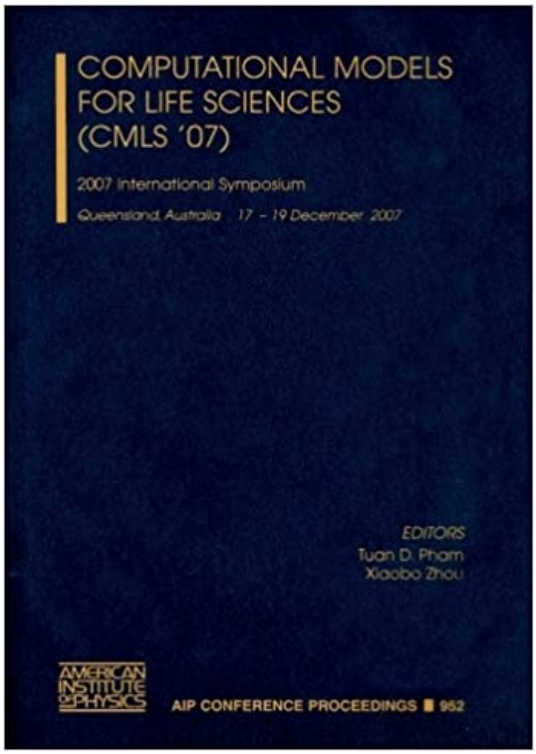
Simulation of RNA silencing pathway for time-dependent transgene transcription rate

Yang, X-D, Mahapatra, D.R., and Melnik, R.V.N.

Computational Models for Life Sciences -CMLS'07: 2007 Intern. Symposium on Computational Models of Life Sciences, AIP (American Institute of Physics) Conf. Proc., Vol 2, 2007, Vol. 952, pp. 229--237, 2007.



COMPUTATIONAL MODELS FOR LIFE SCIENCES—CMLS '07: 2007 International Symposium on Computational Models of Life Sciences

Conference date: 17–19 December 2007
Location: Gold Coast, Queensland (Australia)
ISBN: 978-0-7354-0466-3
Editors: Tuan D. Pham and Xiaobo Zhou
Volume number: 952
Published: Nov 2, 2007

Simulation of RNA Silencing Pathway for Time-Dependent Transgene Transcription Rate

Xiao-Dong Yang[a,b], Debiprosad Roy Mahapatra[b] and Roderick V. N. Melnik[b]

[a] *Department of Engineering Mechanics, Shenyang Institute of Aeronautical Engineering, Shenyang 110136, China*
[b] *Mathematical Modeling & Computational Sciences, Wilfrid Laurier University, Waterloo, Ontario Canada N2L 3C5*

Abstract. The synthesis of dsRNA is analyzed using a pathway model with amplifications caused by the aberrant RNAs. The transgene influx rate is assumed time-decaying considering the fact that the number of transgenes can not be infinite. The dynamics of the transgene induced RNA silencing is investigated using a system of coupled nonautonomous ordinary nonlinear differential equations which describe the model phenomenologically. The silencing phenomena are detected after a period of transcription. Important contributions of certain parameters are discussed with several numerical examples.

Keywords: RNA silencing pathway; Computational models; RNAi; Dynamics
PACS: 87.14.Gg; 82.35.Rs

INTRODUCTION

RNA silencing and associated phenomena of RNA interference (RNAi) and post-transcriptional gene silencing (PTGS), are recognized broadly in biology field. Invading nucleic acids, such as transgenes and viruses, are silenced through the action of small homologous RNA molecules [1,2]. This process is a reflection of a cellular defense mechanism against transposons and viruses paralleling the operation of the vertebrate-specific immune system against harmful elements.

