Dynamics and Complexity in a Time Delay Model of RNA Silencing with Periodic Forcing

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Abstract: *Simple periodic behavior and occurrence of complex oscillatory phenomena underlie of a large number of biochemical system models. In many cases the transition from stable to simple/complex oscillatory behavior can be connected with the appearance of abnormal process likes as cancer. In this paper we propose a time delay model of RNA silencing (also known as RNA interference) with periodic forcing. In organisms with RNA silencing, each cell has a miniature "immune system" able to generate and amplify specific responses to a variety of gene transcripts. The consequences of a time delay on the dynamics of this model are analysed using Hopf's theorem. Our analytical calculations predict that time delay acts as a key bifurcation parameter. From the accomplished numerical results, it becomes clear that model has complexity oscillatory behavior when the amplitude of periodic force (i.e. the confusion in the target mRNA synthesis) is large.*

Keywords: *RNA silencing, Time delay model, Bifurcation analysis.*

Introduction

In modeling in the biological, physical and engineering sciences, it is sometimes necessary to take account of time delays inherent in the phenomena. The inclusion of delays in the equations is often a simplification or idealization that is introduced because a detailed description of underlying processes is too complicated to be modeled mathematically or because some of the details are unknown [3, 5]. In the recent years many models have been used to investigate the role of phosphorylation, positive or negative feedback regulation of transcription factors, and gene expression multistability. For instance, time delay, especially discrete delay, emerges in biochemical system models, models of reduction and regulation of blood cells, and gene regulatory systems [1, 18, 19, 20, 22].

Various authors have previously considered biochemical oscillators with time delay [15] and the usefulness of bifurcation analysis to investigate properties of time delay biochemical networks [14, 22]. Their analyses show that the introduction of a large enough time delay can some times change the unique equilibrium of the system and induce periodic solutions (self-oscillations), which arise from the equilibrium through an Andronov-Hopf bifurcation. From the point of view of the dynamic systems theory, the Hopf bifurcation theorem [12] together with other elements of the bifurcation theory are basic analytical tools to investigate pathological conditions in biological systems. The qualitative knowledge on the dynamics of the systems emerging from this analysis can help in the development of diagnostic methods and the choice of rational therapeutic strategies [8, 24].

To understanding basic biological processes and role of the gene product in a particular disease we must know the function of a gene product. In the recent years a new technique has been described for determining gene function in mammalian cells [23]. This method exploits

the RNA interference (RNAi) pathway [10, 13]. RNAi is induced by the introduction of double stranded RNA (dsRNA) into the cell where it is cleaved by the action of Dicer enzyme into short dsRNA molecules, 21-25 bp in length, called short interfering RNAs (siRNAs). siRNAs interact with proteins in the cytoplasm to form a ribonucleoprotein complex known as dsRNA-induced silencing complex (RISC). Using the antisense strand of the siRNA as a guide, RISC associates with and cleaves the mRNA of identical sequence. The cleaved mRNA is then degraded by nonspecific RNases. This mechanism is shown in Fig. 1. In organisms with RNA silencing, each cell has a miniature "immune system" able to generate and amplify specific responses to a variety of gene transcripts [21, 25]. In other words, by silencing a gene, we can stop or significantly reduce the production of the specific protein encoded by the target gene. In [4], a mathematical description of a conceptual of the RNA silencing process is presented.
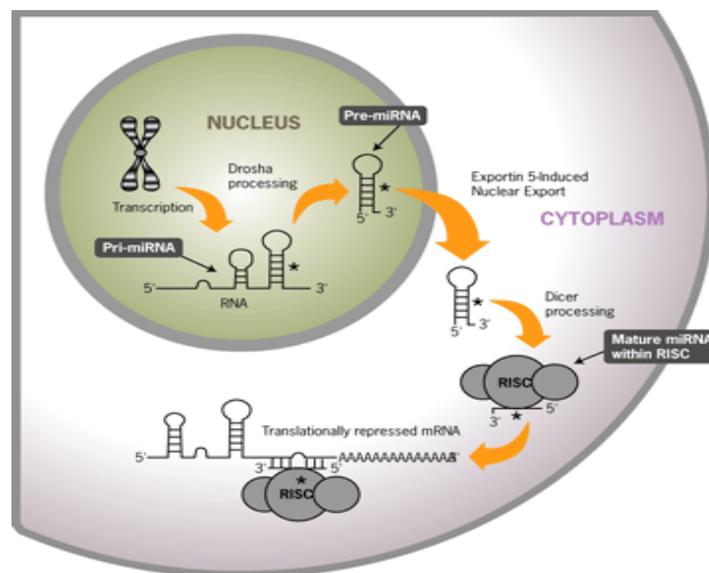

