Improvement and construction of RNA Silencing Model with hybrid functional Petri net

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ABSTRACT

RNA interference, also known as RNAi, RNA silencing, or posttranscriptional gene silencing (PTGS) is a mechanism presented in almost all eukaryotes that functions like miniature immune system. The mechanism of RNA interference is a successive phenomenon taken place from DNA level to protein level. In this paper, we have modified the RNA silencing mechanism by adding four important factors that did not show in other works. Those factors are stochastic translocation of mRNA, stochastic dsRNA synthesis, formation of RISC protein, and randomly functions of primed as well as unprimed dsRNA supply. We also construct our new model using Hybrid functional Petri net. The simulation results of our model show that our findings are strong consistent with known biological phenomena.

Keywords: RNA interference, RNA silencing model, systems biology, HFPN, antisense therapeutics.

1: INTRODUCTION

RNA interference, also known as RNAi, RNA silencing, or posttranscriptional gene silencing (PTGS) is a mechanism presented in almost all eukaryotes that functions like miniature immune system [16]. This naturally occurring phenomenon was first found in the nematode worm *Caenorhabditis elegans* [4]. RNA interference can be applied by variable ways. The most impressive and burgeoning exploit is expected in clinical called antisense therapeutics. Three type of antisense therapeutics can be distinguished. Firstly, the use of single stranded antisense oligonucleotides; secondly, the triggering of RNA cleavage through catalytically active oligonucleotides referred to as ribozymes; and thirdly, RNA interference induced by small interfering RNA molecules [8]. The third one, RNA interference, holds more potential since Elbashir and its colleagues showed that 21 nucleotide-long siRNA duplexes with 3'overhangs can specifically suppress gene expression in mammalian cells [3].

Antisense molecules are stretches of single stranded nucleic acid that target and bind with a specific messenger RNA (mRNA), interfering with and even preventing the translation or over-expression of the protein encoded by the mRNA. The single strand RNA complementary to target mRNA called "antisense", because the "sense" sequence and orientation of an mRNA is the one that directly translates itself into functional protein. This binding prevents target mRNA from carrying out its function, and we describe that the single strand RNA "silence" or "interfere" the target mRNA. The ability to interfere with a critical point along the gene expression and protein synthesis pathway is what makes antisense such an attractive molecular therapeutic platform. Theoretically, antisense molecules could be used to cure any disease which is caused by the expression of a deleterious gene, e.g. viral infections, cancer growth, inflammatory diseases, and heredity disease [15].

In the last few years, important insights have been gained in elucidating the mechanism of RNAi. A combination of results obtained from several in vivo and in vitro experiments have divided into a three-step mechanistic model for RNA interference [6, 7]. The first step, referred to as the RNAi initiating step, involves binding of the RNA nucleases, or Dicer, to dsRNA and cleavage it into discrete 21 to 25 nucleotide RNA fragments (siRNA). In the second step, these siRNAs join multinuclease complex, RISC, which degrades the homologous single-stranded mRNAs. In the third step, RISC is recycled and siRNA undergoes amplification to supply siRNA to maintain silencing reaction. We show a brief step of RNA silencing in Figure 1. Despite the fact that many studies about RNAi have showed a concise procedure of RNA silencing mechanism, there are still lots of problems suspending. Fist, most processes in biology are stochastic instead of deterministic. The RNA silencing phenomenon is without exception. For instance, dsRNA synthesis and mRNA translocation should be the stochastic phenomena. Secondly, RISC is a tetramer proteins, the formation of RISC takes time during RNA silencing. It may influence the formation of RISC-siRNA complex. Thirdly, according to the literature [9, 11], supply of dsRNA has two different ways, primed and unprimed mechanism. However, we still not sure which way is correct. All above problems will be solved by Petri Net in the present paper.

