Original Article

Colored petri net modeling of small interfering RNA-mediated messenger RNA degradation

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Abstract

Background: Mathematical modeling of biological systems is an attractive way for studying complex biological systems and their behaviors. Petri Nets, due to their ability to model systems with various levels of qualitative information, have been wildly used in modeling biological systems in which enough qualitative data may not be at disposal. These nets have been used to answer questions regarding the dynamics of different cell behaviors including the translation process. In one stage of the translation process, the RNA sequence may be degraded. In the process of degradation of RNA sequence, small-noncoding RNA molecules known as small interfering RNA (siRNA) match the target RNA sequence. As a result of this matching, the target RNA sequence is destroyed.

Materials and Methods: In this context, the process of matching and destruction is modeled using Colored Petri Nets (CPNs). The model is constructed using CPNs which allow tokens to have a value or type on them. Thus, CPN is a suitable tool to model string structures in which each element of the string has a different type. Using CPNs, long RNA, and siRNA strings are modeled with a finite set of colors. The model is simulated via CPN Tools.

Results: A CPN model of the matching between RNA and siRNA strings is constructed in CPN Tools environment.

Conclusion: In previous studies, a network of stoichiometric equations was modeled. However, in this particular study, we modeled the mechanism behind the silencing process. Modeling this kind of mechanisms provides us with a tool to examine the effects of different factors such as mutation or drugs on the process.

Key Words: Colored petri nets, colored petri nets tools, modeling, RNA degradation, small interfering RNA

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Received: 13.04.2014, **Accepted:** 05.07.2015

INTRODUCTION

Small interfering RNAs (siRNA) are 20–25 nucleotide, double-stranded RNA molecules which have an important role in RNA interference pathway.[1,2] siRNAs cause degradation of the target messenger RNA (mRNA) by complementary matching.[1] They were first discovered as a part of posttranscriptional gene silencing mechanism in plants.[3] It is reported that synthetic siRNAs are able to induce gene silencing in mammalian cells.[2] The discovery caused an interest in designing siRNAs for biomedical research.

Access this article online	
Quick Response Code:	Website: www.advbiores.net
	DOI: 10.4103/2277-9175.183660

How to cite this article: Nickaeen N, Moein S, Heidary Z, Ghaisari J. Colored petri net modeling of small interfering RNA-mediated messenger RNA degradation. Adv Biomed Res 2016;5:100.

An important step in understanding the mechanism by which siRNAs play their role in gene silencing is uncovering the rules behind complementary matching between siRNA and its target mRNA. Mathematical modeling paves the way for unfolding these relations and rules.

Different mathematical methods have been proposed to model complex biological behaviors. Graphical methods have received much attention due to their ability to be comprehensible both for biologists and engineers. Petri Nets are a graphical and mathematical formalism for the modeling and analyzing of concurrent, asynchronous, and distributed systems.[4] Modeling biological systems via Petri Nets was first proposed in.[5] Many biological systems have been modeled using Petri Nets since.[6,7] Also different analyzing approaches have been introduced using these nets.[8]

The translation process, due to its importance, has been a subject of study. In,[9] the translation process has been modeled using Colored Petri Nets (CPNs) in gene expression level. The model was used to answer questions about transfer RNA binding sites. Authors in[10] have studied the effects of a mutation in translation process using CPN. A structure is proposed to identify the mutations which happened in the translation process.

The present paper models the matching process in which a siRNA matches parts of RNA string to downregulate the gene expression. Because of the structural formation of some important biological molecules such as DNA, RNA, and proteins which have a string-like composition, Strings play an important role in computational biology concepts. Thus, modeling these structures may very well define a huge part of cell activities. Modeling is conducted through CPN Tools, software for modeling systems using CPNs.

MATERIALS AND METHODS

Mathematical basics

Petri Net is a graphical tool for modeling and analyzing concurrent distributed systems. First introduced by Carl Adam Petri in 1965, the main concept, and its extensions are now widely used in modeling step-wise processes, including biological reactions. In a formal definition, a Petri Net is a five-tuple: PN = (P, T, A, W, M_0) where:

- $P = \{p_1, p_2, ..., p_n\}$ is a finite set of places
- $T = \{t_1, t_2, ..., t_m\}$ is a finite set of transitions
- $A \subseteq (P \times T) \cup (T \times P)$ is a set of arcs
- $W: A \rightarrow \{1, 2, ...\}$ is a weight function
- $M_0: P \rightarrow \{0, 1, 2, ...\}$ is the initial marking
- $P \cap T = \Phi$ and $P \cap T = \Phi$.