Fig. 1 miRNA processing and activity

Fig. 2 shows a schematic outline of the basic elements comprising this model. On the basis of the steps denoted in Fig. 2, Bergstrom and co-authors in [4] obtain an autonomous system of four ordinary nonlinear differential equations. Later, Nikolov and Petrov [17], and Nikolov [19] presented and investigated bifurcation behavior of a model of RNA silencing with one and two time delays, where the delay function $C(t-\tau)$ expresses the assumption that the net rate of dsRNA degradation by Dicer and background process as well as the net rate of dsRNA loss are proportional, thus triggering the process of mRNA binding to form the RISC-mRNA complex at the moment $(t-\tau)$.
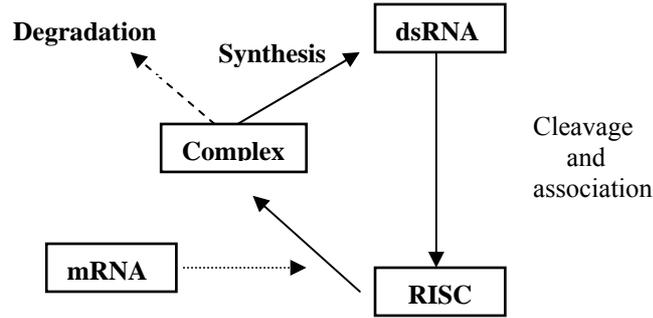
Fig. 2 Schematic diagram of the basic RNA silencing model according to Bergstrom et al. [4]

In [17], in order to make the analytical investigation of time delay system easier, we assume that two times - of the regeneration and degradation of RISC-mRNA are equal. Of course, the finite time τ_1 of the regeneration can be different from that of the degeneration τ_2. Here, we also assume that $\tau = \tau_1 = \tau_2$. Hence, we obtain a system with one time delay in the form

$$\frac{dD}{dt} = -a.D + g.C(t-\tau),$$

$$\frac{dR}{dt} = an.D - d_R.R - b.RM,$$

$$\frac{dC}{dt} = b.RM - (g + d_c).C(t-\tau),$$

$$\frac{dM}{dt} = \Omega - d_M M - b.RM,$$

$$(1)$$

where the state variables D, R, C, M represent the concentrations of the dsRNA, RISC, RISC-mRNA complex, and mRNA respectively, at time t. With $a, b, d_c, d_M, d_R, g, n$ and Ω are denoted the kinetic rate constants. Here we note that constant Ω is periodical function of time t, i.e. external periodic perturbation, which describe the confusion in the target mRNA synthesis. From mathematical point of view it is an example of periodic forcing [16] (and references there). Thus, for Ω we assume

$$\Omega = h + h_1 \sin \varepsilon t \qquad (2)$$

It is well known that nonlinear dynamics systems are sensitive to structural changes of the latter and simple additive perturbations (periodic forcing) can change the global behavior of a given system. Generally, it cannot be predicted in advance what the global response of the system to such changes will be, even in the case of additive periodic perturbations like Ω in the system (1). Hence, to study this effect, in the present paper we investigate the dynamics and bifurcation behavior of the system (1).

The plan of the paper is as follows: in next sections we derive the governing equations of the time delay model, analyze them by nonlinear dynamics methods (when the time delay is a bifurcation parameter), and solve them numerically. Finally, we summarize and discuss our analytical and numerical results.

Bifurcation analysis
Before to start with our analysis of the system (1) let us denote

$$v = \sin \varepsilon t, \quad u = \cos \varepsilon t. \tag{3}$$

Obviously

$$\frac{dv}{dt} = \varepsilon u, \quad \frac{du}{dt} = -\varepsilon v. \tag{4}$$

After substitution of Eq. (3) and Eq. (4) into system (1), the non-autonomous forth first-order delay ordinary differential equations (1) are reduced to autonomous six first order delay differential equations

$$\frac{dD}{dt} = -a.D + g.C(t - \tau),$$

$$\frac{dR}{dt} = an.D - d_R.R - b.RM,$$

$$\frac{dC}{dt} = b.RM - (g + d_c).C(t - \tau),$$

$$\frac{dM}{dt} = h + h_1 v - d_M M - b.RM, \tag{5}$$

$$\frac{dv}{dt} = \varepsilon u,$$

$$\frac{du}{dt} = -\varepsilon v.$$

Hence, system (5) has two steady states: the trivial

$$\left(\bar{D} = \bar{C} = \bar{R} = \bar{v} = \bar{u} = 0, \bar{M} = h/d_M \right) \quad \text{and}$$

