in R^n$, $\vec{y} \in R^m$ and $0 < \epsilon << 1$.

The terminology of the QSSA theorem related to the system (1–2) is as follows. The subsystem of equations having ϵ in the numerator is called *an attached system*, and its variables are fast variables with respect to the other part of equations, which form *a degenerate system*, and its variables are *slow variables*. The set of both subsystems forms the *complete system*. In accordance with this terminology, the QSSA theorem [7] claims that the solution of the *complete system* (1–2) tends to the solution of the *degenerate system* (2) at $\epsilon \to 0$, if the following conditions are satisfied:

a) There is an isolated equilibrium (steady state) solution of the *attached system* (1)(i.e., there is not another solution in its neighborhood).

b) The existing equilibrium solution of the *attached system* is a stable one for each value of the slow variables $\vec{c}_s$.

c) The initial conditions (states) are contained in a region of influence (a basin) of the equilibrium solution of the *attached system*.

d) The solution of the *complete system* is single-valued and its right-hand side is continuous.

The essence of the QSSA theorem is that the character of the solution of (1–2) does not change when the small parameter ϵ converges to zero. Thus, we can assume $\epsilon = 0$ in (1)and instead of differential equations obtain algebraic ones for the steady-state value of fast variables, i.e.,

$$\vec{f}(\vec{x}, \vec{y}) = 0, \ \vec{x} = \vec{\varphi}(\vec{y})$$

$$\frac{d\vec{y}}{dt} = \vec{g}[\vec{\varphi}(\vec{y}), \vec{y}] \quad (3)$$

In this way, the complete system (1–2) is reduced to the degenerate system (3). For every fixed $\vec{y}$, the equation $\vec{f}(\vec{x}, \vec{y})$ has a unique solution that depends continuously (or smoothly, if needed) on $\vec{y}$. Thus the variables $\vec{y}$ play the role of a driver of the subordinated variables $\vec{x}$. According to the QSSA theorem, when the stationary solution of the attached system is isolated and stable, then the solution of the reduced (degenerate) system depends only on the post-initial values of the slow variables. The term "post-initial" is introduced in sense of the considerations of initial and later intervals of validity of the complete and degenerate systems, respectively. Certainly, the complete system (1–2) holds any time, and the degenerate system (3) will be valid from some later period of time. But it can reveal new properties of the investigated processes near their stationary states as we will show in the following paragraphs.

III. REDUCTION OF DIMENSIONALITY OF A MODIFIED MATHEMATICAL MODEL OF MICRORNA-MEDIATED PROTEIN TRANSLATION PROCESS

A. Applying the QSSA Theorem to the Model

We apply the QSSA theorem to a modified ordinary differential equation model of microRNA-mediated protein translation process, presented in [4]. It explicitly takes into account recycling of initiation factors and ribosomal subunits. In accordance with Fig. 1 there are four reactions in the model, all considered to be irreversible:

1) 40S + eIF4F $\longrightarrow$ mRNA - 40S, assembly of the initiation complex, cap-dependent initiation steps (rate k_1).
2) mRNA - 40S $\longrightarrow$ AUG, some later and cap-independent initiation steps, such as scanning the 5'UTR by the start codon AUG recognition (rate k_2).
3) AUG $\longrightarrow$ 80S, assembly of ribosomes and protein translation (rate k_3).
4) 80S $\longrightarrow$ 60S+40S, recycling of ribosomal subunits (rate k_4).