In years, different versions of standard Petri Nets evolved. One of these extensions is CPN. Unlike standard Petri Nets, in CPNs, tokens accept data type and data manipulations.[11] CPNs are a combination of Petri Nets and programming languages. Control structures, synchronization, communication, and resource sharing are described by Petri Nets, and data manipulations are described by functional programming languages.[12,13] CPN Tools is software which supports CPN modeling and simulations.[14]

Computational approaches

The model is represented by CPNs. Simulations were conducted through CPN Tools, an environment for modeling and analyzing systems based on CPNs.[14] Color sets used in the model are presented in Table 1. The model was constructed with 18 places and 9 transitions. Important transitions and places along with their functions are illustrated in Table 2.

RESULTS

The constructed model is illustrated in Figure 1. Although we appreciate the fact that mRNA sequences are commonly longer than 1 Kb, Modeling was conducted for strings of <100 units of bases length due to simplicity but it can easily be extended by using the current model as a hierarchical level in a more sophisticated model structure.

In order to simulate the process, two stochastic strings with different lengths of 100 and 20 units of bases were produced to represent RNA and siRNA strings, respectively. The lengths were controlled by two counters specified by the corresponding places "count 1" and "co". The goal is to determine if the shorter

Table 1: Color sets defined in the model

Name	Color set	Definition
UNIT	Unit	To define single elements
INT	Int	To define integer colors
BOOL	Bool	To define Boolean colors
STRING	String	To define string colors
Gene	With A|C|G|U	To define bases as colors
Codon	Product gene* gene*gene	To define codons with bases A, C, G and U
Smallint	Int with 0.99	To define integer colors within 0-99
Smallint2	Int with 0.19	To define integer colors within 0-19
Codon1	Product smallint*codon	To define colors to specify each codon and its position
Gene1	Product smallint*gene	To define colors to specify each base and its position

*Is the multiplication sign. It is used to define n-tuples in CPNtools

Table 2: Transitions, functions and places used in the model

Name	Type	Comments
P1, P2, P3, P4	Place	Each of these four places, contain the four main bases: A, C, G and U
RNA sequence production	Transition	Whenever this transition fires, one codon of RNA sequence is formed
Compare	Transition	As this transition fires, each base in the RNA sequence along with its position is stored in the place "location"
Compare2	Transition	When this transition fires, if the current base in siRNA string matches with its corresponding base in RNA sequence, the next base of siRNA and RNA sequence will be compared. Otherwise, the Counter associated with siRNA resets and the counter associated with RNA sequence increases by one
siRNA production	Transition	With the firing of this transition, siRNA sequence (or its complement) is formed
siRNA complement	Place	This place contains the complement of siRNA sequence
Inhibition	Place	The place gets a token ("inhibit") each time a perfect match between RNA sequence and siRNA sequence happens
mRNA	Place	This place contains the mRNA sequence
Location	Place	This place stores each base in the RNA along with its position in the sequence
Com1, com2, com3, com4	Function	Com1(g4)=(g4=A); Com2(g4)=(g4=C); Com3(g4)=(g4=U); Com4(g4)=(g4=G); (Com1(g4) is true whenever g4 is equal to A, others are the same)
Counter	Function	Fun Counter (*n*)=(*n*=20) (The function is true when *n*=20)
Similar (i)	Function	Fun similar1(g1, g)=(g1=g); Fun similar2(g2, g)=(g2=g); Fun similar3(g3, g)=(g3=g); Fun similar4(g, g4)=(g=g4); (The function is true when gi=g)
Ok	Function	Fun ok (*n*, 33)=(*n*=32) (The function is true when *n*=32)
Ok final	Function	Fun ok final (*n*1, 20)=(*n*1=20) (The function is true when *n*1=20)

string (or actually its complement) is part of the constructed RNA string. If the matching happens, the corresponding gene is silenced.

The first step in the modeling process is to construct RNA and siRNA strings. P1, P2 and P3 are the three places responsible for the production of RNA string. The color type associated with each of these places is "gene." As introduced earlier, this type is made of four different bases: A, C, G, and U. By firing the transition "RNA sequence production," three bases form a codon. The process continues as long as we have 33 codons (or a string of 99 bases). Similarly, place P4 and transition "siRNA production" produce a string of length 20 which represents siRNA.

During the next level, each base in the RNA sequence along with its position is identified. For this purpose, place "test" is introduced which contains four bases: A, C, G, and U. The identifying mechanism is as follows. One by one, the four main bases are selected from the place "test." Then they are compared with the whole RNA string. Whenever a base in the string is a match with the selected base from the place "test", name and position of the base are transferred to the place "location." At the end of this level, all elements of the RNA string are identified.

At last, RNA and siRNA strings are compared with each other in the following way. One by one, each base in the siRNA string is chosen and compared with the RNA string. Whenever a match happens, the next base in the siRNA string is chosen and compared with the next base of RNA string. The perfect match and consequently the inhibition happen when the number of matches in a row is the same as the size of siRNA string. The place "inhibition" indicates a perfect match if it contains any tokens.