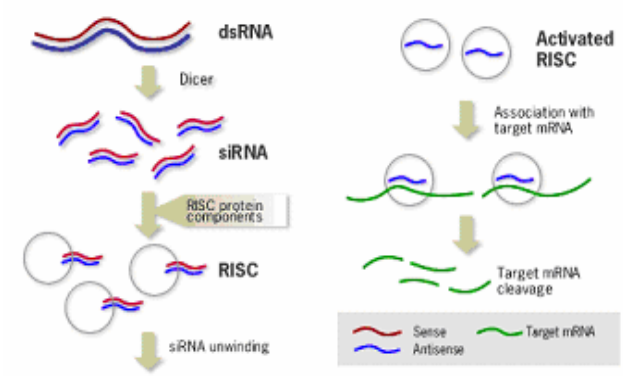

Figure 1. A brief pathway of RNA silencing.

2: CONSTRUCTING A RNA SILENCING MODEL WITH PETRI NET

In this section, we try to elucidate the magnificent advantages in RNA silencing model based on Petri Net. We will first give a concise introduction to Petri Net theory, including its characteristics and basic components with their functions, especially those applied to RNA interference models [2, 14]. Secondly, a basic mathematical model of RNA silencing will be demonstrated. At the last, we present the procedure in constructing RNA silencing model in an intuitive way.

2.1: HYBRID FUNCTIONAL PETRI NET IN BIOLOGICAL SIMULATION

Many theories have been demonstrated for biological pathways, such as E-Cell [10, 19], BioSpice [17] and so forth. Many useful simulating systems are built on the basis of ordinary differential equations (ODEs). Unfortunately, it is rather complicated to use E-Cell for modeling a simple biological pathway. Those who majors in biology won't prefer to learn complicated mathematical equations to construct their own models. It is really a chore for biologists. Nevertheless, Petri Net holds its advantage for constructing model intuitively. In brief, Petri nets is suitable than other mathematical descriptions while simulating biological phenomena.

Petri Net is a theory employed in many fields. Many extensions to the simple Petri Nets model have been developed for various modeling and simulation purposes. The major categories of Petri Net extensions are listed: (a) Hierarchical Petri Nets, which allow the previously defined net to present in a new net as an entity or process. (b) Hybrid Petri Nets, which allow the component to deal with continuous values instead of integer numbers of tokens. (c) Timed Petri Nets, which introduce the concept of deterministic time delays. (d) Stochastic Petri Nets, in which entity and process may be assigned delays which are given by a probability distribution. (e) Colored Petri Nets, which allow more complex firing rules in the processes.

However, it is until recently that Petri Net makes its huge influence on biological application. A novel notion of Petri net called hybrid functional Petri net (HFPN) and the enhanced version, hybrid functional Petri net with extension (HFPNe), have been develop by Masao Nagasaki and his colleague. Both methods extend and combine the notions of different kinds of Petri Nets, making it suitable for solving biological problems. As a result, in this paper, we establish RNA silencing model with HFPNe [12, 13].

HFPN contains several key components: entity (place), process (transition), connector (arc), and tokens (markings). Entity can represent protein, gene, metabolite, and any signal factor. Processes stand for reactions, binding, separation, transcription, translation and ordinary biochemical reactions. In Petri Net, entity and process can be divided into two kinds in accordance with its properties, discrete and continuous. A continuous entity contains positive real number; otherwise, discrete entity contains natural number. Furthermore, a continuous process delivers tokens at every time unit; however, discrete process can deliver tokens every define time interval. Connectors play the roles of connecting each component and making a network. Tokens, or markings, indicate the value held by each entity. With those elements, we can represent some special reaction in RNA silencing model, such as mRNA degradation, mRNA translocation, transcription, complex formation, and so on. Different kind of components and reactions are showed in Figure 2.