The model is described by the following system of nonlinear ordinary differential equations:

$$\begin{aligned}
\frac{dm_1}{dt} &= k_4 m_4 - k_1 m_1 m_6 \\
\frac{dm_2}{dt} &= k_1 m_1 m_6 - k_2 m_2 \\
\frac{dm_3}{dt} &= k_2 m_2 - k_3 m_3 m_5 \\
\frac{dm_4}{dt} &= k_3 m_3 m_5 - k_4 m_4 \\
\frac{dm_5}{dt} &= k_4 m_4 - k_3 m_3 m_5 \\
\frac{dm_6}{dt} &= k_2 m_2 - k_1 m_1 m_6 \\
\frac{dm_7}{dt} &= k_3 m_3 m_5 - k_5 m_7
\end{aligned} \quad (4)$$

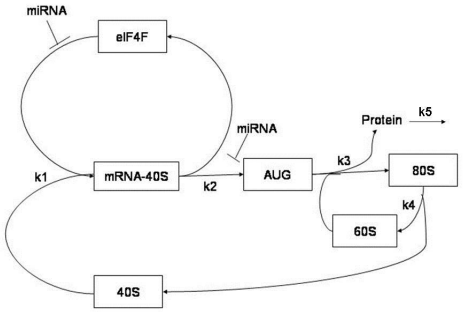

Fig. 1. Biochemical diagram of protein translation process repressed by microRNAs

where m_1 is the concentration of free 40S ribosomal subunits, m_2 is the concentration of 40S subunit bound to the initiation site of mRNA, m_3 is the concentration of AUG, the initiation complex bound to the start codon of mRNA, m_4 is the concentration of 80S, the ribosomes translating protein, m_5 is the concentration of 60S subunit joining factors, m_6 is the concentration of eIF4F, the free translation initiation factors and m_7 is the protein concentration. In fact, our modification consists of adding a term for protein decay in the last equation of (4). Otherwise, the protein will constantly increase, which is biologically impossible.

The notations k_i $(i = 1, 2, ..., 5)$ present the rate constants of bimolecular reactions involved in the process and have the following numerical values

$$k_1 = 2;\ k_2 = 2;\ k_3 = 5;\ k_4 = 1;\ k_5 = 1 \tag{5}$$

The numerical values of k_i $(i = 1, ..., 4)$ are taken from [4], and the rate constant for the protein decay k_5 is taken from [8]. The methodology for separation of the complete system to fast and slow subsystems [6] lies on pure mathematical basis. In principle it involves separate scaling (normalization) of reaction rates as well as chemical concentrations on the basis of well known data of their numerical values. We propose a normalized (scaling) procedure, which is a similar to the dimensionless principle. It requires each term in the right-hand side of the system equations to have an order of one. Unlike most models in systems biology, including cell signaling pathways, immunological processes, etc., where the rate constants differ by at least one numeric order, the rate constants of the protein translation process have one and same order. Therefore we do not normalize them. We will normalize (scale) only the system variables. For the purpose we simulate dynamics of the considered process for a period of 10 seconds taking into account the numerical values of the parameters (4) and the initial values [100, 0, 0, 25,0, 6, 0] of the variables, taken from [4]. Next, we select the values near the settled (steady state) ones in order to use them as characteristic values of state variables in accordance with the QSS assumption They are:

$$\begin{aligned} m_1^0 = 82.04;\ m_2^0 = 5,93;\ m_3^0 = 0,18; \\ m_4^0 = 11,85;\ m_5^0 = 13,14; \\ m_6^0 = 0,07;\ m_7^0 = 11.85; \end{aligned} \tag{6}$$

The parameters and concentration values shown above are given here without units in view of the fact that we do not intend to compare them. What is of interest for us is not to compare parameters or concentrations, but the terms in (4). In accordance with the normalized (scaling) procedure, which we apply here each term in the right-hand side of the system equations must have an order of one. Towards this end, we introduce normalized (scaling) substitutions only for the model variables. They have the form

$$\begin{aligned} m_1 = \frac{x_1}{\epsilon};\ m_2 = x_2;\ m_3 = x_3;\ m_4 = \frac{x_4}{\epsilon}; \\ m_5 = \frac{x_5}{\epsilon};\ m_6 = \epsilon x_6;\ m_7 = \frac{x_7}{\epsilon}; \end{aligned} \tag{7}$$

