Time delay and protein modulation analysis in a model of RNA silencing

Svetoslav Nikolov [1,†], Xin Lai[2,†], Olaf Wolkenhauer[2] and Julio Vera[2,*]

[1] *Institute of Mechanics and Biomechanics-BAS, Acad. G. Bonchev St., bl. 4, 1113 Sofia Bulgaria,*
[2] *Systems Biology and Bioinformatics Group, Department of Computer Science, University of Rostock, 18051 Rostock, Germany*
[†] *Equal contributors*
[*] *Corresponding author: julio.vera@informatik.uni-rostock.de*

Abstract: RNA silencing is a recently discovered mechanism for posttranscriptional regulation of gene expression. Precisely, in RNA interference, RNAi, endogenous expressed or exogenously induced small RNAs promote and modulate the cleavage of complementary messenger RNA involved in the synthesis of targeted proteins. In this paper we investigated the role of time delay and protein regulation in the posttranslational protein regulation through RNA interference. Towards this end, we used and modified a simple model accounting for RNAi and used qualitative bifurcation analysis, sensitivity analysis and predictive simulations to analyze it. Our results suggest that some processes in the system, like Dicer-mediated mRNA degradation or non specific mRNA degradation, play an important role in the modulation of RNA silencing, whereas silencing seems virtually independent of modulation in other processes.

Keywords: Andronov-Hopf bifurcation, delay differential equations, RNA silencing, sensitivity analysis.

1. Introduction

In a panorama of increasing complexity in the regulation of biochemical systems, a new control mechanism is the posttranscriptional regulation of gene expression regulation through RNA silencing [22]. RNA silencing accounts for the downregulation (or full suppression) of a given gene through the introduction of a complementary (antisense) RNA, which blocks the final steps of the protein expression process. Interesting enough, RNA silencing is not a unique (perfectly defined) mechanism, but a family of them. The best known among them is RNA interference (RNAi), in which either endogenous expressed microRNAs (miRNA, [23]) or exogenously promoted small interfering RNA (siRNA, [11]) promote and modulate the degradation of complementary messenger RNA involved in the synthesis of targeted proteins.

The basics of RNA silencing through small RNAs are depicted in Figure 1. The process has high specificity, in a way a specific small RNA, FDS_{RNA}, forms the RNA-induced silencing complex, *RISC*, together with some endonucleases called argonaut proteins [23]. This highly organized complex is capable of recognizing the target messenger RNA, mRNA, by hybridization (establishing the complex *mRNA/RISC*) and inducing its endonucleolytic cleavage together with other proteins like Dicer. In animals, 21- to 22- length nucleotides miRNA targets RISC to mRNA with partial sequence complementarity [20]. The RISC-mRNA interaction results in translational repression that may be accompanied by mRNA destruction. The precise factors that determine the extent to which normal mRNA decay versus translational repression contributes to the net silencing are not yet well understood [21].

In recent times some mathematical models accounting for RNA silencing dynamics have been proposed. C. Bergstrom et al. derived a simple model accounting for RNAi and tested several hypotheses to find the precise model structure accounting for the phenomenon as described in the biological literature [7]. Arciero et al. integrated in a unique ODE model a description accounting for tumour growth, the immunological response and a rough description of potential treatments based on the use of siRNA [8]. Raab and Stephanopoulos investigated basic features of RNAi, such as RNA dose level or RNA complex exposure time, and developed a model intending to explore alternative gene silencing protocols [9]. Groenenboom et al. proposed and analyzed additional mechanisms associated to the basic RNAi processes and used these extensions to investigate dynamical differences between various types of silencing phenomena [10]. Finally, Bartlett and Davis used non-invasive bioluminescent data and a mathematical modelling

to investigate the effects of target-specific and treatment-specific parameters on siRNA-mediated gene silencing [6].
In our work, we started from the model proposed in [7] and modified it to include the potential effect of time delays associated to gene expression. Furthermore, we used sensitivity analysis and predictive simulations to investigate the effect of modulation in critical processes involved in RNAi.

2. Material and methods

In order to perform our analysis, we used the strategy presented in [26], based on previous experiences suggested by [24], [25]. The strategy we proposed is composed by the following sequence of steps: a) a mathematical modelling containing the essential dynamical features of the system is set up; b) bifurcation analysis is used to investigate the role of time-delay in the dynamics of the system; c) the sensitivities of the selected model outcomes with respect to changes in the model parameters are computed and the parameters are ranked according to their sensitivity; and d) according with the previous steps, we select the critical parameters in the model and investigate the qualitative behaviour of the system when the values of these parameters are modified using systematic predictive simulations. We think that the strategy proposed permits to point out the critical processes in the model able to modulate its biological features. In the context of biomedical research, these critical processes can be considered potential new drug targets.

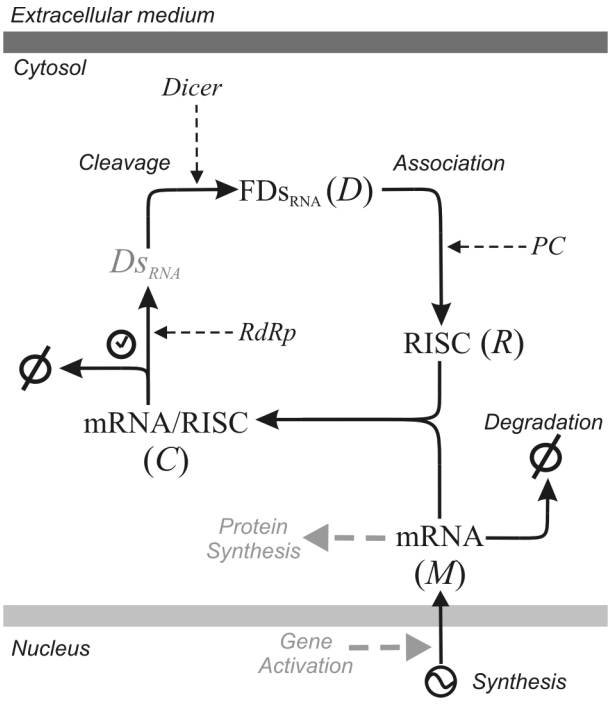


Fig.1. Schematic diagram of the basic RNA silencing model according to [7]. Processes included in the model appear in black while grey arrows indicating ultimate input and output signal of the system.

3. Results

3.1 Model of RNA silencing with two delays

In this work we used and expanded the model proposed in [7]. Figure 1 shows a schematic outline of the model. A certain small RNA fragment, FDS_{RNA}, (D in the model) binds to specific endonucleases called argonaut proteins, establishing the RNA-induced silencing complex, RISC (R). This complex recognizes the target messenger RNA, mRNA (M), hybridizes it (establishing the complex mRNA/RISC, C) and induces its endonucleolytic cleavage together with the protein Dicer. Several of the fragments resulting from the cleavage are integrated in new RNA-induced silencing complex, in a way there is close loop and signal gets amplification every cycle. In previous works[1], we investigated the bifurcation behaviour of the system under the assumption that there is a delay in the critical step of the silencing process, where the delay function $C(t-\tau)$ accounts for the triggering process of mRNA binding to form the RISC-mRNA complex at the moment $(t-\tau)$. Here, we assumed that this effect occurs in different times for the multiplication of small RNA fragment (τ_1) and for the effective break down of the mRNA/RISC complex (τ_2), obtaining a system with two delays in the form:

$$\begin{aligned}
\frac{dD}{dt} &= -a \cdot D + g \cdot C(t-\tau_1),\\
\frac{dR}{dt} &= a \cdot n \cdot D - d_R \cdot R - b \cdot R \cdot M,\\
\frac{dC}{dt} &= b \cdot R \cdot M - (g + d_c) \cdot C(t-\tau_2),\\
\frac{dM}{dt} &= h - d_M \cdot M - b \cdot R \cdot M
\end{aligned} \qquad (1)$$

where D, R, C, M are the already defined state variables and a, b, d_C, d_M, d_R, g, h and n are the kinetic rate constants. According to [7], the original set of model parameters defines a scenario where a) the value of D cannot reach zero level, not admissible from a biological perspective; and b) it is not dependent on the initial dsRNA dose. In order to surmount these problems, our strategy was to re-estimate the values of the model parameters using the SBToolbox [27] in a way the model satisfies the expected biological behaviour described in [7]. Model parameters and data fitting figures are included in the Appendix.