$$\bar{D} = \frac{g}{a}\bar{C}, \bar{R} = \frac{\varsigma}{d_R}\bar{C}, \bar{C} = \frac{h}{g + d_c} - \frac{d_M d_R}{b\varsigma}, \bar{M} = \frac{(g + d_c)d_R}{b\varsigma}, \bar{v} = \bar{u} = 0,$$

where $\varsigma = [g(n-1) - d_c]$. Furthermore, we investigate the bifurcation behavior - particularly the Andronov-Hopf bifurcation - for system (5), using time delay τ as bifurcation parameter. First, we obtain the characteristic equation for linearization of system (5) near the equilibrium $\bar{E}\left(\bar{D} > 0, \bar{C} > 0, \bar{R} > 0, \bar{M} > 0, \bar{v} = \bar{u} = 0 \right)$, i.e. when the silencing reaction controls the level of mRNA below its normal level. Next, we consider a small perturbation about the equilibrium level, i.e. $D = \bar{D} + x, R = \bar{R} + y, C = \bar{C} + z, M = \bar{M} + w$. Substituting these into the differential equations in system (5), we have

$$\frac{dx}{dt} = -ax + g\ell^{-\tau\chi}z,$$

$$\frac{dy}{dt} = anx - a_1 y - a_2 w - byw,$$

$$\frac{dz}{dt} = a_3 y - a_4\ell^{-\tau\chi}z + a_2 w + byw,$$

(6)

$$\frac{dw}{dt} = -a_3 y - a_5 w - byw + h_1 v,$$

$$\frac{dv}{dt} = \varepsilon u,$$

$$\frac{du}{dt} = -\varepsilon v,$$

where $a_1 = d_R + b\bar{M}$, $a_2 = b\bar{R}$, $a_3 = b\bar{M}$, $a_4 = g + d_C$, $a_5 = d_M + b\bar{R}$. Hence, we obtain the stability matrix in the form

$$\begin{pmatrix} -a & 0 & g\ell^{-\tau\chi} & 0 & 0 & 0 \\ an & -a_1 & 0 & -a_2 & 0 & 0 \\ 0 & a_3 & -a_4\ell^{-\tau\chi} & a_2 & 0 & 0 \\ 0 & -a_3 & 0 & -a_5 & h_1 & 0 \\ 0 & 0 & 0 & 0 & 0 & \varepsilon \\ 0 & 0 & 0 & 0 & -\varepsilon & 0 \end{pmatrix}$$

(7)

The stability matrix (7) leads to the following characteristic equation:
$$\chi^6 + K_1\chi^5 + K_2\chi^4 + K_3\chi^3 + K_4\chi^2 = \ell^{-\tau\chi}\left(T_1\chi^5 + T_2\chi^4 + T_3\chi^3 + T_4\chi^2 + T_5\chi + T_6\right)$$

(8)

where
$$K_1 = a + a_1 + a_5,$$
$$K_2 = a(a_1 + a_5) + a_1 a_5 + \varepsilon^2 - a_2 a_3,$$
$$K_3 = \varepsilon^2(a + a_1 + a_5) + a(a_1 a_5 - a_2 a_3),$$
$$K_4 = \varepsilon^2(a_1 a_5 - a_2 a_3 + aa_5),$$
$$T_1 = -a_4,$$
$$T_2 = -a_4(a + a_1 + a_5),$$
$$T_3 = anga_3 + a_4(a_2 a_3 - aa_1 - a_1 a_5 - aa_5 - \varepsilon^2),$$
$$T_4 = a(a_2 a_3 a_4 + nga_3 a_5 - a_1 a_4 a_5 - nga_2 a_3) - a_4 a_5 \varepsilon^2,$$
$$T_5 = a_4 a_5 \varepsilon^2(a - a_1),$$
$$T_6 = a\varepsilon^2\left[a_2 a_3(a_4 - ng) + a_5(a_1 a_4 - nga_3)\right]$$

(9)