where $\epsilon = 0.01$. After replacing (7) in (4) we obtain the following system in a normalized form

$$\frac{dx_1}{dt} = k_4 x_2 - \epsilon k_1 x_1 x_6 \tag{8}$$

$$\frac{dx_2}{dt} = k_1 x_1 x_6 - k_2 x_2 \tag{9}$$

$$\epsilon \frac{dx_3}{dt} = \epsilon k_2 x_2 - k_3 x_3 x_5 \tag{10}$$

$$\frac{dx_4}{dt} = k_3 x_3 x_5 - k_4 x_4 \tag{11}$$

$$\frac{dx_5}{dt} = k_4 x_4 - k_3 x_3 x_5 \tag{12}$$

$$\epsilon \frac{dx_6}{dt} = k_2 x_2 - k_1 x_1 x_6 \tag{13}$$

$$\frac{dx_7}{dt} = k_3 x_3 x_5 - k_5 x_7 \tag{14}$$

Here x_i $(i = 1, 2, ..., 7)$ are scaling state variables of order of unity. This means that, in accordance with the terminology of QSSA theorem, we can say that the equations (10) and (13) form an attached system, and the

equations (8), (9), (11), (12) and (14) form a degenerated one. The set of both systems is called a complete system. In this way AUG, the initiation complex bound to the start codon of mRNA and the free translation initiation factors eIF4F are fast participants in the microRNA-mediated protein translation process and the free 40S ribosomal subunits, the 40S - mRNA complex, the ribosomes translating protein 80S, the subunit joining factors 60S and the protein are its slow components.

Next, following the QSSA theorem for the equilibrium values x_3^0 and x_6^0 the following expressions are valid:

$$x_3^0 = \frac{\epsilon k_2 x_2}{k_3 x_5} > 0 \qquad x_6^0 = \frac{k_2 x_2}{k_1 x_1} > 0 \tag{15}$$

As it is seen in the right-hand sides of (15) the slow varying (not equilibrium) concentrations x_i $(i = 1, 2, 5)$ are involved in accordance with the QSSA theorem. The equilibrium solution (x_3^0, x_6^0) is unique and stable one for the attached system of equations in view of the fact that the variational equations

$$\frac{d\xi}{dt} = -\frac{a_3}{\epsilon} x_5^0 \xi; \quad \frac{d\eta}{dt} = -\frac{a_1}{\epsilon} x_1^0 \eta \tag{16}$$

tend asymptotically to zero, where ξ and η are variations around the stationary values x_3^0 and x_5^0, respectively. Thus the assertions (a) and (b) of the theorem are satisfied. That allows us to substitute the expressions (15) in the five equations of the degenerated system. As a result the following QSSA of the original model (4) is obtained by using reverse substitutions (7) :

$$\begin{aligned} \frac{dm_1}{dt} &= k_4 x_4 - k_2 m_2^i \\ \frac{dm_2}{dt} &= k_2 x_2 - k_2 m_2 = 0 \\ \frac{dm_4}{dt} &= k_2 m_2^i - k_4 m_4 \\ \frac{dm_5}{dt} &= k_4 m_4 - k_2 m_2^i \\ \frac{dm_7}{dt} &= k_2 m_2^i - k_5 m_7 \end{aligned} \tag{17}$$

It is seen the last system consists already four linear ordinary differential equations. After our mathematical transformations the variable m_2, representing dynamics of the [40S-mRNA] complex appears to be a constant. By this reason we express it by its initial (post-initial) value in the other equations of (17). So far, our theoretical result related to the persistent behaviour of the [40S-mRNA] complex has not reported in similar investigations of the microRNA-mediated protein translation dynamics. In this way it could be experimentally verified. However, as it can be seen from the complete (4) and the degenerate (17) systems, it determines the behaviour of the fast varying components, as well as slow varying concentrations. Coincidence between the complete and degenerate systems observes after the 1/10 second from the beginning of the protein translation process as it can be seen from Fig. 2. Coincidence between the graphs of m_4 and m_7 exists too (see Fig. 2).