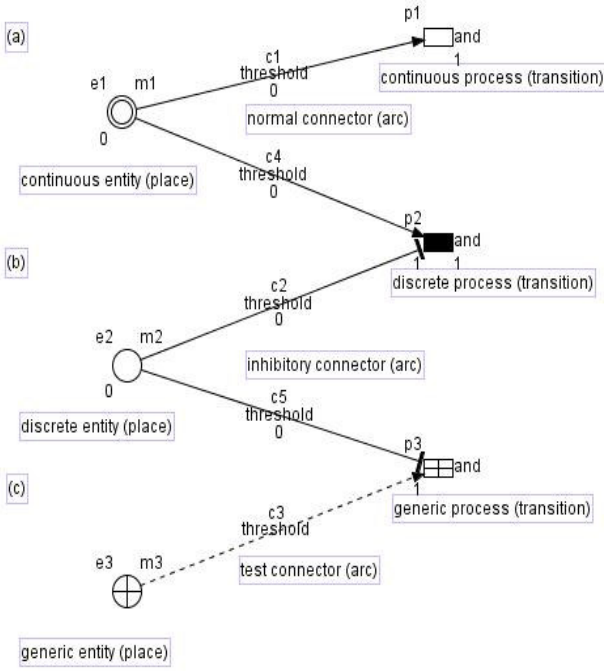

Figure 2. The different kinds of entities, processes, and connectors in HFPN. (a) continuous entity, normal connector, and continuous process. (b) discrete entity, inhibitory connector, and discrete process. (c) generic entity, test connector, generic process.

2.2: OUR MODIFIED MATHEMETICAL MODEL FOR RNA SILENCING

In order to run simulation of RNA silencing, we need to introduce firing rules into every process. In the present paper, we have modified the original modes [1, 5] by adding four important factors that did not show in other works. Those factors are stochastic translocation of mRNA, stochastic dsRNA synthesis, formation of RISC protein, and randomly functions of primed as well as unprimed dsRNA supply. We divide RNA silencing into four steps; therefore, it can be described quantitatively as a system of differential equations. The four equations are:

$$\frac{dm1}{dt} = -anm1 + gum3 + pm4 \quad (1)$$

$$\frac{dm2}{dt} = anm1 - bm2m4 - gpm2m4 - dsm2 \quad (2)$$

$$\frac{dm3}{dt} = bm2m4 - gum3 - dcm3 \quad (3)$$

$$\frac{dm4}{dt} = i - bm2m4 - pm4 - dmm4 \quad (4)$$

The four variables, m1, m2, m3, m4, stand for concentrations of dsRNA, RISC-siRNA complex, RISC-siRNA-mRNA complex, and mRNA in cytoplasm, respectively. Moreover, the parameters in above equation are listed in Table 1.

2.3: CONSTRUCTING AND RUNNING RNA SILENCING MODEL

We have already given a brief introduction to basic components in Petri Net. Therefore, how to use those components adequately to represent biological reactions in real world is what we concern. In this section, we will describe the process of building a RNA silencing model step by step. On the other hand, those problems which have not been taken into account in previous works will be solved in this Petri Net model.

First, we need to decide the number of nodes in our Petri Net model. Considering the mechanism of RNA silencing, we put seven elements, so called entities in Petri Net, in this model, including dsRNA (m1), RISC-siRNA complex (m2), RISC-siRNA-mRNA complex (m3), mRNA in cytoplasm (m4), mRNA in nuclei (m5), monomer of RISC (m6), and ssRNA (m8). Secondly, continuous processes, however, represent the specific reactions occurred in this pathway, such as combination, degradation, translocation, and synthesis. Finally, the normal arcs connect each component to complete a model. After the main structure has been established, we apply parameters, as lists in Table 1 and processes firing rules, as lists in Table 2, to complete the whole model of the RNA silencing. In Figure 3, we can see a model of RNA silencing with all the firing rules (the functions present in every processes).