PTGS is related to the RNA interference in which double-stranded RNA (dsRNA) is used to target homologous messenger RNAs (mRNAs) for destruction. As shown in pathway of RNA silencing in Fig. 1, the mRNAs origin from the invaded transgenes. The RNA-directed RNA polymerase (RDR) helps synthesis of dsRNAs from mRNA. The structure of RNA polymerase has been explained by Westover et al [3]. The cleavage of dsRNA by Dicer, a RNase III-class enzyme, into small interfering RNAs (siRNAs) 21-25 nucleotides long initiates the process. Then, siRNAs can be incorporated into RNA induced silencing complex (RISC). The complex is guided to cleave the target mRNA into aberrant pieces of RNA (garbage RNA), via antisense base-pairing. After the entire process, contrary to expectations, the increased gene dosage does not result in enhanced expression but gene silencing [4]. This activation of transgene induced RNA silencing is linked to transgenes invaded into the cell and the transcription rate to mRNA. The pathway prescribed here may include two types

CP952, *Computational Models for Life Sciences—CMLS '07, 2007 International Symposium*
edited by T. D. Pham and X. Zhou

of RNA silencing [4]. The first is induced by highly transcribed sense transgenes (S-PTGS) [5] and the second by trangene loci producing or injected dsRNA due to the presence of inverted repeats (IR-PTGS) [6].

Also the mechanism of RNA silencing is not very clear, some mathematical models have been proposed to describe the phenomenon. In [7] the authors introduced extended models to describe transgene induced silencing. The dynamics and solution bifurcations were investigated for three different models. Inspired by their results, we study the model with amplification of aberrant DNAs, in which the mRNA transcription rate is assumed to be an exponentially decaying function of time. It is natural to assume such a time varying transcription rate because the transcription rate of the numbers of mRNA cannot tend to be infinite in one cell. The parameters' contributions to the two kinds of RNA silencing caused by high transcription rates of mRNA from transgenes (S-PTGS) and by high initial values of injected dsRNA (IR-PTGS) are demonstrated respectively. The results of this research have not been validated by biologists. But the phenomenon of RNA silencing can be predicted and described well qualitatively by some mathematical equations proposed in this article.

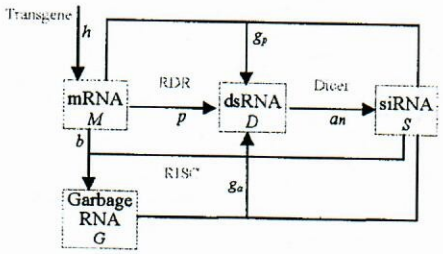


FIGURE 1. The pathway of RNA silencing

MODEL DESCRIPTION

There are different types of RNA silencing pathways based on the experiments (See, e.g., ref. [8]). In ref. [9], the authors extended the basic model to explain what prevents a mistaken reaction from silencing vital organismal genes. According to the pathways illustrated in Fig. 1, the basic model can be interpreted by the four coupled ordinary differential equations. Since the basic model does not lead to the transgene induced RNA silencing as elucidated in ref. [7], an extended model is needed to interpret the phenomenon. As discussed in [10], the small interfering RNAs (siRNAs) serve as primers to transforming the target mRNAs into dsRNAs, in which siRNAs act as RDR in synthesis of dsRNAs from mRNA. Then dsRNAs are cleaved by activity of Dicer into siRNAs to eliminate the target mRNAs to produce aberrant RNAs. It is obvious that this process amplifies the pathway of eliminating mRNAs. If the aberrant RNAs are assumed to incorporate the target mRNAs into dsRNA, a model termed as 'garbage model', as mentioned in ref. [7], can be obtained. Figure 1 interprets this model including all the pathways. The RNA silencing pathway can be described with four nonlinear coupled differential equations as follows:

$$\begin{cases} \dfrac{dM}{dt} = h - d_m M - pM - bSM - g_p SM, \\ \dfrac{dD}{dt} = pM - aD + g_p SM + g_a SG, \\ \dfrac{dS}{dt} = anD - d_s S - bSM - g_p SM - g_a SG, \\ \dfrac{dG}{dt} = bSM - d_g G - g_a SG, \end{cases} \tag{1}$$

where M, D, S, and G denote the number of mRNA, dsRNA, siRNA, and garbage pieces of RNA. The parameters of d_m, d_s, and d_g are the degradation rates of mRNA, siRNA, and garbage RNA. dsRNA is synthesized from mRNA by RDR with small rate p, and is cleaved into n siRNAs with rate a. The term bSM results from the binding of mRNA and RISC. mRNA is transcribed with rate h, which may be expressed as

$$h = ri \tag{2}$$

in which r is the transcription rate of every transgene and i is the copy number of transgenes.