Bifurcation analysis. Hence, system (1) has two steady states: the trivial $\left(\bar{D}=\bar{C}=\bar{R}=0, \bar{M}=h/d_M\right)$ and $\left(\bar{D}=\frac{g}{a}\bar{C}, \bar{R}=\frac{\varsigma}{d_R}\bar{C}, \bar{C}=\frac{h}{g+d_C}-\frac{d_M d_R}{\varsigma}, \bar{M}=\frac{(g+d_C)d_R}{b\varsigma}\right)$, where $\varsigma=[g(n-1)-d_C]$. We investigated the bifurcation structure of the system using time delays τ_1 or τ_2 as bifurcation parameters. First, we obtain the characteristic equation for the linearization of system (1) near the equilibrium $\bar{E}\left(\bar{D}>0, \bar{C}>0, \bar{R}>0, \bar{M}>0\right)$ and consider a small perturbation about the equilibrium level $D=\bar{D}+x, R=\bar{R}+y, C=\bar{C}+z, M=\bar{M}+w$. Substituting these into the differential equations in system (1), we have

$$\begin{aligned}
\frac{dx}{dt}&=-ax+g\ell^{-\tau_1\chi}z,\\
\frac{dy}{dt}&=anx-a_1y-a_2w-byw,\\
\frac{dz}{dt}&=a_3y-a_4\ell^{-\tau_2\chi}z+a_2w+byw,\\
\frac{dw}{dt}&=-a_3y-a_5w-byw,
\end{aligned} \tag{2}$$

with the characteristic equation:

$$\begin{aligned}
&\chi^4+K_1\chi^3+K_2\chi^2+K_3\chi=\\
&\ell^{-\tau_1\chi}\left(T_5\chi+T_6\right)+\ell^{-\tau_2\chi}\left(T_1\chi^3+T_2\chi^2+T_3\chi+T_4\right)
\end{aligned} \tag{3}$$

The new defined coefficients a_i, K_i and T_i are defined in the Appendix. The presence of two different delays in (1) becomes a direct approach to investigate of the sign of the real parts of eigenvalues unfeasible [4]. Thus, we used an alternative strategy, consisting of determining the stability of steady state when one delay is equal to zero similar as [13, 14] and deriving additional theorems to investigate the case of two delays.

Case $\tau_1=0$ and $\tau_2>0$. Firstly, we assume that the finite time delay τ_2 of degeneration is longer than the time of regeneration of RISC-mRNA complex setting $\tau_1=0$ in (3). The characteristic equation becomes:

$$\begin{aligned}
&\chi^4+K_1\chi^3+K_2\chi^2+K_{31}\chi-T_6=\\
&\qquad\qquad\ell^{-\tau_2\chi}\left(T_1\chi^3+T_2\chi^2+T_3\chi+T_4\right)
\end{aligned} \tag{4}$$

where $K_{31}=K_3-T_5$. If $\ell^{-\chi\tau_2}\approx 1-\chi\tau_2$ (linear approximation, when $\tau_2<1$), then equation (4) becomes:

$$h(\chi,\tau_2)=\chi^4+p\chi^3+q\chi^2+r\chi+s=0. \tag{5}$$

By the Hopf bifurcation theorem and Routh-Hurwitz criteria [19], an Andronov-Hopf bifurcation occurs at a value $\tau_2=\tau_b$ where:

$$\begin{aligned}
&p=\frac{K_1+T_2\tau_2-T_1}{\delta}>0, q=\frac{K_2+T_3\tau_2-T_2}{\delta}, s=-\frac{T_4+T_6}{\delta}>0,\\
&r=\frac{K_{31}+T_4\tau_2-T_3}{\delta}, l=pqr-sp^2-r^2=0,
\end{aligned} \tag{6}$$

where $\delta=1+T_1\tau_2$ and the condition $T_1\tau_2\neq -1$ is valid. Evaluating $h(\tau_b,\chi(\tau_b))=0$ at $\tau_2=\tau_b$ we get the eigenvalues $\chi_{1,2}=\pm ik=\pm\sqrt{\frac{r}{p}}$. For the other two eigenvalues we have two possibilities: $\chi_{3,4}=-\frac{p}{2}\pm\Delta_2 i$ if $\Delta_1>0$ and $\chi_{3,4}=-\frac{p}{2}\pm\Delta_2$ if $\Delta_1<0$, where $\Delta_1=\frac{sp}{r}-\frac{p}{4}$ and $\Delta_2^2=\frac{sp}{r}-\frac{p^2}{4}(\Delta_2>0)$. After additional derivations (see Appendix), we obtained that the derivative $\frac{d\chi_1(\tau_b)}{d\tau}$ is always positive when the following conditions are satisfied:

$$\begin{vmatrix} p_1k^2>r_1\\ q>2k^2\\ s_1>q_1k^2 \end{vmatrix} \quad or \quad \begin{vmatrix} p_1k^2<r_1\\ q<2k^2\\ s_1>q_1k^2 \end{vmatrix}$$

For a larger time delay τ_2, linear stability analysis is no longer effective and we need to use another approach [3, 4, 13-16]. The stability of equilibrium state depends on the sign of the real parts of the roots of (4). We let $\chi=m+in\ (m,n\in R)$, and rewrite (4) in terms of its real and imaginary parts. To find the first bifurcation point we look for purely imaginary roots $\chi=\pm in, n\in R$, of (4), i.e. we set $m=0$ Then, we obtain:

$$\begin{vmatrix} n^4-K_2n^2-T_6=\left(-T_1n^3+T_3n\right)\sin n\tau_2+\left(-T_2n^2+T_4\right)\cos n\tau_2,\\ -K_1n^3+K_{31}n=\left(-T_1n^3+T_3n\right)\cos n\tau_2+\left(T_2n^2-T_4\right)\sin n\tau_2, \end{vmatrix} \tag{7}$$

Note that $n=0$ can be a solution if $T_4=T_6$. If the first bifurcation point is $\left(n_b^0,\tau_b^0\right)$, then the other bifurcation points (n_b,τ_b) satisfy $n_b\tau_b=n_b^0\tau_b^0+2v\pi,\quad v=1,2,...,\infty$. By squaring the two equations into system (7) and then adding them, it follows that

$$\begin{aligned}
&n^8+\left(K_1^2-2K_2-T_1^2\right)n^6+\left[K_2^2-T_2^2+2\left(T_1T_3-K_1K_{31}-T_6\right)\right]n^4+\\
&\quad+\left[K_{31}^2-T_3^2+2\left(K_2T_6+T_2T_4\right)\right]n^2-T_4^2+T_6^2=0.
\end{aligned} \tag{8}$$

Since this is a quartic equation on n^2 and the left side is positive for large values of n^2 and negative for $n=0$ if and only if $T_4^2>T_6^2$, i.e. the equation (8) has at least one positive real root. Moreover, to apply the Hopf bifurcation theorem, according to [17], the following theorem in this situation applies:

Theorem 1. *Suppose that* n_b *is the least positive simple root of (8). Then,* $in(\tau_b) = in_b$ *is a simple root of (4) and* $m(\tau_2) + in(\tau_2)$ *is differentiable with respect to* τ_2 *in a neighbourhood of* $\tau_2 = \tau_b$.