This characteristic Eq. (8) is transcendental and can not be solved analytically. Moreover, it has an indefinite number of roots [6, 7, 19]. The stability of equilibrium state depends on the

sign of the real parts of the roots of Eq. (8). We let $\chi = m + in$ $(m, n \in R)$ and rewrite Eq. (8) in terms of its real and imaginary parts as

$$
\begin{vmatrix}
m^6 - n^6 - 15m^4n^2 + 15m^2n^4 + K_1\left(m^5 - 10m^3n^2 + 5mn^4\right) + K_2\left(m^4 + n^4 - 6m^2n^2\right) + \\
+ K_3\left(m^3 - 3mn^2\right) + K_4\left(m^2 - n^2\right) = \\
= \ell^{-m\tau}\left\{T_1\left[\left(m^5 - 10m^3n^2 + 5mn^4\right)\cos n\tau + \left(n^5 + 5m^4n - 10m^2n^3\right)\sin n\tau\right] + \right. \\
+ T_2\left[\left(m^4 + n^4 - 6m^2n^2\right)\cos n\tau + 4\left(m^3n - mn^3\right)\sin n\tau\right] + \\
+ T_3\left[\left(m^3 - 3mn^2\right)\cos n\tau + \left(3m^2n - n^3\right)\sin n\tau\right] + T_4\left[\left(m^2 - n^2\right)\cos n\tau + 2mn\sin n\tau\right] + \\
+ T_5\left[m\cos n\tau + n\sin n\tau\right] + T_6\cos n\tau\right\}, \\
6m^5n - 20m^3n^3 + 6mn^5 + K_1\left(n^5 + 5m^4n - 10m^2n^3\right) + 4K_2\left(m^3n - mn^3\right) + K_3\left(3m^2n - n^3\right) + \\
+ 2K_4mn = \ell^{-m\tau}\left\{T_1\left[\left(n^5 + 5m^4n - 10m^2n^3\right)\cos n\tau - \left(m^5 - 10m^3n^2 + 5mn^4\right)\sin n\tau\right] + \right. \\
+ T_2\left[4\left(m^3n - mn^3\right)\cos n\tau - \left(m^4 + n^4 - 6m^2n^2\right)\sin n\tau\right] + \\
+ T_3\left[\left(3m^2n - n^3\right)\cos n\tau - \left(m^3 - 3mn^2\right)\sin n\tau\right] + T_4\left[2mn\cos n\tau - \left(m^2 - n^2\right)\sin n\tau\right] + \\
+ T_5\left(n\cos n\tau - m\sin n\tau\right) - T_6\sin n\tau\right\},
\end{vmatrix}
\tag{10}
$$

To find the first bifurcation point we look for purely imaginary roots $\chi = \pm in$, $n \in R$, of Eq. (8), i.e. we set $m = 0$. Then the above two equations are reduce to

$$
\begin{vmatrix}
-n^6 + K_2n^4 - K_4n^2 = \left(T_1n^5 - T_3n^3 + T_5n\right)\sin n\tau + \left(T_2n^4 - T_4n^2 + T_6\right)\cos n\tau, \\
K_1n^5 - K_3n^3 = \left(T_1n^5 - T_3n^3 + T_5n\right)\cos n\tau + \left(-T_2n^4 + T_4n^2 - T_6\right)\sin n\tau
\end{vmatrix}
\tag{11}
$$

or another one

$$
\begin{aligned}
\cos n\tau &= \frac{n^2\left[\left(-n^4 + K_2n^2 - K_4\right)\left(T_2n^4 - T_4n^2 + T_6\right) + n^2\left(T_1n^4 - T_3n^2 + T_5\right)\left(K_1n^2 - K_3\right)\right]}{n^2\left(T_1n^4 - T_3n^2 + T_5\right)^2 + \left(-T_2n^4 + T_4n^2 - T_6\right)^2}, \\
\sin n\tau &= \frac{n^3\left[\left(K_1n^2 - K_3\right)\left(-T_2n^4 + T_4n^2 - T_6\right) + \left(T_1n^4 - T_3n^2 + T_5\right)\left(-n^4 + K_2n^2 - K_4\right)\right]}{n^2\left(T_1n^4 - T_3n^2 + T_5\right)^2 + \left(-T_2n^4 + T_4n^2 - T_6\right)^2}
\end{aligned}
\tag{12}
$$

Note that $n = 0$ can be a solution of Eqs. (12) if $T_6^2 = 0$. If the first bifurcation point is $\left(n_b^0, \tau_b^0\right)$ satisfy $n_b\tau_b = n_b^0\tau_b^0 + 2\upsilon\pi$, $\upsilon = 1, 2, ..., \infty$.