In Eq. 1, g_pSM represents the amplification caused by synthesis of dsRNA by the presence of siRNA and mRNA. The g_aSG term denotes dsRNA synthesis of garbage RNA associated with siRNA. Therefore, dsRNAs are produced by three ways indicated in Fig. 1 where three arrows are pointed towards the square of dsRNA.

Usually, the number of transgenes is assumed constant, that is, the transcription rate of mRNA does not vary along time. In fact, the number of transgenes in eukaryotic cell can not be infinite. So it is natural to assume that the number of transgenes is an exponentially decaying function of time, and so the mRNA transcription rate h can be replaced by

$$h = r\exp(-d_e t),\ d_e > 0. \tag{3}$$

As a particular example of exponential distributions, we consider also the case of gene operation in the course of medical remedy where the transcription rate is given in the following Gaussian form:

$$h = r\exp\left(-d_e \frac{(t-t_0)^2}{\tau^2}\right), \tag{4}$$

where t_0 is the beginning time when dsRNA is injected into the cell and τ is the parameter denotes the decaying speed.

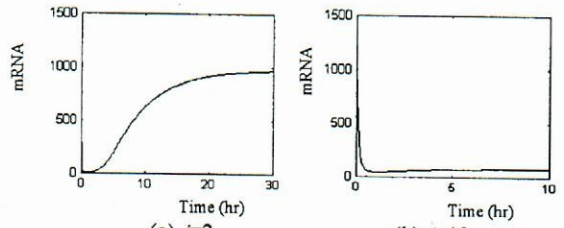
RNA SILENCING ANALYSIS WITH CONSTANT TRANSGENE INFLUX RATE

First we consider the dynamics governed by Eq. 1, where the numbers of transgenes are assumed constant. By the Runge-Kutta method, the solutions can be obtained to demonstrate RNA silencing induced by transgenes. All the values of the parameters present in Table I are identical to those used in [7].

TABLE I Parameter Values

Parameters	Values	Parameters	Values
d_m	0.14 hr^{-1}	d_s	2 hr^{-1}
d_g	2.8 hr^{-1}	r	160 $hr^{-1}cell^{-1}$
p	0.002 hr^{-1}	a	2 hr^{-1}
n	10	b	0.008 cell mol^{-1} hr^{-1}
g_a	0.0008cell mol^{-1} hr^{-1}	g_p	0.0008 hr^{-1}

Figure 2 shows the time histories of mRNA after the transgenes invading the cell for various values of transgene numbers when the transgene number is constant. When the transgene number is small, it shows a transient silencing after dsRNA is introduced into the cell and then the mRNA is restored to a steady state value; otherwise silencing is triggered and the default equilibrium is brought to the basin of attraction of silenced equilibrium. It is quite evident that higher transgene influx values induce the RNA silencing (S-PTGS). In this computation and also in those of Fig. 3, the initial values for [mRNA(M), dsRNA(D), siRNA(S), aberrant RNA(G)] are adopted as [1000, 1000, 0, 0].



(a) i=2 (b) i=12

FIGURE 2. Time histories of mRNA for constant transcription rate

The equilibrium solutions of Eq. 1 can be obtained by letting time-dependent terms equal to zero:

$$\begin{cases} h - d_m M - pM - bSM - g_p SM = 0, \\ pM - aD + g_p SM + g_a SG = 0, \\ anD - d_s S - bSM - g_p SM - g_a SG = 0, \\ bSM - d_g G - g_a SG = 0. \end{cases} \tag{5}$$

Eliminating the other variables in Eq. 5, we can get the following algebraic equation of S:

$$\begin{aligned} & d_s(b+g_2)g_3S^3+\left[(2bh-nbh+hg_2-nhg_2+d_sd_m+d_sp)g_3+\right. \\ & \left.d_s(b+g_2)(d_g+g_1)\right]S^2+\left[(bh+g_2h-ng_2h+d_sd_m+d_sp)(d_g+g_1)+\right. \\ & \left.-pnhg_3-nbhg_1\right]S-pnh(d_g+g_1)=0. \end{aligned} \tag{6}$$