Table 1. Properties of each entry in the model we construct. (all variables are described per molecule per time unit)

Name	Type	Variable	Initial Value
dsRNA	Double	m1	0
RISC-siRNA complex	Double	m2	0
RISC-siRNA-mRNA complex	Double	m3	0
mRNA_cytoplasm	Double	m4	0
mRNA_nuclei	Double	m5	1000
RISC_monomer	Double	m6	1000
ssRNA	Double	m7	10000
Unprimed amplification rate	Double	gu	0.4
Primed amplification rate	Double	gp	0.002
Decay rate of RISC-siRNA-mRNA complex	Double	dc	0.0001
Decay rate of mRNA	Double	dm	0.1
Decay rate of siRNA	Double	ds	0.0001
Rate of dsRNA cleavage	Double	a	20
Rate of RISC-siRNA-mRNA complex formation	Double	b	0.008
Rate of mRNA synthesis	Double	i	1000
Number of siRNAs cleaved from one dsRNA	Double	n	5
Rate of dsRNA synthesis from mRNA	Double	p	0.002

Now we discuss in detail about the processes in our model:

1. The dsRNA is formed from ssRNA, which will be translocated from nuclei to cytoplasm, stochastically by RNA-dependent polymerase. In our model, a process with stochastic activity (if (rand()<0.5){return true;}else {return false;}) works for this reaction.
2. The target mRNA for RNA silencing should be transcribed in nuclei and translocate into cytoplasm. We set a stochastic process to handle this phenomenon.
3. After the complex finish silencing works, dsRNA will be supplied through two pathways, primed and unprimed. For these two pathways are possible in RNA silencing, we use two processes with stochastic activity to represent them.

4. RISC is a protein with four subunits. It must work after the four subunits are combined. We set a process called tetramerization to represent this reaction. The kinetic style of this process is set to stochastic mass (standard deviation=0.2, c6 stoichiometry=4, c5 stoichiometry=1, coefficient1=0.01, coefficient2=0.1).

These four setting should improve this RNA silencing model to be more closer to the real world phenomena.

Table 2. Firing rules and properties of each process

Name	Type	Firing Style	Kinetic Script
synthesis_p1	continuous	and	10
Dicer_cleavage_p2	continuous	and	a*n*m1
Tetramerization_p3	continuous	and	SMass(m7 *0.0010,0.2)
combination_p4	continuous	and	b*m2*m4
translocation_p5	continuous	and	100
synthesis_p6	continuous	and	p*m4
unprimed_ amplification_p7	continuous	and	gu*m3
primed_ amplification_p8	continuous	and	gp*m2*m4
degradation_p9	continuous	and	ds*m2
degradation_p10	continuous	and	dc*m3
synthesis_p11	continuous	and	i
degradation_p12	continuous	and	dm*m4

3: RESULTS

We have constructed our model of RNA silencing. The simulation results are compared with some known experimental RNA silencing phenomena. In order to see the differences in outputs, we have adjusted several levels of dsRNA. Before running the simulation, we set the running time to be 50pt (pt is a time unit in GON, here we assume that a time unit is one hours) .In accordance with the model we have constructed, we can get the simulation results showed in Figure 4 to 7. After simulation, we compared the results with some known biological phenomena below:

1. During RNA silencing process, concentration of dsRNA will exhibits a strong initial drop.
2. Rapid generation of sequence-specific siRNA makes RISC rise in early stage.
3. While RdRp functions normally, or there are enough dsRNA for target mRNA, silencing continues and mRNA will decline sharply.
4. RNA silencing is "dosage dependence", which means that process will be influenced by initial level of dsRNA.

All above biological phenomena match to the results presented in Figure4-7. In Figure 4, the initial levels of ssRNA=10000. We can see that dsRNA drops down and the target mRNA is silenced. In Figure 5, we changed the level of ssRNA to be 1000; however, the phenomenon of dosage dependence is not obvious. In Figure 6, we changed the level of ssRNA again (the 1/10th of Figure 5), we then find that target mRNA does not drop dramatically (dosage dependence). In Figure 7, we set the input of dsRNA; therefore, we can observe that silencing function even more efficiently.