Otherwise, if a chosen base of siRNA string does not match its corresponding base in the RNA string, siRNA goes to its first base, and RNA moves a base forward and the comparison starts all over again.

DISCUSSION

In the present paper, the process during which siRNA matches with parts of mRNA string was modeled.

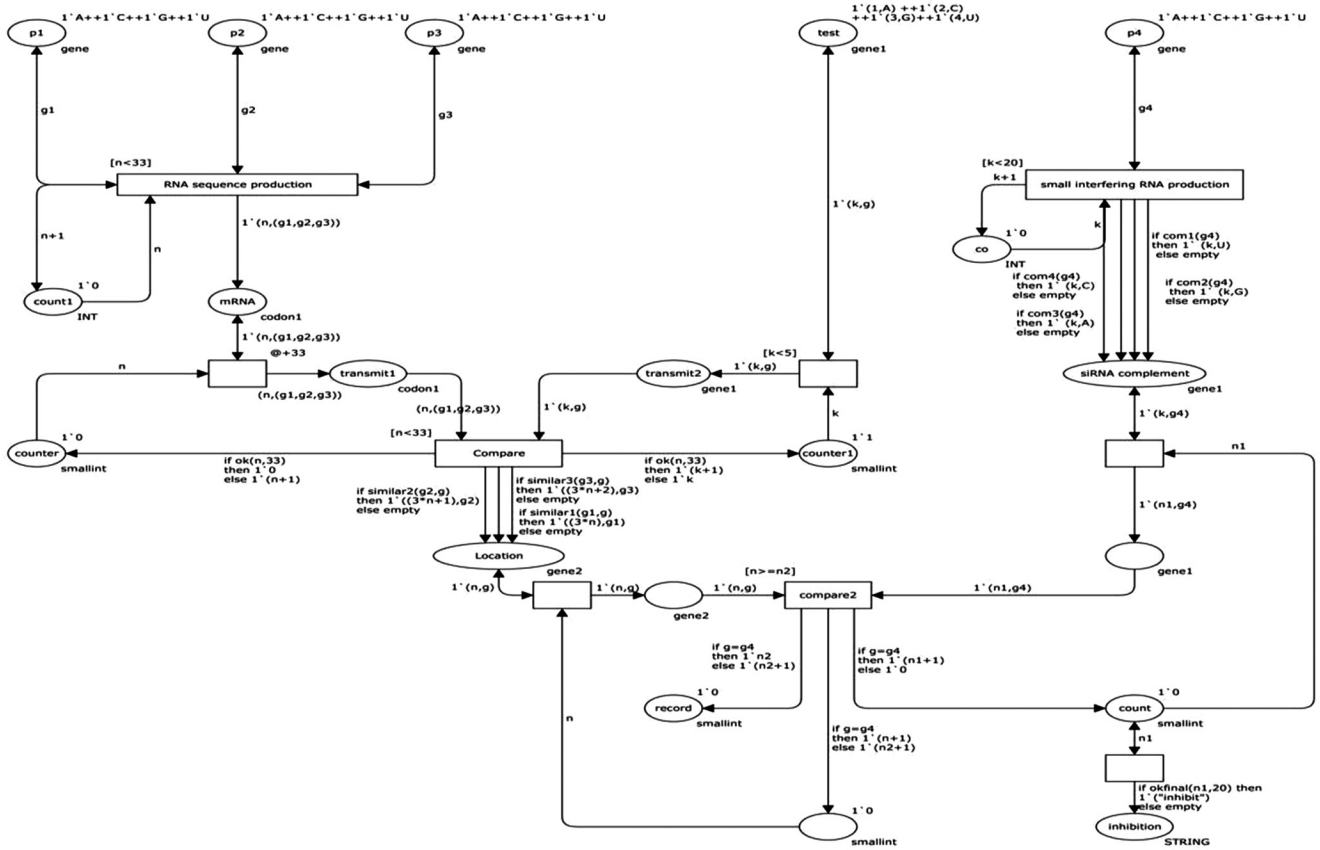

Figure 1: Colored Petri model of the gene silencing process through small interfering RNA matching. Figure was constructed in the colored petri nets tools environment

Modeling was conducted using CPNs in CPN Tools modeling environment. The model of the matching process is actually a string mining program with siRNA as the target.

In previous studies,[6,7,9] a network of stoichiometric equations was modeled. However, in this particular study, we modeled the mechanism behind the silencing process. Modeling this kind of mechanisms provides us with a tool to examine the effects of different factors such as mutation or drugs on the process. In others words, instead of modeling the components of the network, we modeled the logic behind the process.

This model may serve as a basis for mutation recognition or in a more advanced application, and it may actually help to design appropriate drugs to halt gene expression in desired cases. Future models may include more sophisticated details of the process, especially in RNA structure and matching rules.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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