The equilibrium solution for S can be obtained from Eq. 6 numerically and then the other variables in equilibrium position can be derived one by one from the following relations:

$$M=\frac{h}{d_m+p+(b+g_2)S} \tag{7}$$

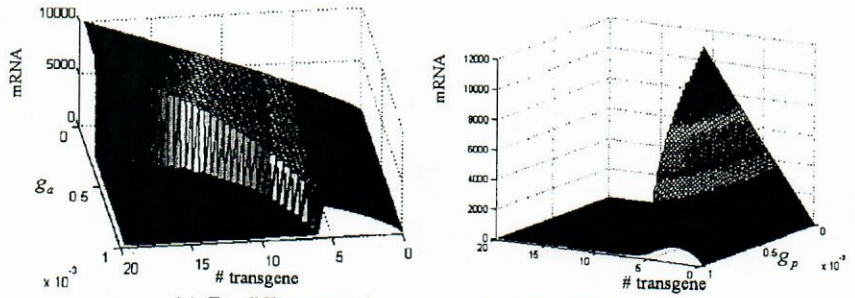
$$G=\frac{bSM}{d_g+g_1+g_3S} \tag{8}$$

$$D=\frac{pM+g_2SM+g_sSG+g_1G}{a} \tag{9}$$

For different transgene numbers by using Eq. 6, we can find the right parameters where the transgene triggered RNA silencing phenomenon occurs. Also, with the same method, we can study the effect of each of the parameters g_a and g_p, respectively, for various transgene numbers with the other parameters fixed.

In Fig. 3(a), we present the mRNA numbers in one cell when it has attained the steady equilibrium for different initial transgene numbers and different values of parameter g_a. When g_a is nonzero, the increase of copy number of transgenes leads to an increase in mRNA levels. Then after transgene copy number surpasses a critical value RNA silencing occurs. If the amplification caused by the incorporation of aberrant RNA and target mRNA is not considered, that is g_a=0, then the RNA silencing does not happen for any initial transgene copy number. An increase in the parameter g_a results in a decrease in the critical value of the transgene copy number required to initiate the RNA silencing.

Figure 3(b) demonstrates the mRNA levels for a series of values of parameter g_p, which denotes the amplification of the pathway by the synthesis of dsRNA, siRNA and mRNA. The transgene induced RNA silencing happens when the parameter g_p is zero or small. Same as the effects of g_a, an increase in g_p also leads to a decrease in the critical transgene number to trigger the RNA silencing. When the value of g_p is rather high, the transgene induced RNA silencing does not happen at all.



(a) For different g_a values (b) For different g_p values

FIGURE 3. Contributions of nonlinear parameters to RNA silencing for different transgene numbers

In the numerical examples shown in Fig. 3, the parameter values are taken from Table 1 unless mentioned otherwise.

In the case that the transgene number is constant, the discontinuity for different transgene copy numbers can be explained by analyzing the equilibria of the nonlinear differential equations. To obtain the steady state solutions of Eq. 1, we can equate the right sides of the equations to zero. After substitutions we get the cubic algebraic equation with respect to the dsRNA variable S

$$A_3 S^3 + A_2 S^2 + A_1 S + A_0 = 0, \tag{10}$$

where

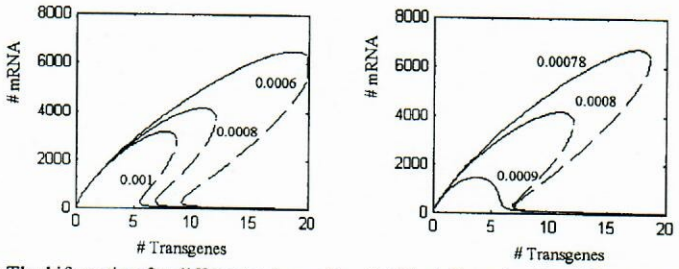
$$\begin{aligned} A_3 &= d_s(b + g_a)g_p, \\ A_2 &= (bi + g_a i + d_s d_m + d_s p)g_p + \\ &\quad d_s d_g(b + g_a) + g_p i(b - bn - g_a n), \\ A_1 &= (bi + g_a i + d_s d_m + d_s p)d_g \\ &\quad - pnig_p - g_a nid_g, \\ A_0 &= -pnid_g. \end{aligned} \tag{11}$$