To establish an Andronov-Hopf bifurcation at $\tau_2 = \tau_b$, we need to show that the following transversality condition $\left.\frac{dm}{d\tau_2}\right|_{\tau=\tau_b} \neq 0$ is satisfied. Hence, by denoting

$$H(\chi, \tau_2) = \chi^4 + K_1\chi^3 + K_2\chi^2 + K_3\chi - e^{-\tau_2\chi}\left(T_1\chi^3 + T_2\chi^2 + T_3\chi + T_4\right), \quad (9)$$

deriving with respect to τ_2 and evaluating the real part of this equation we obtain:

$$\left.\frac{dm}{d\tau_2}\right|_{\tau_2=\tau_b} = \mathrm{Re}\left(\frac{d\chi}{d\tau_2}\right)_{\tau_2=\tau_b} = \frac{n_b^2\left\{4n_b^6 + 3\left(K_1^2 - 2K_2 - T_1^2\right)n_b^4\right\}}{L_1^2 + I_1^2} + \frac{2\left[K_2^2 - T_2^2 + 2\left(T_1T_3 - K_1K_{31} - T_6\right)\right]n_b^2 + K_{31}^2 - T_3^2 + 2\left(T_2T_4 + K_2T_6\right)}{L_1^2 + I_1^2}$$

where the new coefficients are defined in the Appendix. Let $\theta = n_b^2$; then, the equation (8) reduces to

$$g(\theta) = \theta^4 + \left(K_1^2 - 2K_2 - T_1^2\right)\theta^3 + \left[K_2^2 - T_2^2 + 2\left(T_1T_3 - K_1K_{31} - T_6\right)\right]\theta^2 + \left[K_{31}^2 - T_3^2 + 2\left(K_2T_6 + T_2T_4\right)\right]\theta - T_4^2 + T_6^2.$$

Deriving this equation with respect to the new variable θ we obtain that $\left.\frac{dg}{d\tau_2}\right|_{\theta=n_b^2} > 0$ where n_b is the least positive simple root of (8). In that case, $\left.\frac{dm}{d\tau_2}\right|_{\tau_2=\tau_b} = \mathrm{Re}\left(\frac{d\chi}{d\tau_2}\right)_{\tau_2=\tau_b} = \frac{n_b^2 g'\left(n_b^2\right)}{L_1^2 + I_1^2} > 0$ and according to the Hopf bifurcation theorem [18], we define the following:

Theorem 2. *If* n_b *is the least positive root of (8), then an Andronov-Hopf bifurcation occurs as* τ_2 *passes through* τ_b.

(**Corollary 2.1.** *When* $\tau_2 < \tau_b$, *then the steady state* $\bar{E}$ *of system (1) is locally asymptotically stable.*)

This means that the non trivial steady-states of the system are locally asymptotically stable for values of time delay τ_2 smaller than the bifurcation point τ_b of the system, whereas sustained oscillations emerge when passing through τ_b.

Case $\tau_1, \tau_2 > 0$**.** In order to investigate the local stability of the equilibrium state $\bar{E}$ of the system (1), we derived and proved the following theorem accounting for the real parts of characteristic roots of (3):

Theorem 3. *If all roots of (4) are with negative real parts for* $\tau_2 > 0$, *then there exists a* $\tau_1^{bif}(\tau_2) > 0$ *such that all roots of characteristic equation (3) have negative real parts at* $\tau_1 < \tau_1^{bif}(\tau_2)$, *i.e. when* $\tau_1 \in \left[0, \quad \tau_1^{bif}(\tau_2)\right)$.

The demonstration of this theorem is discussed in the Appendix. Under this condition, we can enunciate the following:

Corollary 3.1. *If* τ_2^{bif} *is defined as in Theorem 2, then for any* $\tau_2 \in [0, \tau_b)$, *there exists a* $\tau_1^{bif}(\tau_2) > 0$ *such that the steady state* $\bar{E}$ *of system (1) is locally asymptotically stable when* $\tau_1 \in \left[0, \quad \tau_1^{bif}(\tau_2)\right)$.

Thus, this ensures that the non trivial steady-states of the system will keep their locally asymptotically stable nature at least for values of τ_1 smaller than the bifurcation point τ_1^{bif}, whose value depend on the current value of τ_2, whereas sustained oscillations emerge when passing through τ_1^{bif}. Our analysis suggests that the stability of the solution in terms of oscillatory behaviour will depend on the interplay between the values of τ_1 and τ_2. In addition, numerical calculation reveals that bifurcation points of the system occur for values of of τ_1 and τ_2 that are in principle non-feasible from a biological perspective, which in turn ensures the stability of the solutions in the feasible region for time delay (data not shown).

3.2 Sensitivity analysis and predictive simulations

We calculated the local sensitivities of the state variables with respect to the model parameters, whose corresponding values are represented in a normalized scale [26] in Figure 2. *D, R,* and *C* show an almost identical sensitivity pattern, in which parameter *a* (Dicer-mediated FD_{SRNA} degradation) is the most influential parameter together with *n* (ratio of siRNA produced from each secondary FD_{SRNA} molecule). This last is in accordance with the results in [7], where authors claimed that *n* is the critical parameter accounting for RNAi amplification. The other parameters show a similar sensitivity value. Interesting enough, sensitivity associated to parameter *dR* (rate of RISC dissociation) is very small, which suggests a reduced importance of changes in its value in the modulation of the RNAi process. In case of *M*, our local sensitivity analysis emphasizes the importance of the primal parameters accounting for mRNA turnover, *dM* (normal, non RNAi-mediated, mRNA degradation) and *h* (gene-mediated mRNA synthesis), but it is unable to detect the effect that modulation in the processes involved in RNA interference has in the mRNA stability.

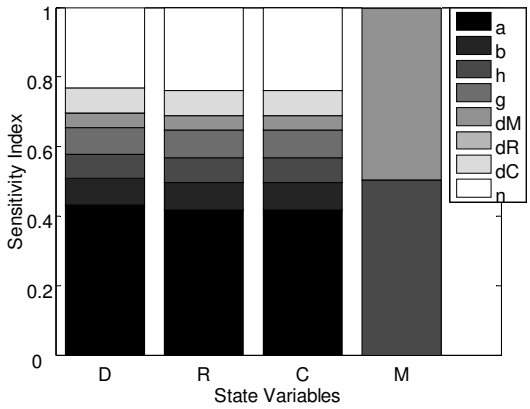


Fig. 2. Local sensitivities of the state variables with respect to the model parameters.

We furthermore, investigated the effect that modulation of selected parameters has in the dynamics of RNA silencing. Towards this end, we systematically modified the value of selected pairs of parameters around their nominal values (from 0.1 to 10 times the nominal value) and compute the value of some variables accounting for RNA silencing dynamics. We therefore define: a) **silencing time**, τ_s, as the time that the system takes to reach the silencing level for mRNA (assumed here as the 15% of the initial concentration of mRNA in our simulations); and b) **silencing intensity**, m_s, as the minimum (steady-state) concentration of the mRNA for a given set of parameter values (see Figure 3 for further explanations).

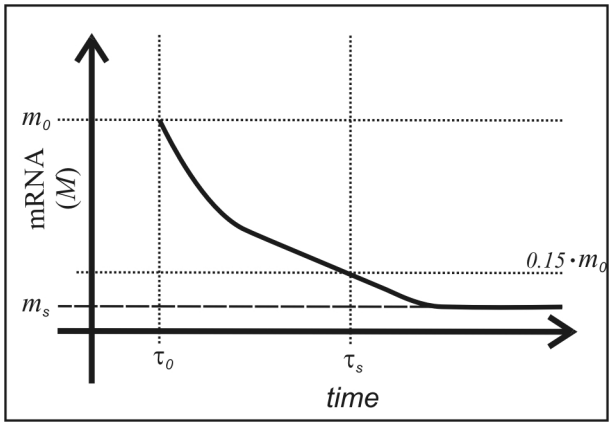


Fig. 3. Definition of silencing dynamics (silencing time, τ_s, and silencing intensity, $m_{s)}$ used in our analysis.

In case of the couple *a* (rate of dsRNA degradation) and *g* (rate of dsRNA synthesis from RISC-mRNA complex) (Figure 4), our simulations show that silencing is not reachable when both parameters are extremely downregulated; parameter values for which this occurs are represented by a blank area in Figure 4 (Top), indicating that the silencing time is infinite. Interestingly, the silencing time for most of the other values is quite stable and ranges between 1 and 4 hours, which suggests fast silencing. In addition, when *g* exceeds a given value (≈ 0.8 of its initial value), the silencing time remains almost constant and will not change with the variation of *a* and *g*. In case of silencing intensity (Figure 4 Bottom), we can distinguish two domains: weak silencing (mRNA approx. 12%) for downregulation and weak upregulation of *a,* and intense silencing (mRNA<5%) for higher values. Interesting enough, silencing intensity (once satisfied the threshold of 15%) seems independent of the level of modulation for *g*.