One can notice that if n is a solution of Eqs. (12) (or system (11)), then so is $-n$. Hence, in the following, we only look for positive solutions n of system (11), or Eqs. (12) respectively. By squaring the two equations into system (11) and then adding them, it follows that n must be a root of the following equation:

$$
\begin{aligned}
&n^{12} + \left(K_1^2 - T_1^2 - 2K_2\right)n^{10} + \left[K_2^2 - T_2^2 + 2\left(K_4 - K_1K_3 + T_1T_3\right)\right]n^8 + \\
&\quad + \left[K_3^2 - T_3^2 + 2\left(T_2T_4 - K_2K_4 - T_1T_5\right)\right]n^6 + \left[K_4^2 - T_4^2 + 2\left(T_3T_5 - T_2T_6\right)\right]n^4 + \\
&\quad + \left(2T_4T_6 - T_5^2\right)n^2 - T_6^2 = 0.
\end{aligned}
\tag{13}
$$

Here, we note that this is a hexatic equation about n^2 and that the left side is positive for large values of n^2 and negative for $n = 0$ because $-T_6^2$ is always negative, i.e. Eq. (13) has at least one positive real root. Moreover, to apply Hopf bifurcation theorem the following theorem in this situation applies:

Theorem 1.

Suppose that n_b is the least positive simple root of Eq. (13). Then, $in(\tau_b) = in_b$ is a simple root of Eq. (8) and $m(\tau) + in(\tau)$ is differentiable with respect to τ in a neighborhood of $\tau = \tau_b$.

The proof of this theorem in details can be founded in [11].

To establish an Andronov-Hopf bifurcation at $\tau = \tau_b$, we need to show that the following transversality condition $\left. \dfrac{dm}{d\tau} \right|_{\tau=\tau_b} \neq 0$ is satisfied.

Hence, if we denote
$$H(\chi, \tau) = \chi^6 + K_1\chi^5 + K_2\chi^4 + K_3\chi^3 + K_4\chi^2 - \ell^{-\tau\chi}\left(T_1\chi^5 + T_2\chi^4 + T_3\chi^3 + T_4\chi^2 + T_5\chi + T_6\right) \quad (14)$$

then

$$\frac{d\chi}{dt} = -\left. \frac{\partial H}{\partial \tau} \middle/ \frac{\partial H}{\partial \chi} \right. = \frac{-\chi\ell^{-\tau\chi}\left(T_1\chi^5 + T_2\chi^4 + T_3\chi^3 + T_4\chi^2 + T_5\chi + T_6\right)}{6\chi^5 + 5K_1\chi^4 + 4K_2\chi^3 + 3K_3\chi^2 + 2K_4\chi + P_1 + P_2} \quad (15)$$

where
$$\begin{aligned}
P_1 &= \tau\ell^{-\tau\chi}\left(T_1\chi^5 + T_2\chi^4 + T_3\chi^3 + T_4\chi^2 + T_5\chi + T_6\right), \\
P_2 &= -\ell^{-\tau\chi}\left(5T_1\chi^4 + 4T_2\chi^3 + 3T_3\chi^2 + 2T_4\chi + T_5\right).
\end{aligned} \quad (16)$$

Evaluating the real part of this equation at $\tau = \tau_b$ and setting $\chi = in_b$ yield
$$\begin{aligned}
\left. \frac{dm}{d\tau} \right|_{\tau=\tau_b} &= \left. \mathrm{Re}\left(\frac{d\chi}{d\tau}\right) \right|_{\tau=\tau_b} = \\
&= \frac{n_b^2\left\{6n_b^{10} + 5\left(K_1^2 - T_1^2 - 2K_2\right)n_b^8 + 4\left[K_2^2 - T_2^2 + 2\left(K_4 - K_1K_3 + T_1T_3\right)\right]n_b^6\right\}}{L^2 + I^2} + \\
&\quad + \frac{n_b^2\left\{3\left[K_3^2 - T_3^2 + 2\left(T_2T_4 - K_2K_4 - T_1T_5\right)\right]n_b^4 + 2\left[K_4^2 - T_4^2 + 2\left(T_3T_5 - T_2T_6\right)\right]n_b^2 + 2T_4T_6 - T_5^2\right\}}{L^2 + I^2},
\end{aligned} \quad (17)$$

where
$$\begin{aligned}
L &= n_b^2\left(5K_1n_b^2 - 3K_3\right) + \tau_b\left(-n_b^4 + K_2n_b^2 - K_4\right)n_b^2 - \left(5T_1n_b^4 - 3T_3n_b^2 + T_5\right)\cos n_b\tau_b + \\
&\quad + 2n_b\left(2T_2n_b^2 - T_4\right)\sin n_b\tau_b
\end{aligned}$$
and
$$\begin{aligned}
I &= \tau_b\left(K_1n_b^2 - K_3\right)n_b^3 + 2n_b\left(3n_b^4 - 2K_2n_b^2 + K_4\right) + \\
&\quad + 2n_b\left(T_2n_b^2 - T_4\right)\cos n_b\tau_b + \left(5T_1n_b^4 - 3T_3n_b^2 + T_5\right)\sin n_b\tau_b.
\end{aligned}$$