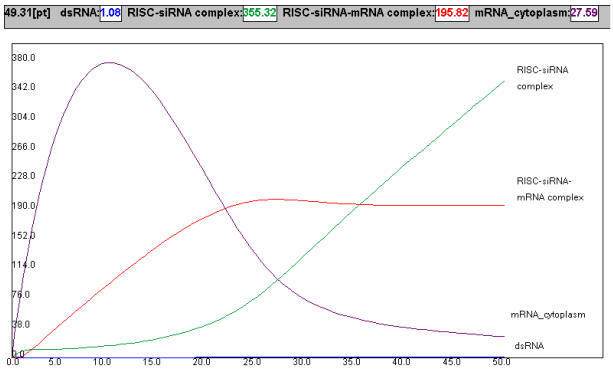

Figure 4.The simulation results of our RNA silencing model when initial ssRNA =10000.

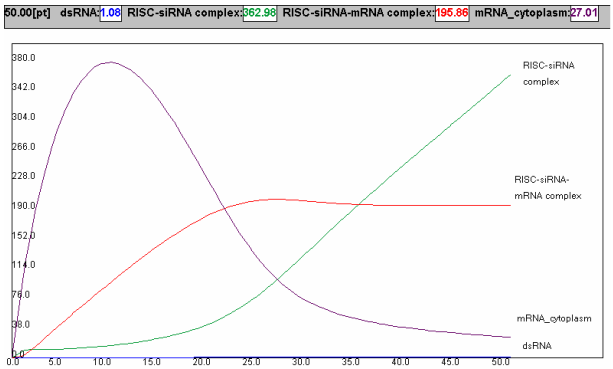

Figure 5. The simulation results of our RNA silencing model when initial ssRNA=1000.

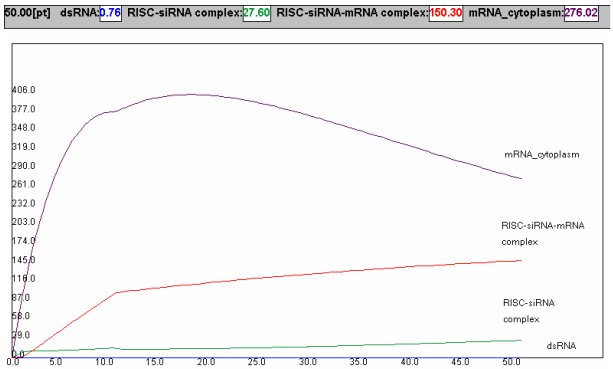

Figure 6. The simulation results of our RNA silencing model when initial ssRNA=100.

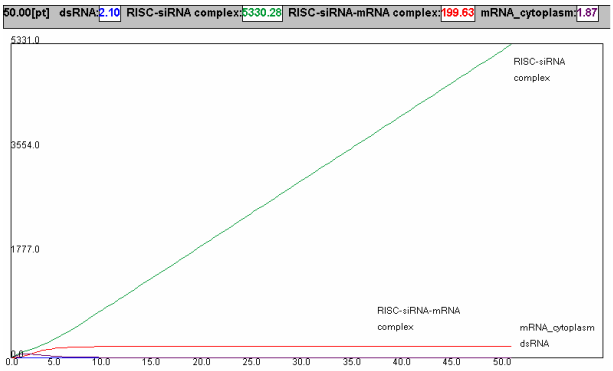

Figure 7. The simulation results of our RNA silencing model when initial ssRNA=10000 and the dsRNA input is at rate of 100/h. It may represent chemically synthesized or in vitro transcribed siRNA duplexes which can be transfected into cells

4: CONCLUSION

In the present work, we strive to use the simple way, which biologists can easily accept, to construct a modified model with Petri Net. Future studies will encompass more of variables involved in this reaction and hopefully further elucidate RNA silencing pathway and the other predictions which can be applied to antisense therapeutics. KEGG [18] provides many biological pathways that may be helpful while considering the whole-cell simulation. RNA interference is still a new phenomenon, so that the mechanism still holds lots of unknown variables. Furthermore, the full-size simulation is a challenging endeavor and we intend to do in our model in the near future. On the other hand, RNA silencing has a clinical application called antisense therapeutics. In other works, we have constructed a model of methionine cycle which plays important role in liver. We intend to combine these two models, simulating every component's concentration in using antisense therapeutics to treat liver disease.