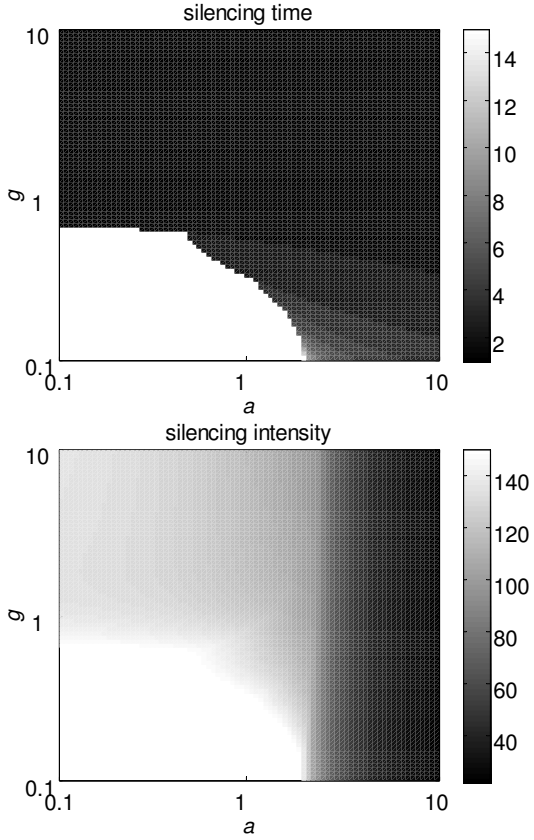


Fig. 4. Variation of silencing time (top), τ_s, and silencing intensity (bottom), $m_{s,}$ when the values of parameters *a* and *g* are modified in the interval [0.1, 10] times of their nominal value.

For *a* and *dM* (rate of non RNAi-mediated mRNA degradation) (Figure 5), our simulations show that silencing is not reachable when both parameters are extremely downregulated, but also that extreme overexpression of *a* (enhancement of dsRNA degradation) is enough to compensate a loose in the rate of non RNAi-mediated mRNA degradation. In accordance with results in Figure 4, the

silencing time for most of the other values is quite stable and ranges between 1 and 2 hours, which suggests an even faster silencing. In this case the silencing intensity strongly depends on the interplay between *a* and *dM* (Figure 5 Bottom). Furthermore, we can distinguish a systematic increase in the silencing intensity when the solutions leave the vicinity of the silencing boundary. In addition, silencing intensity depends strongly on the value of *a*, ranging from weak silencing (mRNA approx. 14%) for downregulation to intense silencing (mRNA<5%) for overexpression.

When modulation of *dM* and *dC* (rate of mRNA/RISC complex degradation) are considered (Figure 6), the behaviour is somehow reverse. With extreme downregulation of mRNA/RISC degradation and upregulation of mRNA non-specific degradation the silencing is not reachable, but in all the other cases the silencing time is independent of regulation of these two parameters and stands in a value of 1 hour (Figure 6 Top). In case of the silencing intensity, we can distinguish two regions (Figure 6 Bottom): a) for strong repression of non specific mRNA degradation the system reaches an intense silencing, independent of regulation of in the mRNA/RISC degradation rate; and b) for low downregulation or upregulation, silencing intensity becomes strongly dependent of the mRNA/RISC degradation rate, ranging from weak (for *dC* downregulation) to intermediate silencing (for intense upregulation).

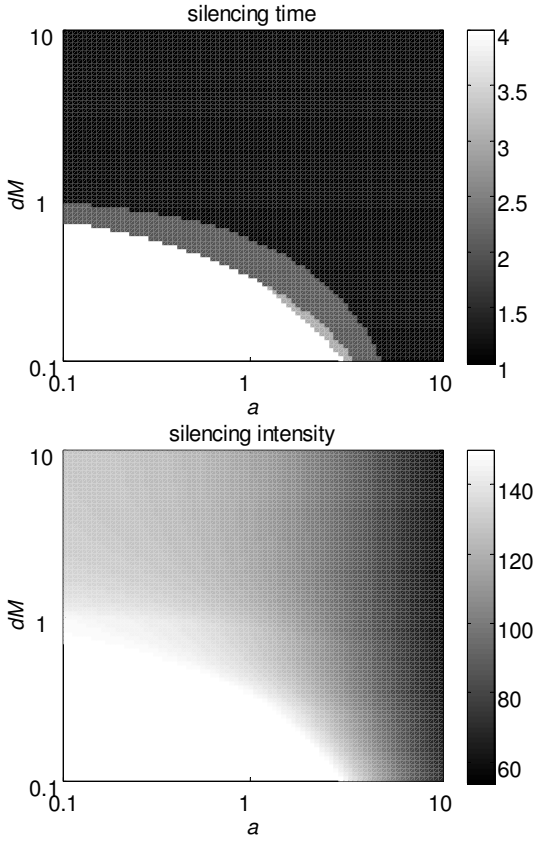


Fig. 5. Variation of silencing time (top), τ_s, and silencing intensity (bottom), m_s, when the values of parameters *a* and *dM* are modified in the interval [0.1, 10] times of their nominal value.

We furthermore investigated the effect of time delays τ_1 and τ_2 in the dynamics of RNA silencing. In order to get meaningful results, we constrained the interval of values for both time delays to the one that seem biologically feasible and perform predictive simulations computing the silencing time and silencing intensity for couple of values of τ_1 and τ_2. The system reaches effective silencing in all the simulated cases, but displays a very irregular pattern with silencing times in the interval 14-26 hours (data not shown). In case of silencing intensity (Figure 7), increase in τ_1 reduces silencing intensity while increase in τ_2 makes reverse. The lowest silencing intensity is reached for long τ_1 and short τ_2 (mRNA approx. 14%) and the highest for short τ_1 and long τ_2 (mRNA approx. 10%). We noticed that outside the time delay framework displayed, the system becomes in some cases unstable and biologically unfeasible solutions appeared.

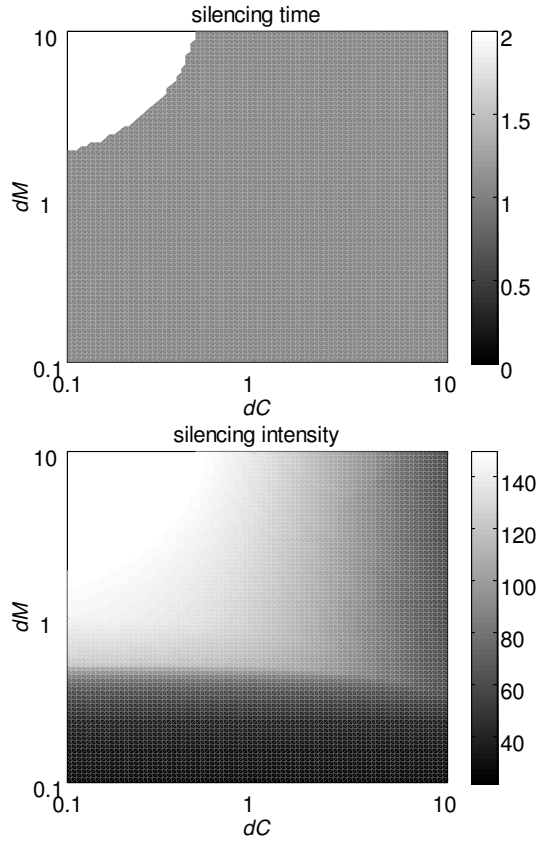


Fig. 6. Variation of silencing time silencing time (top), τ_s, and silencing intensity(bottom), m_s, when the values of

parameters *dM* and *dC* are modified in the interval [0.1, 10] times of their nominal value.

4. Discussion and Conclusions

In our work we investigated the role of time delay and protein regulation in the posttranslational protein regulation through RNA interference. Towards this end, we combined qualitative bifurcation analysis, sensitivity analysis and predictive simulations.

We modified the model proposed by [7] to include the potential effect of time delays in the process accounting for the triggering of mRNA binding to form the RISC-mRNA. Our bifurcation analysis suggests that the stability of the solutions in terms of oscillatory behaviour will depend on the interplay between the time delay values of τ_1 and τ_2.