The steady state solution of mRNA M can be obtained by using Eq. 5 and

$$M = \frac{i}{d_m + p + (b + g_a)S}. \tag{12}$$

After solving Eq. 10 and using Eq. 11 for different parameters, we can plot the bifurcation diagrams of the mRNA for different transgene numbers. The nonlinear nature of the system has been shown in the bifurcation diagrams of Fig. 4 where the solid lines denote the stable solutions and the dash lines denote unstable solutions. From Fig. 4, we can verify the previous conclusion we have obtained that increasing g_a or g_p both leads to a decrease in the critical transgene copy number value. But the difference is when g_p is large enough, there exists no unstable solution as shown in Fig.

4(b) for the case g_p=0.0009, as indicated that the RNA silencing does not happen when larger g_p is considered in Fig. 3(b).



(a) The bifurcation for different values of g_a (b) The bifurcation for different values of g_p
FIGURE 4. The bifurcation diagrams

RNA SILENCING ANALYSIS FOR TIME-DECAYING TRANSGENE INFLUX RATE

For the case of decaying transgene influx rate, the system of ordinary differential Eq. 1 is not autonomous. So we can not obtain the steady state solutions directly from an algebraic equation. We can solve non-autonomous nonlinear Eq. 1 by a computational method to obtain the mRNA values at any time after transgenes' invading. Figure 5 demonstrates the mRNA values after 400 hours of reaction for various initial transgene numbers and exponential indices. The time-decaying transgene influx rate has made the RNA silencing not as intense as it happened when the transgene influx rate was assumed constant. An increase of exponential index value results in the increase of the critical transgene copy number of triggering the RNA silencing.

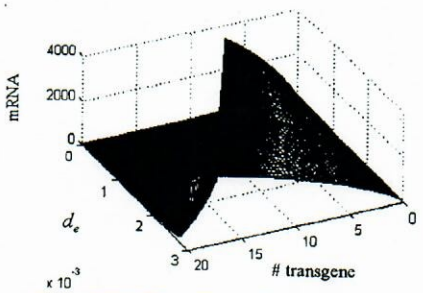
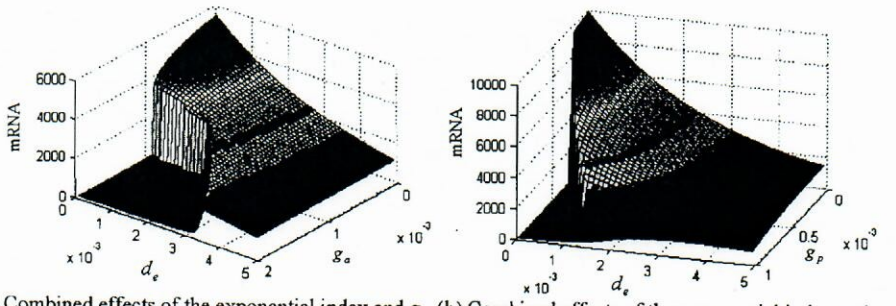


FIGURE 5. The effects of exponential index

Figure 6 (a) and (b) demonstrate the combined effects of exponential index d_e with g_a and g_p respectively. The effect of exponential index value is twofold. Firstly, the increasing exponential index value makes the RNA silencing difficult to be triggered.

It means that if we want to trigger the silencing for the exponential index values, we must adopt higher transgene number, higher g_a or higher g_p, as indicated in Fig. 5 and Fig. 6. Secondly, an increase of the exponential index leads to a decrease of mRNA levels at a fixed time for all cases. When the exponential index is large enough, it is hard to detect the phenomenon of RNA silencing.