Let $\theta = n_b^2$. Then, Eq. (13) reduces to

$$f(\theta) = \theta^6 + (K_1^2 - T_1^2 - 2K_2)\theta^5 + [K_2^2 - T_2^2 + 2(K_4 - K_1K_3 + T_1T_3)]\theta^4 +$$
$$+ [K_3^2 - T_3^2 + 2(T_2T_4 - K_2K_4 - T_1T_5)]\theta^3 + [K_4^2 - T_4^2 + 2(T_3T_5 - T_2T_6)]\theta^2 +$$
$$+ (2T_4T_6 - T_5^2)\theta - T_6^2 = 0.$$

Then, for $f'(\theta)$ we have

$$f'(\theta)\big|_{\tau=\tau_b} = \frac{df}{d\theta}\bigg|_{\tau=\tau_b} = 6\theta^5 + 5(K_1^2 - T_1^2 - 2K_2)\theta^4 + 4[K_2^2 - T_2^2 + 2(K_4 - K_1K_3 + T_1T_3)]\theta^3 +$$
$$+ 3[K_3^2 - T_3^2 + 2(T_2T_4 - K_2K_4 - T_1T_5)]\theta^2 + 2[K_4^2 - T_4^2 + 2(T_3T_5 - T_2T_6)]\theta + 2T_4T_6 - T_5^2$$

If n_b is the least positive simple root of Eq. (13), then

$$\frac{df}{d\tau}\bigg|_{\theta=n_b^2} > 0. \tag{18}$$

Hence,

$$sign\left(\frac{dm}{d\tau}\bigg|_{\tau=\tau_b}\right) = sign\left\{\frac{d\chi}{d\tau}\bigg|_{\tau=\tau_b}\right\} = \frac{n_b^2 f'(n_b^2)}{L^2 + I^2} > 0. \tag{19}$$

According to the Hopf bifurcation theorem [12], we define the following theorem:
Theorem 2.
   If n_b is the least positive root of Eq. (13), then an Andronov-Hopf bifurcation occurs as τ passes through τ_b.

As a consequence of our analysis, we can predict that a limit cycle will emerge if the time delay is higher than τ_b, while the limit cycle will vanish if the time delay is smaller. In other words, we may conclude that in this case the time delay has a destabilizing role because it changes drastically the properties of the system when pass through the bifurcation point provoking the emergence of a limit cycle.

Numerical analysis
In this section, we illustrate numerically the different stability results obtained for time delay system (1) in previous section. We also focus on periodic and complex solutions appearing through an Andronov-Hopf bifurcation. The corresponding numerical values of the model parameters are those in [2, 4, 17], i.e.
$$a = 10, b = 0.1, h = 2, g = 0.1, d_M = 0.5, d_R = 0.1, d_C = 0.05, n = 5, \tau \in [1, 13]. \tag{20}$$

The dimension of rate constants into (20) is $hours^{-1}$ and of time delay - $hours$. In view of lack of data for the parameters h_1 and ε we assume to vary h_1 and fix $\varepsilon = 0.01$.

Fig. 3 depicts the solution of system (1), in the case when parameter τ is smaller than the bifurcation value. It is seen that the variables describing dsRNA, D, RISC, R, RISC-mRNA complex, C and mRNA, M approach to constant values that describe a steady state of the