In order to investigate the dynamics of RNA silencing, we defined the variables silencing time (time to reach the silencing level for mRNA) and silencing intensity (steady-state concentration of mRNA for a given set of parameter values). We found that modulation in Dicer-mediated FD_{SRNA} degradation, represented by the parameter *a*, plays an important role in the modulation of RNA silencing; we distinguished a weak silencing regime for downregulation and weak upregulation of *a,* and intense silencing for higher values. In addition, modulation of the non specific mRNA degradation, *dM*, seems also a crucial process in a way the system reaches intense silencing with strong repression of non specific mRNA degradation, whereas low downregulation or upregulation in *dM* makes the system strongly dependent of other processes like mRNA/RISC degradation. Interesting enough, silencing seems independent of other model parameters and, for example, modulation of dsRNA synthesis from RISC-mRNA complex, *g,* seems to play a minor role in the regulation of the system. In future works we want to integrate this module accounting for RNAi into more complex, pathway specific, models describing the interplay between gene activation, RNA interference, protein expression and stress mediated regulation of the silencing process.

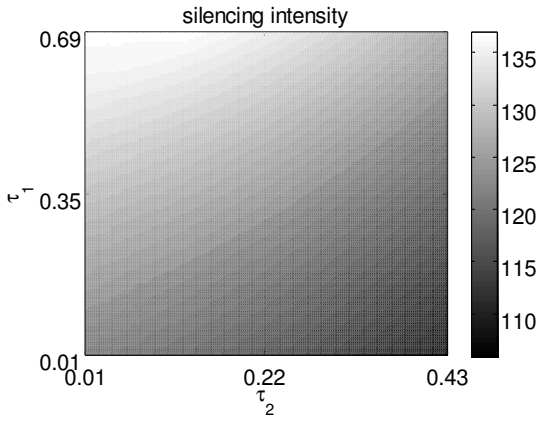


Fig. 7. Variation of silencing intensity, m_s, when the values of τ_1 and τ_2 are modified in the interval of biologically feasible values.

Acknowledgements

J.V. and S.N. designed the study and set up the mathematical model. X.L. performed the calculations concerning the sensitivity analysis and the predictive simulations under the supervision of J.V. S.N. performed the bifurcation analysis. The biological interpretation of the results was conducted by J.V. Finally, all the authors including O.W. drafted the manuscript. This work was supported by the German Federal Ministry of Education and Research (BMBF) as part of the project CALSYS-FORSYS under contract 0315264 (www.sbi.uni-rostock.de/calsys). Supplementary information included in the Appendix can be downloaded from: www.sbi.uni-rostock.de/software.

References

[1] S. Nikolov, V. Petrov, **Time delay model of RNA silencing. J. of Mech. in Med. and Biol.** Vol. 7, No. 3, 2007, pp. 297.

[2] M. Adimy, F. Crauste, Sh. Ruan, **Periodic oscillations in leucopoiesis models with two delays.** J. of Theor. Biol,Vol. 242, 2006, pp. 288.

[3] S. Nikolov, J. Vera, V. Kotev, O. Wolkenhauer, V. Petrov, **Dynamic properties of a delayed protein cross talk model BioSystems .** Vol. 91, 2008, pp. 51.

[4] R. Bellman, K. Cooke, **Differential-Difference Equations, Academic Press.** New York, 1963

[5] S. Nikolov, **Stability and bifurcation behaviour of genetic regulatory systems with two delays.** Comptes Rendus de l'Acad. Bulg. des Sci, Vol. 61, No. 5, 2008, pp. 585.

[6] D. Bartlett, M. Davis, **Insights into the kinetics of siRNA-mediated gene silencing from live-cell and live animal bioluminescent imaging.** Nucleic Acids Research, Vol. 34, 2006, pp. 322.

[7] C. Bergstrom, E. McKittrick, R. Anita, **Mathematical models of RNA silencing: unidirectional amplification limits accidental self-directed reactions.** Proc. Natl. Acad. Sci USA, Vol. 100, 2003, pp. 11511.

[8] J. Arciero, T. Jackson, D. Kirschner, **A mathematical model of tumor-immune evasion and siRNA treatment.** Discrete and Continuous Dynamical Systems, Vol. 4, 2004, pp. 39.

[9] R. Raab, G. Stephanopoulos, **Dynamics of gene silencing by RNA interference.** Biotechnol. Bioeng, Vol. 88, 2004, pp. 121.

[10] M. Groenenboom, A. Maree, P. Hogeweg, **The RNA silencing pathway: the bits and pieces that matter.** PLos Comput. Biol, Vol. 1, 2005, pp. 155.

[11] T. Rana, **Illuminating the silence: understanding the structure and function of small RNAs.** Molecular Cell Biology, Vol. 8, 2007, pp. 23.

[12] Ch. Li, A. Parker, et.al., **Delivery of RNA interference.** Cell Cycle, Vol. 5, No. 18, 2006, pp. 2103.

[13] Sh. Ruan, J. Wei, **Dynamics of Continuous, Discrete and Impulsive Systems.** Series A: Math. Analysis, Vol. 10, 2003, pp. 863.

[14] J. Wei, Sh. Ruan, **Stability and bifurcation in a neural network model with two delays.** Physica D, Vol. 130, 1999, pp. 255.

[15] K. Cooke, Z. Grossman, **Discrete delay, distributed delay and stability switches.** J. of Math. Analysis and Applications, Vol. 86, 1982, pp. 592.

[16] E. Beretta, Y. Kuang, **Geometric stability switch criteria in delay differential systems with delay dependent parameters.** SIAM J. Math. Analysis, Vol. 33, No. 5, 2002, pp. 1144.

[17] Q. Khan, D. Greenhagh, **Hopf bifurcation in epidemic models with a time delay in vaccination.** IMA J. Math. Appl. Med. Biol, Vol. 16, 1999, pp. 133.

[18] J. Marsden, M. McCracken, **The Hopf Bifurcation and its Applications.** Springer-Verlag, New York, 1976.

[19] N. Bautin, **Behaviour of Dynamical Systems Near the Boundary of Stability.** Nauka, Moscow, 1984.

[20] F. Karginov, C. Conaco, Zh. Xuan, et al., **A biochemical approach to identifying microRNA targets.** Proc Natl Acad Sci USA, Vol. 104, No. 49, 2007, pp. 19291.

[21] S. Hammond, E. Bernstein, D. Beach, G. Hannon, **Double-stranded RNA as a template for gene silencing.** Nature, Vol. 404, 2000, pp. 293.

[22] H. Hermeking, **p53 enters the mircoRNA world.** Cancer Cell, Vol. 12, No. 5, 2007, pp. 414-418.

[23] RS. Pillai, SN. Bhattacharyya, W. Filipowicz, **Repression of protein synthesis by miRNAs: how many mechanisms.** Trends Cell Biol, Vol. 17, No. 3, 2007, pp. 118-126.

[24] H. Yue, M. Brown, J. Knowles, H. Wang, DS. Broomhead, DB. Kell, **Insights into the behaviour of systems biology models from dynamic sensitivity and identifiability analysis: a case study of an NF-kappaB signalling pathway.** Mol Biosyst, Vol. 2, No. 12, 2006, pp. 640-649.

[25] J. Joo, S. Plimpton, S. Martin, L. Swiler, JL. Faulon, **Sensitivity analysis of a computational model of the IKK NF-kappaB IkappaBalpha A20 signal transduction network.** Ann N Y Acad Sci, No. 1115, 2007, pp. 221-39.

[26] S. Nikolov, X. Lai, U. Liebal, O. Wolkenhauer and J.Vera, **Integration of sensitivity and bifurcation analysis to detect critical processes in a model combining signalling and cell population dynamics.** Submitted, 2009.

[27] H. Schmidt, M. Jirstrand, **Systems Biology Toolbox for MATLAB: A computational platform for research in Systems Biology.** Bioinformatics, Vol. 22, No. 4, 2006, pp. 514-515.

Time delay and protein modulation analysis in a model of RNA silencing

Svetoslav Nikolov[1,†1 *], Xin Lai[2,†], Olaf Wolkenhauer[2] and Julio Vera[2,*]

[1]*Institute of Mechanics and Biomechanics-BAS, Acad. G. Bonchev St., bl. 4, 1113 Sofia Bulgaria,*
[2]*Systems Biology and Bioinformatics Group, Department of Computer Science, University of Rostock, 18051 Rostock, Germany*

Appendix

Abstract

RNA silencing is a recently discovered mechanism for posttranscriptional regulation of gene expression. Precisely, in RNA interference, RNAi, endogenous expressed or exogenously promoted small RNAs promote and modulate the degradation of complementary messenger RNA involved in the synthesis of targeted proteins. In this paper we investigated the role of time delay and protein regulation in the posttranslational protein regulation through RNA interference. Towards this end, we used and modified a simple model accounting for RNAi and used qualitative bifurcation analysis, sensitivity analysis and predictive simulations to analyze it. Our results suggest that some processes in the system, like Dicer-mediated FD_{SRNA} mRNA degradation or non specific mRNA degradation, play an important role in the modulation of RNA silencing, whereas silencing seems virtually independent of modulation in other processes.

