Article

Effects of a silenced gene in Boolean network models

Emir Haliki, **Nadide Kazanci**
Faculty of Science, Department of Physics, Ege University, Bornova 35040, Turkey
E-mail: emir.haliki@ege.edu.tr, ehaliki@gmail.com, nadide.kazanci@ege.edu.tr

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Abstract
Gene regulation and their regulatory networks are one of the most challenging research problems of computational biology and complexity sciences. Gene regulation is formed by indirect interaction between DNA segments which are protein coding genes to configure the expression level of one another. Prevention of expression of any genes in gene regulation at the levels of transcription or translation indicates the gene silencing event. The present study examined what types of results in gene silencing would bring about in the dynamics of Boolean genetic regulatory mechanisms. The analytical study was performed in gene expression variations of Boolean dynamics first, then the related numerical analysis was simulated in real networks in the literature.

Keywords gene silencing; gene regulation; Boolean network; cell-cycle; gene expression; cellular phenotype.

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1 Introduction

Gene silencing is a usual term used in blocking expression of any genes inside a cell, implying prevention of a gene to express. In complete set of genes of a cell, most of them are important for a variety of reasons (Bouchard, 1994; Cavalli-Sforza et al., 1994; Giaever et al., 2002). However, some of them can have mutations, leading to them not functioning in a normal way, which is undesirable for the cell. Several techniques exist to eradicate the mutated genes. A gene can be cut out from a cell, by recombinant technologies with several methods, which is called the gene knock-out (Colot et al., 2006; Han et al., 2002). There is another way named the gene knock-down, blocking only the expression of a gene while gene is still present (Szulc et al., 2006; Tiscornia et al., 2003). A gene knock-down mechanism is called the gene silencing, meaning the degradation of that gene for the DNA, after which a gene can no longer produce protein no products can be made in the absence of RNA (Herman and Baylin, 2003). These processes are different but both of them have similar objectives. Although gene silencing can emerge at transcriptional and translational levels, transcriptional regulatory networks provide an investigation platform for its effects in a cellular phenotype (Agustino-Martinez, 2011; Hammond et al., 2001).

The expression of most protein-coding genes in eukaryotes is regulated predominantly at the

transcriptional level (Johnson and McKnight, 1989). Transcription mechanism includes transcription factors bound to promoter sites around a gene (Mermelstein et al., 1989). Gene regulatory networks (GRN) or transcriptional regulatory networks indicate that sets of genes encoding transcriptional regulators mutually regulate expression level of each other and determine the very basis of any cell's fate (Wagner, 1994). On the other hand, many genes are regulated by the RNA interference (RNAi). For example, miRNAs of mammals are predicted to control the activity of $\sim 50\%$ of all protein-coding genes. Functional studies point out that miRNAs take part in the regulation of almost every cellular process which has been investigated so far. RNA interference is a gene silencing mechanism that can also naturally appears during or after the transcription throughout the life (Haynes et al., 2012). If gene silencing occurred naturally as the result of evolution or selection or a random process, it could account for significant changes in sustainability of an organism.

It is very crucial impression of modeling the qualitative behavior of biological networks where molecules are represented as nodes and the molecular interactions are so called edges (Din, 2014; Zhang, 2012, 2015, 2016a, 2016b). Thus, investigation of gene silencing requires that an appropriate gene regulatory network model should be selected. Gene regulatory network models are mainly categorized into three groups namely, logical models, continuous models and single-molecule models. Those that fall into logical category are discrete models so that they can explain the existing network qualitatively, allowing a basic knowledge of the dynamics and functions of a network under different conditions (Bolouri and Davidson, 2002). Their applicability covers a wide range of systems including biological phenomena, one of which is the Boolean modeling technique introduced by Kauffman (Glass and Kauffman, 1973; Kauffman, 1993). Under the Boolean model, the state of the genes, which are Boolean variables and the phenotypic transitions which they can make are determined by the states of the other genes in the network with the Boolean logic functions governing each gene (Albert, 2004). One of the aspects of all Boolean model is that microarray experiments must first be processed to binary in the experimental data from time series as the Boolean functions of the networks can only process binary data (Hakamada et al., 2001). Successfully applied to several different organisms, Boolean GRN models are a simple and useful model to describe genetic regulatory systems (Hickman and Hodgman, 2009).