5: ACKNOWNLEDGEMENT

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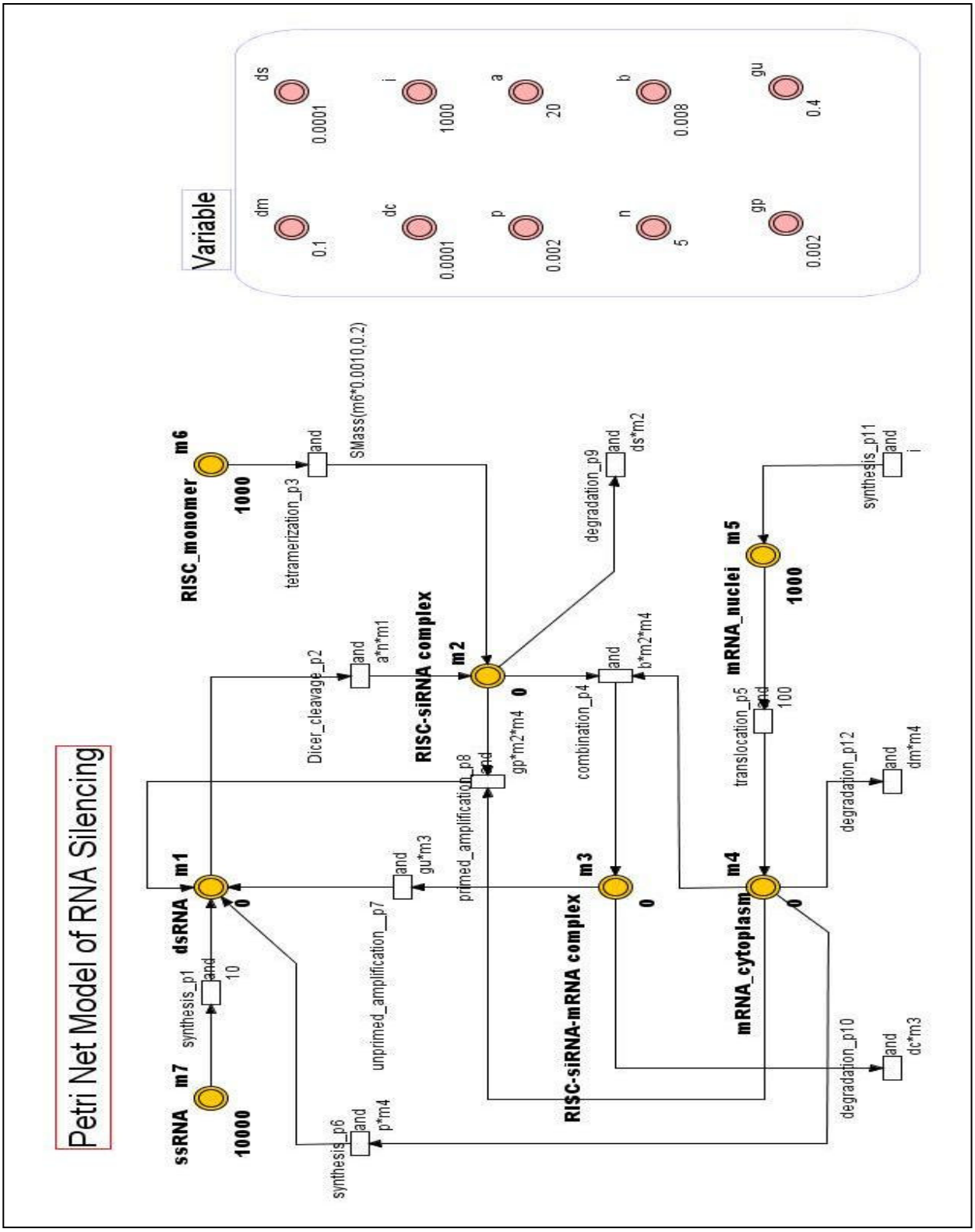

Figure 3. Our RNA silencing model constructed by HFPN. (Entities inside the left frame represent variables.)