system. In other words the system possesses a stable equilibrium state which corresponds to a normal silencing process.
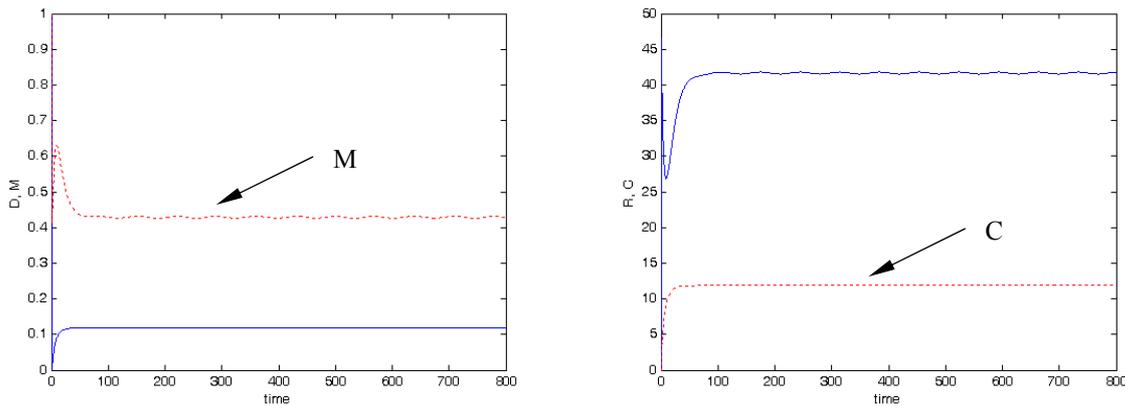

Fig. 3 Stable solution of system (1) at $h_1 = 0.01$ and $\tau = 1$

Fig. 4 elucidates the effect of destabilization of the time delay on the behavior of system (1). It is evident that for the same values of the rate constants (only time delay is larger than the bifurcation value τ_b), after the Andronov-Hopf bifurcation, the stable limit cycle with period close to one occurs and system (1) has periodic solutions. Thus, we may conclude that the time delay has a destabilization effect on the RNA silencing process. These results are in accordance with the analytical results obtained in previous section.
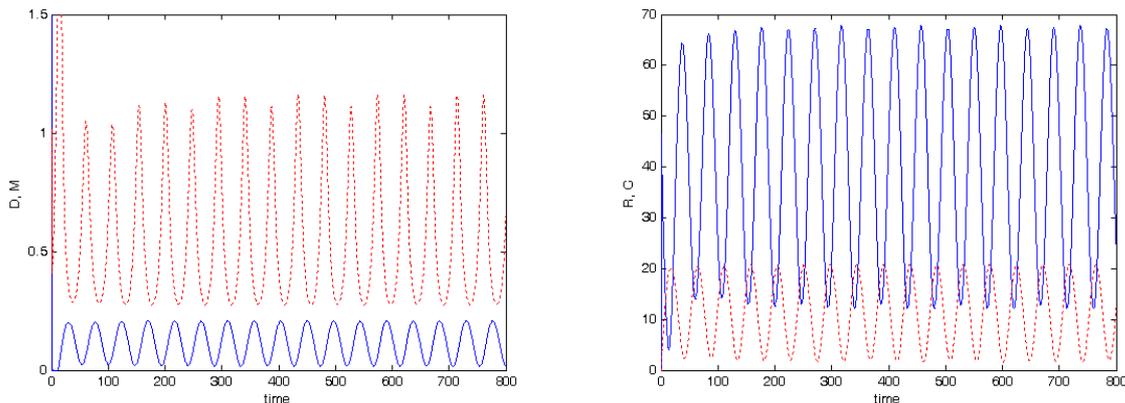

Fig. 4 Periodic (self-oscillation) solution of the system (1) at $h_1 = 0.01$ and $\tau = 12.4$

Fig. 5 illustrates the influence of the amplitude h_1 on the period of oscillations. The amplitude h_1 was increased 25-fold to 0.25. Here, we see that system has periodic solution with period three. Comparing Figs. 4 and 5, we conclude that for larger values of amplitude h_1 the system (1) has more complex behavior. In other words, if the rate of target mRNA synthesis is periodic function with large amplitude then the silencing process is also abnormal but with more complexity.
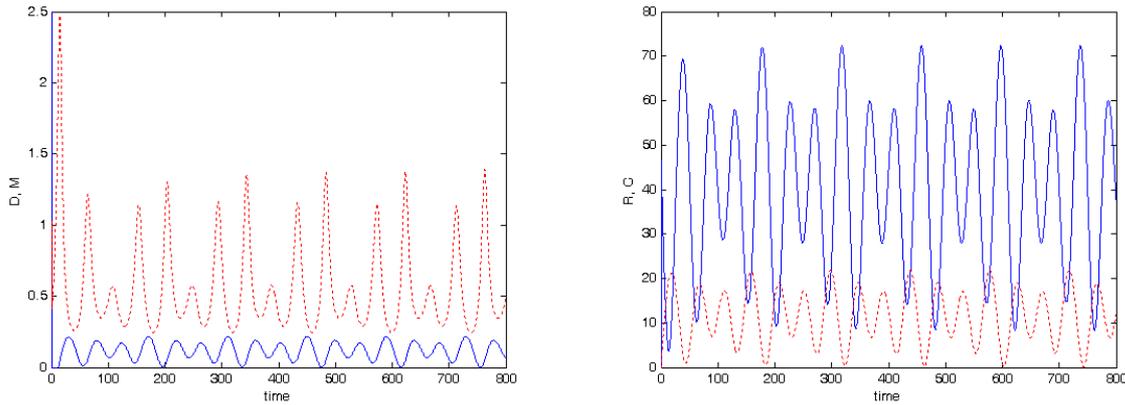
Fig. 5 Oscillations with period three of system (1) at $h_1 = 0.25$ and $\tau = 12.4$