The different effects of the mRNA values of g_a and g_p can also be observed from Fig. 6 as discussed in the constant transgene influx case. The nonlinearity characteristic disappears as shown in Fig. 6(b), where the discontinuity is smoothed out when g_p is large enough. But for the case of g_a, this phenomenon never happens.



(a) Combined effects of the exponential index and g_a (b) Combined effects of the exponential index and g_p

FIGURE 6. Combined effects of the exponential index and nonlinear parameters

CONCLUSIONS

The transgene and dsRNA induced RNA silencing has been investigated using the four coupled ordinary differential equations which describe the process with amplifications of the pathways of RNA silencing from the incorporation of siRNAs and aberrant RNAs. By using numerical examples, we have analyzed the contributions of the amplification parameters in the case where the transgene transcription rate is assumed time-dependent. The two kinds of RNA silencing, S-PTGS and IR-PTGS, have been explained by this model well, giving the dynamical interpretation of the silencing phenomenon.

ACKNOWLEDGMENTS

The authors would like to thank Gabriel Moreno-Hagelsieb of the Department of Biology, Wilfrid Laurier University for useful discussions and Marian A. C. Groenenboom of Theoretical Biology and Bioinformatics, University of Utrecht for insightful correspondence on transgenes.

REFERENCES

1. T. A. Volpe et al, "Regulation of heterochromatic silencing and histone H3 Lysine-9 methylation by

RNAi," *Science* **297**, 1833-1837 (September 2002)
2. M. Pal-Bhadra, U. Bhadra and J. A. Birchler, "RNAi related mechanisms affect both transcriptional and post-transcriptional transgene silencing in *Drosophila*," *Mol. Cell* **9**, 315-327 (February 2002)
3. K. D. Westover, D. A. Bushnell, R. D. Kornberg, "Structural basis of transcription: separation of RNA from DNA by RNA polymerase II", *Science* **303**, 1014-1016 (February 2004)
4. C. Béclin, S. Boutet, P. Waterhouse, and H. Vaucheret. " A branched pathway for transgene-induced RNA silencing in plants," *Current Biology* **12**, 684-688 (April 2002)
5. H. Vaucheret, L. Nussaume, J.-C. Palauqui, I. Quillere, and T. Elmayan, "A transcriptionally active state is required for post-transcriptional silencing (cosuppression) of nitrate reductase host genes and transgenes," *Plant Cell* **9**, 1495-1504 (August 1997)
6. P. M. Waterhouse, and M.-B. Wang. "Virus resistance and gene silencing in plants can be induced by simultaneous expression of sense and antisense RNA," *Proc. Natl. Acad. Sci. USA* **95**, 13959-13964 (November 1998)
7. M. A. C. Groenenboom, A. F. M. Maree and P. Hogeweg, "The RNA silencing pathway: the bits and pieces that matter," *PLoS Computational Biology* **1**, e21 (July 2005)
8. P. Brodersen and O. Voinnet, "The diversity of RNA silencing pathways in plants," *TRENDS in Genetics* **22**, 268-280 (May 2006)
9. C. T. Bergstrom, E. McKittrick, and R. Antia, "Mathematical models of RNA silencing: Unidirectional amplification limits accidental self-directed reactions," *PNAS* **100**, 11511-11516 (September 30, 2003)
10. C. Lipardi, Q. Wei, and B. M. Paterson, "RNAi as random degradative PCR: siRNA primers convert mRNA into dsRNAs that are degraded to generate new siRNAs," *Cell* **107**, 297-307 (November 2, 2001)

Titre: COMPUTATIONAL MODELS FOR LIFE SCIENCES(CMLS '07): 2007 International Symposium on Computational Models of Life Sciences; DOI:10.1063/1.2816627

Auteur: American Institute of Physics Conference Proceedings, Volume 952,

Adresse: American Institute of Physics, Issue Date: November 2, 2007

Pages: Volume 952, Issue 1, pp. 229-237

Titre article: Simulation of RNA Silencing Pathway for Time-Dependent Transgene Transcription Rate

Auteur article: Xiao-Dong Yang,a,b Debiprosad Roy Mahapatra,b and Roderick V. N. Melnikb

ISBN: 9780735404663