Keywords: delay differential equations; RNA silencing; Andronov-Hopf bifurcation; sensitivity analysis

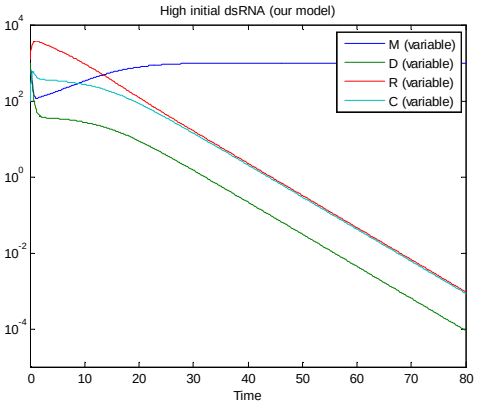
Model calibration

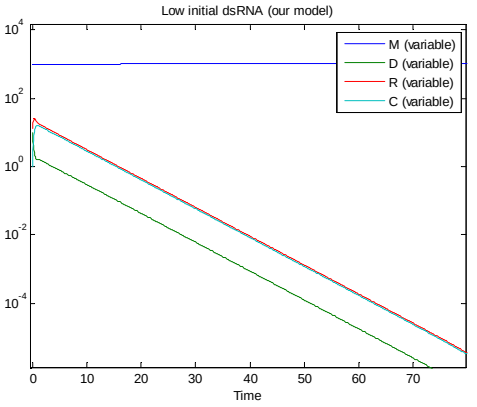
Parameter	Calculated value[1]	Original value[2]
a	4	10
b	0.002	0.001
h	1000	1000
g	0.4	1
d_M	1	1
d_R	0.1	0.1
d_C	2	1
n	5	5

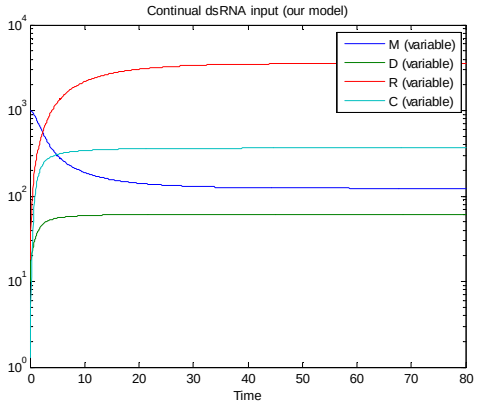
1. Values estimated using model calibration in the way discussed in the text.
2. Values used in Bergstrom et al. 2003.

[1] [†] Equal contributors.
[*]Corresponding author: *E-mail address:* julio.vera@informatik.uni-rostock.de. URL:www.sbi.uni-rostock.de.

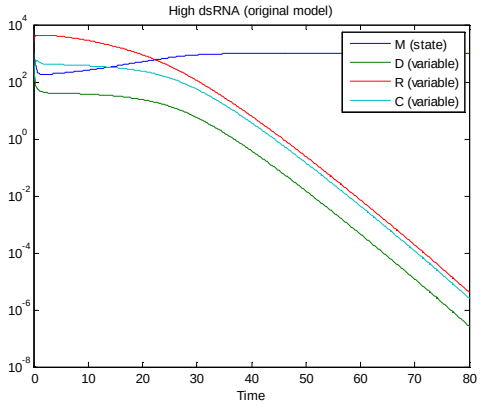
Calculated parameters

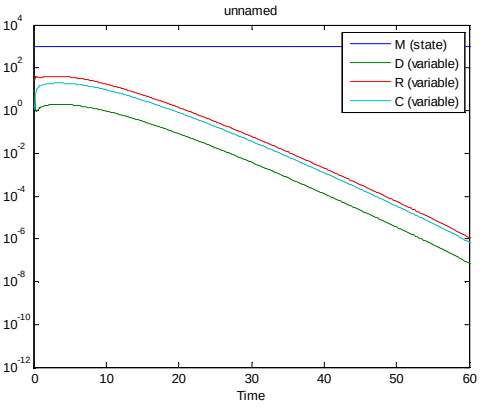


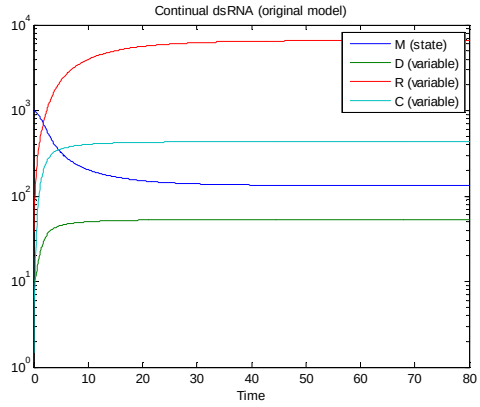




Original simulations (Bergstrom et al. 2003)







Complete derivation used in our qualitative bifurcation analysis

In Nikolov and Petrov [6] we investigated the bifurcation behavior of a model of RNA silencing with one time delay, where the delay function $C(t-\tau)$ express 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 $(t-\tau)$. In [6], in order to make the analytical investigation of time delay system easier, we assume that the two times –of the regeneration and degradation of the RISC-mRNA are equal. Of course, the finite time τ_1 of regeneration can be different from that of degeneration τ_2 [12, 22, 23]. Hence, we obtain a system with two delays in the form:

$$\begin{aligned}
\frac{dD}{dt} &= -a.D + g.C(t-\tau_1),\\
\frac{dR}{dt} &= an.D - d_R.R - b.RM,\\
\frac{dC}{dt} &= b.RM - (g+d_c).C(t-\tau_2),\\
\frac{dM}{dt} &= h - d_M M - b.RM,
\end{aligned} \tag{4}$$

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 $a, b, d_C, d_M, d_R, g, h$ and n are noted the kinetic rate constants. Hence, system (4) has two steady states: the trivial $\left(\bar{D}=\bar{C}=\bar{R}=0, \bar{M}=h/d_M\right)$ and $\left(\bar{D}=\frac{g}{a}\bar{C}, \bar{R}=\frac{\varsigma}{d_R}\bar{C}, \bar{C}=\frac{h}{g+d_C}-\frac{d_M d_R}{b\varsigma}, \bar{M}=\frac{(g+d_C)d_R}{b\varsigma}\right)$, where $\varsigma=[g(n-1)-d_C]$. Here we note that the original ODE system has the same fixed points which are always stable.

Furthermore, we investigate the bifurcation structure- particularly the Andronov-Hopf bifurcation- for system (4), using time delays τ_1 or τ_2 as bifurcation parameters. First, we obtain the characteristic equation for the linearization of system (4) near the equilibrium $\bar{E}\left(\bar{D}>0, \bar{C}>0, \bar{R}>0, \bar{M}>0\right)$, i.e. all are positive and 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 (4), we have

$$\begin{aligned}
\frac{dx}{dt} &= -ax + g\ell^{-\tau_1\chi}z,\\
\frac{dy}{dt} &= anx - a_1 y - a_2 w - byw,\\
\frac{dz}{dt} &= a_3 y - a_4\ell^{-\tau_2\chi}z + a_2 w + byw,\\
\frac{dw}{dt} &= -a_3 y - a_5 w - byw,
\end{aligned} \tag{5}$$

where $a_1 = d_R + b\bar{M}$, $a_2 = b\bar{R}$, $a_3 = b\bar{M}$, $a_4 = g + d_C$, $a_5 = d_M + b\bar{R}$. The associated characteristic equation of (5) has the following form

$$\chi^4 + K_1\chi^3 + K_2\chi^2 + K_3\chi = \ell^{-\tau_1\chi}\left(T_5\chi + T_6\right) + \ell^{-\tau_2\chi}\left(T_1\chi^3 + T_2\chi^2 + T_3\chi + T_4\right), \tag{6}$$

where

$$\begin{aligned} &K_1 = a + a_1 + a_5,\ K_2 = a\left(a_1 + a_5\right) + a_1a_5 - a_2a_3,\ K_3 = a\left(a_1a_5 - a_2a_3\right), \\ &T_1 = -a_4,\ T_2 = -K_1a_4,\ T_3 = -a_4\left[a\left(a_1 + a_5\right) + a_1a_5 - a_2a_3\right], \\ &T_4 = aa_4\left(a_2a_3 - a_1a_5\right),\ T_5 = aa_3ng,\ T_6 = aa_3ng\left(a_5 - a_2\right). \end{aligned} \tag{7}$$

Because of the presence of two different delays in (4) the analysis of the sign of the real parts of eigenvalues is very complicated and a direct approach cannot be considered [10]. Thus, in our analysis we will use a method consisting of determining the stability of steady state when one delay is equal to zero similar as [24, 25].