In Fig. 6, we show the bifurcation diagram for system (1): values of C coordinate, $(C)_n$, are plotted against τ regarded as a continuously varying bifurcation (control) parameter. As one decreases τ from $\tau = 12.4$ (till approximately $\tau = 12.3$) the system (1) has periodic solutions with different finite number periods. As τ decreases further, the behavior of the system becomes quasi-periodic. Note that inverse bifurcations also take place. This complex behavior in terms of our model can be connected with absolutely destroyed silencing process.
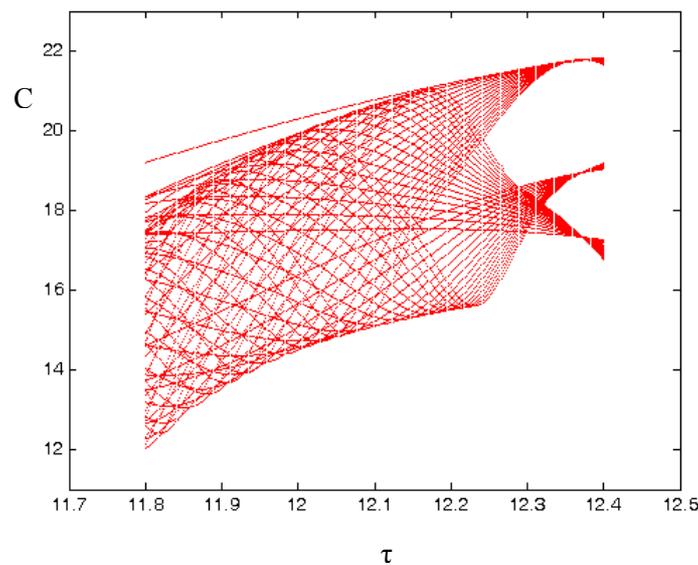

Fig. 6 Bifurcation diagram $(C)_n$ versus τ generated by the computer solutions
of system (1) at $a = 10, b = 0.1, h = 2, g = 0.1, d_M = 0.5,$
$d_R = 0.1, d_C = 0.05, n = 5, \tau \in [11.8, 12.4]$ and $h_1 = 0.25$

Conclusion

The purpose of this paper was to investigate the transitions from stable to simple periodic behavior and from simple to complex oscillatory phenomena in a delayed RNA silencing model with periodic forcing. Because the signalling and cell function are dynamic processes, the analysis is primarily a matter of finding the number of steady states, their nature (stable/unstable) and to characterize the transitions between them. Biochemical reaction networks are complex systems. The complexity arises from both the presence of feedback

loops in the cell, a relatively large number of molecules involved, and the nonlinear nature of interactions between molecules [9]. Thus, the construction and investigation of theoretical (mathematical) models of physiological systems is a powerful tool for understanding complex physiological dynamics. Certainly, the modelling should have a concrete application in the experimental and clinical systems.

If the system (1) possesses a stable equilibrium state, then this corresponds to a normal silencing process. On the other hand, the existence of unstable equilibrium states, stable limit cycles (self-oscillations), or complex oscillatory behaviour in this case corresponds to a pathology, i.e. an abnormal silencing process. From the accomplished analytical and numerical calculations, it becomes clear that time delay τ is a key factor in the behavior of system (1); here, it has a destabilizing effect on the silencing process. In terms of dynamical systems, τ plays the role of a bifurcation parameter. If τ (i.e. the time necessary for the regeneration (or degradation) of the RISC-mRNA complex) is greater than a certain (bifurcation) value in system (1), through Andronov-Hopf bifurcation a self-oscillation related to an abnormal silencing mechanism appears.

From the simulations made in Figs. 5 and 6, it is seen that at larger amplitude of periodic force, i.e. the confusion in the target mRNA synthesis, the periodic oscillation with period three occurs. Also, when time delay τ decreases from 12.4 to 11.8 the transition from simple to complex oscillatory behavior take place.

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