Our purpose here is to investigate the effects of gene silencing on a cellular phenotype using Boolean GRN models. What are its effects on a phenotype if it happens incidentally? We made an analysis for the probability of changes at the expression levels of other genes, obtaining some numerical results for its effects in cell cycles of some real Boolean GRNs in literature.

2 Method

Under the working principle of Boolean GRNs, genes (and also their product proteins) are nodes of the network assigned to a binary value $g_i(t) \in \{0,1\}$ with 1 for active and 0 for inactive. Any cellular phenotypes are represented by their expression patterns $\Phi(t) = \{g_1(t), g_2(t), \dots, g_N(t)\}$ where N is the number of genes. Gene interactions are directed edges. Since genes are described as either active or inactive independently of their RNA levels, edge weights do not have to be quantified biologically. Such interactions are captured by the network adjacency matrix W, which is the GRN itself, with elements w_{ij} representing an interaction arrow from gene j to gene i (Lau et al., 2007), and the allowed values are given by $w_{ij} \in \{-1,0,1\}$. For the two genes i and j, if an interaction exist, it can be either activating (1) or inhibiting (-1). The change in the expression state of each gene $g_i(t)$ of the phenotype $\Phi(t)$ as time $t$progresses in discrete timesteps under the condition below

$$g_i(t+1) = \begin{cases} 1, & \sum_j w_{ji} g_j(t) > 0 \\ 0, & \sum_j w_{ji} g_j(t) < 0 \\ g_i(t), & \sum_j w_{ji} g_j(t) = 0 \end{cases}$$

which reflects the regulation of gene i's expression by other genes. Under the gene regulation rule, and after its completion for each gene, phenotype goes to the next timestep. A sequence of updating phenotypes forms the trajectory. The phenotype which repeats itself in the trajectory is the stable state once it has been reached. There can be some limit cycles as well (Glass and Kauffman, 1973; Kauffman, 1969).

In order to investigate the effects of gene silencing on other genes, we first have to define that event (Jablonka and Lamb, 2005) in Boolean manner. Gene silencing can be considered a type of loss-of-function mutation in which the altered gene product lacks the molecular function of the silenced gene (Nowak, 2006). In our model one particular active gene is chosen and its state is fixed at zero, regardless of the state what remains of the GRN. To examine the effect of the silenced gene on the expression of other genes, such a path can be traced.

By the silencing of active k^{th} gene

$$\Phi(t) = \{g_1(t), g_2(t), \dots, g_k(t), \dots, g_N(t)\}, \quad g_k(t) = 0 \text{ (fixed)}$$

we define a new threshold function for the i^{th} gene, which counted as a target of g_k, by subtracting the contribution of silenced gene from the sum of $w_{ji} g_j(t)$

$$I_i(t) = \sum_j w_{ji} g_j(t) - w_{ki} g_k(t)$$

with the gene regulation condition becoming as follows

$$g_i(t+1) = \begin{cases} 1, & I_i(t) > 0 \\ 0, & I_i(t) < 0 \\ g_i(t), & I_i(t) = 0 \end{cases}$$

3 Application

A silenced gene still sustains its existence in the regulatory network compared to the gene knock-out process. Thus, only its expression is inactivated. Here are two different things to be considered. First, change of at least one gene's expression means that of the phenotype, in which case silenced gene changes the phenotype already by turning its active expression level into inactive. Second, change in the expression level of the silenced gene can induce that in other gene's expression levels. Therefore, the number of regulatory connections of silenced gene is of great importance. More evidently, other affected genes also indicate a contribution to the phenotypic change with the effect of the silenced gene. If there is a connection between silenced g_k and g_i, change in the expression (from now on we have called it alteration) of target g_i can be demonstrated by a flow chart considering all the unknown transcriptional connection conditions (Fig. 1).