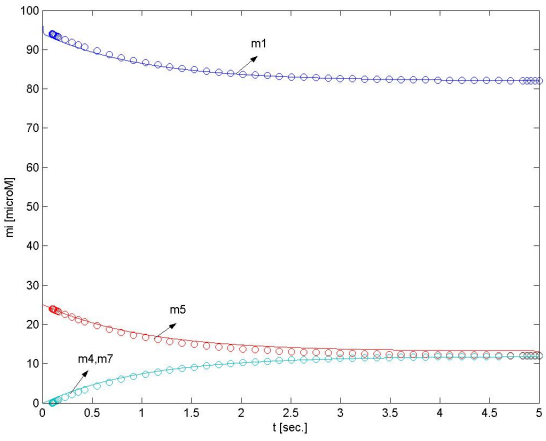

Fig. 2. Coincidence of the graphs of complete (solid lines) and reduced degenerate (dotted lines) system solutions

B. A Consequence of the Quasi-Stationary System on the Dynamical Behaviour of the MicroRNA-mediated Protein Translation Process

In this section we will demonstrate the advantages from the made quasi-steady state approximation for understanding of the main reaction mechanism of microRNA -mediated protein translation in details. In the simplest case, the reduced (degenerate) system is a subsystem of the complete system, as it can be seen from equations (1–2). However, from a biological point of view, it also includes new driving reactions with kinetic rates expressed through the parameters of the complete model, and rates of some reactions are renormalized. Moreover, the quasi-stationary system is conducted by limited number variables (slow variables), playing the role of “drivers” of the subordinated fast variables of the original system according the terminology of the QSSA theorem. In the concrete case the driving reactions in the quasi-stationary protein translation process are the scanning the 5’UTR by the start codon AUG recognition (reaction 2 from the subsection A,

promoting by the rate k_2) and the recycling of the ribosomal subunits (reaction 4 from the same subsection, promoting by the rate k_4). Therefore we can conclude that microRNAs act on one (or both) of these later stages of the translation initiation. Analysis of the reduced system can also answer an important question: Which are control parameters in the microRNA - mediated protein translation process? Many of the parameters of the original model (complete system) are no longer presented and we can ignore them in some later time period. Parameters presented in the reduced system (control parameters) can provoke changes in the dynamical behavior of the quasi-stationary system as well as of the original model. In this case the control parameters reduce to two: k_2 and k_4. It is of interest to see their influence mainly on the protein behaviour. For the purpose numerical simulations of the protein dynamics for different values of k_2 and k_4 are presented in Fig. 3 and Fig. 4. As we mentioned above, although k_2 and k_4 are not presented in the corresponding equation for the protein production of the complete model they influence on the protein dynamics. Fig. 3 and Fig. 4 show that the protein production decreases at essentially lower values of the rate constants. Moreover, unlike the results shown in Fig. 3, changes in protein production, depending on k_4 differ by at least one order (The changes in the protein dynamics start to observe just at $k_4 = 0.3$). In addition, the reduced system is guided only by the variables m_2 (its post-initial value) and m_4, in view of the fact that they involve in the right-hand sides of the equations (17) excepting the equation of protein production, where m_4 is not presented.