2.1. The case $\tau_1 = 0$ and $\tau_2 > 0$.

Hence, we assume that the finite time delay τ_2 of degeneration is longer than the time of regeneration of RISC-mRNA complex, τ_1.

Setting $\tau_1 = 0$ in (6), the characteristic equation becomes

$$\chi^4 + K_1\chi^3 + K_2\chi^2 + K_{31}\chi - T_6 = \ell^{-\tau_2\chi}\left(T_1\chi^3 + T_2\chi^2 + T_3\chi + T_4\right) \tag{8}$$

where $K_{31} = K_3 - T_5$. For small delay $\tau_2 < 1$, we use linear stability analysis. Thus, let $\ell^{-\chi\tau_2} \approx 1 - \chi\tau_2$; then, the eigenvalue equation becomes

$$\chi^4 + p\chi^3 + q\chi^2 + r\chi + s = 0. \tag{9}$$

By the Hopf bifurcation theorem and Routh-Hurwitz criteria [30], an Andronov-Hopf bifurcation occurs at a value $\tau = \tau_b$ where

$$\begin{aligned} &p = \frac{K_1 + T_2\tau_2 - T_1}{\delta} > 0, \quad q = \frac{K_2 + T_3\tau_2 - T_2}{\delta}, \quad s = -\frac{T_4 + T_6}{\delta} > 0, \\ &r = \frac{K_{31} + T_4\tau_2 - T_3}{\delta}, \quad l = pqr - sp^2 - r^2 = 0, \end{aligned} \tag{10}$$

where $\delta = 1 + T_1\tau_2$ and the condition $T_1\tau_2 \neq -1$ is valid. Let

$$h\left(\chi, \tau_2\right) = \chi^4 + p\chi^3 + q\chi^2 + r\chi + s. \tag{11}$$

Evaluating h at $\tau_2 = \tau_b$ yields

$$h\left(\tau_b, \chi\left(\tau_b\right)\right) = \chi^4 + p\chi^3 + q\chi^2 + k^2p\chi + k^2\left(q - k^2\right), \tag{12}$$

where $k^2 = \frac{r}{p}$. The eigenvalues of (9) at τ_b are

$$\chi_{1,2} = \pm ik = \pm\sqrt{\frac{r}{p}}, \tag{13}$$

and the type of the other root pair depends on the sign of the equality $\Delta_1 = \frac{sp}{r} - \frac{p}{4}$. Here i is an imaginary unit. If $\Delta_1 > 0$, then

$$\chi_{3,4} = -\frac{p}{2} \pm \Delta_2 i, \tag{14}$$

where $\Delta_2^2 = \frac{sp}{r} - \frac{p^2}{4} (\Delta_2 > 0)$; if $\Delta_1 < 0$, then

$$\chi_{3,4} = -\frac{p}{2} \pm \Delta_2, \tag{15}$$

where now $\Delta_2 = \sqrt{-\Delta_1}$. Implicitly differentiating $h(\tau_b, \chi(\tau_b))$ yields

$$\frac{d\chi}{d\tau} = -\frac{\frac{\partial h}{\partial \tau}}{\frac{\partial h}{\partial \chi}} = -\frac{p_1\chi^3 + q_1\chi^2 + r_1\chi + s_1}{4\chi^3 + 3p\chi^2 + 2q\chi + k^2 p}, \tag{16}$$

where

$$\begin{aligned} p_1 &= \frac{T_2\delta - T_1(K_1 - T_1 + T_2\tau_2)}{\delta^2}, \quad q_1 = \frac{T_3\delta - T_1(K_2 - T_2 + T_3\tau_2)}{\delta^2}, \\ r_1 &= \frac{T_4\delta - T_1(K_{31} - T_3 + T_4\tau_2)}{\delta^2}, \quad s_1 = \frac{T_1(T_4 + T_6)}{\delta^2}. \end{aligned} \tag{17}$$

Evaluating the required derivatives of h at τ_b, we obtain

$$\frac{d\chi_1(\tau_b)}{d\tau} = \frac{2k^2 N + 2k\left[\left(s_1 - q_1k^2\right)\left(q - 2k^2\right) + pk^2\left(r_1 - p_1k^2\right)\right]i}{L^2 + I^2}, \tag{18}$$

where $L = -2pk^2$, $I = 2k\left(q - 2k^2\right)i$, and $N = \left(p_1k^2 - r_1\right)\left(q - 2k^2\right) + p\left(s_1 - q_1k^2\right)$. The real part of (18) has the form

$$\mathrm{Re}\left(\frac{d\chi_1(\tau_b)}{d\tau}\right) = \frac{2k^2 N}{L^2 + I^2}. \tag{19}$$

and is always positive if $N > 0$, i.e. if the following conditions are valid:

$$\begin{vmatrix} p_1k^2 > r_1 \\ q > 2k^2 \\ s_1 > q_1k^2 \end{vmatrix} \quad or \quad \begin{vmatrix} p_1k^2 < r_1 \\ q < 2k^2 \\ s_1 > q_1k^2 \end{vmatrix} \tag{20}$$

It is well known that for a larger time delay τ_2, linear stability analysis is no longer effective and we need to use another approach [8, 10, 24-27]. The stability of equilibrium state depends on the sign of the real parts of the roots of (8). We let $\chi = m + in$ $(m, n \in R)$, and rewrite (9) in terms of its real and imaginary parts as

$$\begin{vmatrix} m^4+n^4-6m^2n^2+K_1m\left(m^2-3n^2\right)+K_2\left(m^2-n^2\right)+K_{31}m-T_6=\ell^{-m\tau_2}\left\{T_1\left[m\left(m^2-3n^2\right)\cos n\tau_2+\right.\right. \\ \left.+n\left(3m^2-n^2\right)\sin n\tau_2\right]+T_2\left[\left(m^2-n^2\right)\cos n\tau_2+2mn\sin n\tau_2\right]+T_3\left(m\cos n\tau_2+n\sin n\tau_2\right)+T_4\cos n\tau_2\Big\}, \\ 4mn\left(m^2-n^2\right)+K_1\left(3m^2-n^2\right)n+2K_2mn+K_{31}n=\ell^{-m\tau_2}\left\{T_1\left[n\left(3m^2-n^2\right)\cos n\tau_2+m\left(3n^2-m^2\right)\sin n\tau_2\right]+\right. \\ \left.+T_2\left[2mn\cos n\tau_2+\left(n^2-m^2\right)\sin n\tau_2\right]+T_3\left(n\cos n\tau_2-m\sin n\tau_2\right)-T_4\sin n\tau_2\right\}. \end{vmatrix}$$

(21)

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

$$\begin{vmatrix} n^4-K_2n^2-T_6=\left(-T_1n^3+T_3n\right)\sin n\tau_2+\left(-T_2n^2+T_4\right)\cos n\tau_2, \\ -K_1n^3+K_{31}n=\left(-T_1n^3+T_3n\right)\cos n\tau_2+\left(T_2n^2-T_4\right)\sin n\tau_2, \end{vmatrix} \tag{22}$$

or another

$$\begin{aligned} \cos n\tau_2&=\frac{\left(n^4-K_2n^2-T_6\right)\left(T_2n^2-T_4\right)-\left(-K_1n^3+K_{31}n\right)\left(-T_1n^3+T_3n\right)}{\left(T_2n^2-T_4\right)^2+\left(-T_1n^3+T_3n\right)^2}, \\ \sin n\tau_2&=\frac{\left(-K_1n^3+K_{31}n\right)\left(T_2n^2-T_4\right)+\left(n^4-K_2n^2-T_6\right)\left(-T_1n^3+T_3n\right)}{\left(T_2n^2-T_4\right)^2+\left(-T_1n^3+T_3n\right)^2}. \end{aligned} \tag{23}$$

Note that $n=0$ can be a solution of (23) if $T_4=T_6$. If the first bifurcation point is $\left(n_b^0,\tau_b^0\right)$, then the other bifurcation points $\left(n_b,\tau_b\right)$ satisfy $n_b\tau_b=n_b^0\tau_b^0+2v\pi,\quad v=1,2,...,\infty$.

One can notice that if n is a solution of (22) (or (23)), then so $-n$. Hence, in the following we only investigate for positive solutions n of (22), or (23) respectively. By squaring the two equations into system (22) and then adding them, it follows that

$$\begin{aligned} &n^8+\left(K_1-2K_2-T_1^2\right)n^6+\left[K_2^2-T_2^2+2\left(T_1T_3-K_1K_{31}-T_6\right)\right]n^4+ \\ &\quad+\left[K_{31}^2-T_3^2+2\left(K_2T_6+T_2T_4\right)\right]n^2-T_4^2+T_6^2=0. \end{aligned} \tag{24}$$

Here, we note that this is a quartic equation on n^2 and that the left side is positive for large values of n^2 and negative for $n=0$ if and only if $T_4^2>T_6^2$, i.e Eq. (24) has at least one positive real root. Moreover, to apply the Hopf bifurcation theorem, according to [28], the following theorem in this situation applies:

Theorem 1. *Suppose that n_b is the least positive simple root of (24). Then, $in\left(\tau_b\right)=in_b$ is a simple root of (8) and $m\left(\tau_2\right)+in\left(\tau_2\right)$ is differentiable with respect to τ_2 in a neighborhood of $\tau_2=\tau_b$.*

To establish Andronov-Hopf bifurcation at $\tau_2=\tau_b$, we need to show that the following transversality condition $\left.\frac{dm}{d\tau_2}\right|_{\tau=\tau_b}\neq 0$ is satisfied.

Hence, we if denote

$$H\left(\chi,\tau_2\right)=\chi^4+K_1\chi^3+K_2\chi^2+K_3\chi-\ell^{-\tau_2\chi}\left(T_1\chi^3+T_2\chi^2+T_3\chi+T_4\right), \tag{25}$$

then

$$\frac{d\chi}{d\tau_2} = -\frac{\frac{\partial H}{\partial \tau_2}}{\frac{\partial H}{\partial \chi}} = \tag{26}$$

$$= \frac{-\chi \ell^{-\chi\tau_2}\left(T_1\chi^3 + T_2\chi^2 + T_3\chi + T_4\right)}{4\chi^3 + 3K_1\chi^2 + 2K_2\chi + K_{31} + \tau_2\ell^{-\tau_2\chi}\left(T_1\chi^3 + T_2\chi^2 + T_3\chi\right) + T_4 - \ell^{-\tau_2\chi}\left(3T_1\chi^2 + 2T_2\chi + T_3\right)}$$

Evaluating the real part of this equation at $\tau_2 = \tau_b$ and setting $\chi = in_b$ yield

$$\left.\frac{dm}{d\tau_2}\right|_{\tau_2=\tau_b} = \mathrm{Re}\left(\frac{d\chi}{d\tau_2}\right)\Bigg|_{\tau_2=\tau_b} =$$

$$= \frac{n_b^2\left\{4n_b^6 + 3\left(K_1^2 - 2K_2 - T_1^2\right)n_b^4 + 2\left[K_2^2 - T_2^2 + 2\left(T_1T_3 - K_1K_{31} - T_6\right)\right]n_b^2 + K_{31}^2 - T_3^2 + 2\left(T_2T_4 + K_2T_6\right)\right\}}{L_1^2 + I_1^2} \tag{27}$$

where $L_1 = -3K_1n_b^2 + K_{31} + \tau_2\left(n_b^4 - K_2n_b^2 - T_6\right) - \left(-3T_1n_b^2 + T_3\right)\cos n_b\tau_2 - 2T_2n_b\sin n_b\tau_2$ and $I_1 = 4n_b^3 - 2K_2n_b - \tau_2\left(-K_1n_b^3 + K_{31}n_b\right) + 2T_2n_b\cos n_b\tau_2 - \left(-3T_1n_b^2 + T_3\right)\sin n_b\tau_2$.

Let $\theta = n_b^2$; then, (28) reduces to

$$g(\theta) = \theta^4 + \left(K_1 - 2K_2 - T_1^2\right)\theta^3 + \left[K_2^2 - T_2^2 + 2\left(T_1T_3 - K_1K_{31} - T_6\right)\right]\theta^2 + \\ + \left[K_{31}^2 - T_3^2 + 2\left(K_2T_6 + T_2T_4\right)\right]\theta - T_4^2 + T_6^2. \tag{28}$$

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

$$g'(\theta)\Big|_{\tau_2=\tau_b} = \left.\frac{dg}{d\theta}\right|_{\tau_2=\tau_b} = \tag{29}$$

$$= 4\theta^3 + 3\left(K_1 - 2K_2 - T_1^2\right)\theta^2 + 2\left[K_2^2 - T_2^2 + 2\left(T_1T_3 - K_1K_{31} - T_6\right)\right]\theta + K_{31}^2 - T_3^2 + 2\left(K_2T_6 + T_2T_4\right).$$

If n_b is the least positive simple root of (24), then

$$\left.\frac{dg}{d\tau_2}\right|_{\theta=n_b^2} > 0. \tag{30}$$

Hence,

$$\left.\frac{dm}{d\tau_2}\right|_{\tau_2=\tau_b} = \mathrm{Re}\left(\frac{d\chi}{d\tau_2}\right)\Bigg|_{\tau_2=\tau_b} = \frac{n_b^2 g'\left(n_b^2\right)}{L_1^2 + I_1^2} > 0. \tag{31}$$

According to the Hopf bifurcation theorem [29], we define the following Theorem 2:

Theorem 2. *If n_b is the least positive root of (24), then an Andronov-Hopf bifurcation occurs as τ_2 passes through τ_b.*

Corollary 2.1. *When $\tau_2 < \tau_b$, then the steady state $\bar{E}$ of system (4) is locally asymptotically stable.*

2.2. The case $\tau_1, \tau_2 > 0$. We return to the study of (6) with $\tau_1, \tau_2 > 0$. In order to investigate the local stability of the equilibrium state $\bar{E}$ of system (4), we first prove a result regarding the sign of the real parts of characteristic roots of (6) in the next Theorem.

Theorem 3. *If all roots of (8) are with negative real parts for $\tau_2 > 0$, then there exists a $\tau_1^{bif}(\tau_2) > 0$ such that all roots of characteristic equation (6) have negative real parts at $\tau_1 < \tau_1^{bif}(\tau_2)$, i.e. when $\tau_1 \in [0, \quad \tau_1^{bif}(\tau_2))$.*

Proof. Similar to [7], let we assume that (8) has no roots with nonnegative real part when $\tau_2 > 0$. Therefore, characteristic equation (6) has no root with nonnegative real part when $\tau_1 = 0$ and $\tau_2 > 0$. Regard τ_1 as parameter, then (6) is analytic about χ and τ_1. By Theorem 2.1 of [24], when τ_1 varies, then the sum of the multiplicity of zeros of (6) in the open right half plane can only change if a zero appears on or crosses the imaginary axis. Because (6) (with $\tau_1 = 0$) has no root with nonnegative real part, there exists a $\tau_1^{bif}(\tau_2) > 0$ such that all roots of (10) with $\tau_1 < \tau_1^{bif}(\tau_2)$ have negative real part.

Corollary 3.1. *If τ_2^{bif} is defined as in Theorem 2, then for any $\tau_2 \in [0, \tau_b)$, there exists a $\tau_1^{bif}(\tau_2) > 0$ such that the steady state $\bar{E}$ of system (4) is locally asymptotically stable when $\tau_1 \in [0, \quad \tau_1^{bif}(\tau_2))$.*