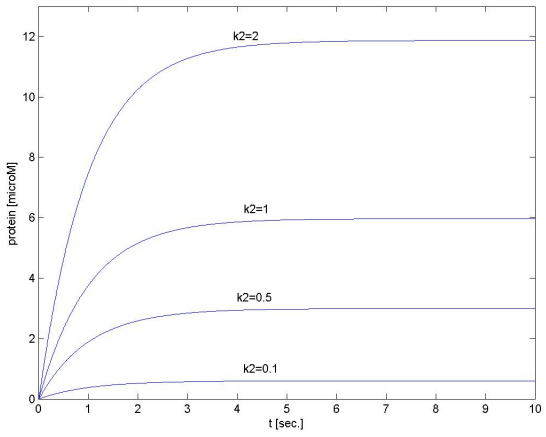

Fig. 3. Graphs of protein production for k_2=0.1; 0.5; 1; 2

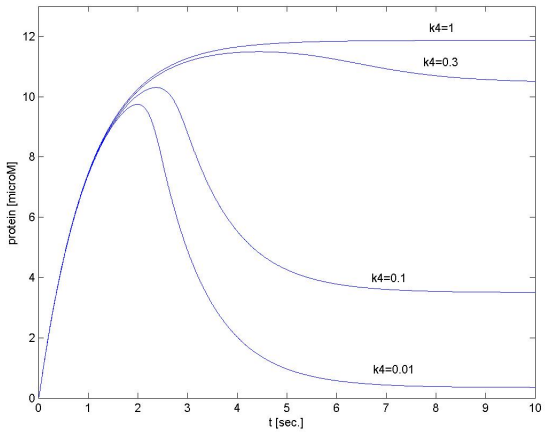

Fig. 4. Graphs of protein production for k_4=0.01; 0.3;0.1; 1

C. Analytical Derivation of the Degenerate System Solution

Here we will support the arguments made in the previous paragraph in a pure mathematical aspect. For the purpose the degenerate system (17) is analytically solved. Further we consider its solution in infinity and derive analytical relationships between steady-state (denoted by '0' upper indexes) and initial (denoted by 'i' upper indexes) values of the slow varying genetic concentrations. They are:

$$m_1^0 = m_1^i + (m_4^i - \frac{k_2 m_2^i}{k_4}; m_2^0 = m_2^i; m_4^0 = \frac{k_2 m_2^i}{k_4};$$
$$m_5^0 = m_5^i + (m_4^i - \frac{k_2 m_2^i}{k_4}); m_7^0 = \frac{k_2 m_2^i}{k_5} \quad (18)$$

The last values, however, take a place in the formulas for the steady-state values of the fast varying concentrations m_3 and m_6. The corresponding formulas are easy to obtain from (15) by substituting there the corresponding relations (7) and taking into account the relations (18). They are:

$$m_3^0 = \frac{k_2 m_2^i}{k_3(m_5^i + m_4^i - k_2 m_2^i/k_4)}$$
$$m_6^0 = \frac{k_2 m_2^i}{k_3(m_1^i + m_4^i - k_2 m_2^i/k_4)} \quad (19)$$

It is seen that the control parameters k_2 and k_4 as well as the "driver" concentrations m_2 and m_4 are involved in (18–19). This means, by changing their values we can essentially control the quasi-stationary genetic process

in terms of input (initial values) and output (stationary values) relationships.

IV. Conclusion

It is shown that the considerations of time hierarchy in biomolecular reactions allows us to find the simplest model of the microRNA-mediated protein translation process, which can substitute a multiscale genetic network such that the dynamics of the complete network can be approximated by the simpler one. This is achieved by applying a well-known QSSA theorem as a basic approach for system reduction. Analysis of the degenerate model help us to derive the following conclusions: 1) MicroRNAs act on the later stages of the translation initiation, such as AUG recognition (cap-independent initiation steps) or recycling of the ribosomal subunits; 2) The rate constants k_2 and k_4 can be considered as control parameters of the protein translation system; 3) The post-initial concentrations of 40S subunit bound to the initiation site of mRNA and ribosomes translating protein [80S] become an important factors when the genetic process approaches its quasi-stationary state; 4) The obtained relationships between the steady-state and initial values of the biomolecular concentrations can be considered as restrictions on participants in the protein translation process, repressed by microRNA. They can be experimentally verified and can be used for direct computation of steady states of the genetic components, especially when kinetic information is incomplete.

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