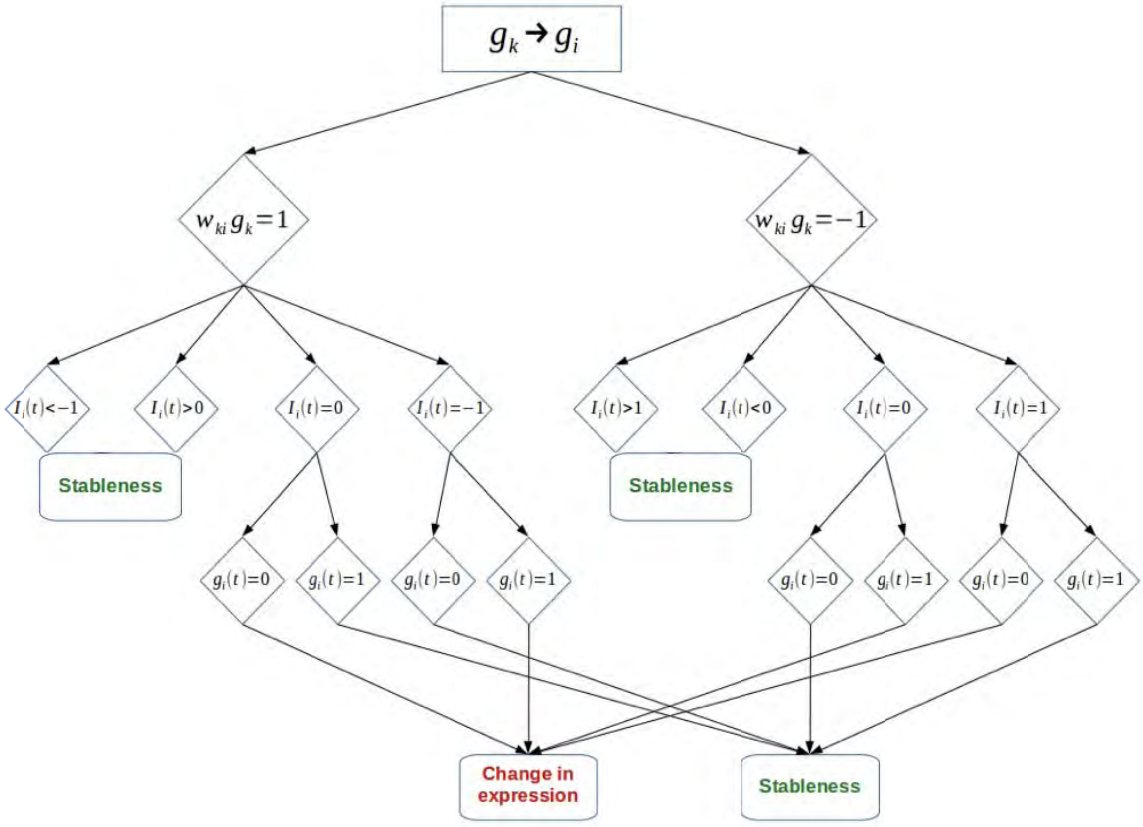


Fig. 1 A generalized flow chart demonstrating g_i's alteration conditions under the influence of the silenced g_k. Left branch shows that if the connection is activatory(1) state and right branch that if it is inhibitory (-1) before the g_k has been silenced. Inner branches indicate values of the new threshold function which is the regulatory contributions of non-silenced genes to the target g_i including itself (self regulation loop). As for some given values of $I_i(t)$, earlier expression levels of target gene ($g_i(t)$s) determine the alteration.

Probability of affected g_i's alteration can be obtained from the generalized scheme of gene silencing in Boolean GRN model. First, we observe that two branches provide us with 1/2 probability for each. Then, for any second layer branch, it is $2/(2N-1)$. The denominator $2N-1$ is number of possible threshold function sums ($I_i(t)$) for g_i, or accessible states. In more detail, negative $I_i(t)$ can be $N-1$ maximum (contribution of silenced gene is subtracted), so does the positive $I_i(t)$. And an accessible state, which is zero, need to be counted. Thus the total number of accessible states is $2N-1$. Finally, on the third layer 2/4 brings us the alteration of the affected gene. With the addition of the products of probabilities for two branches which are exactly the same, total probability for one gene is calculated as following

$$P_{g_i} = \frac{1}{2N-1}$$

The effect of silenced gene on multiple genes depends on the number of connections. If there is no out-connection (a transcription factor arrow from silenced gene to a target gene) of the silenced gene or its isolated from the network, no alterations can be expected. On the other hand, it can be out-connected to all other genes. If we do not have any idea of the silenced gene, number of its out-connections and the structure

of network in which it is, the following equation would be achieved

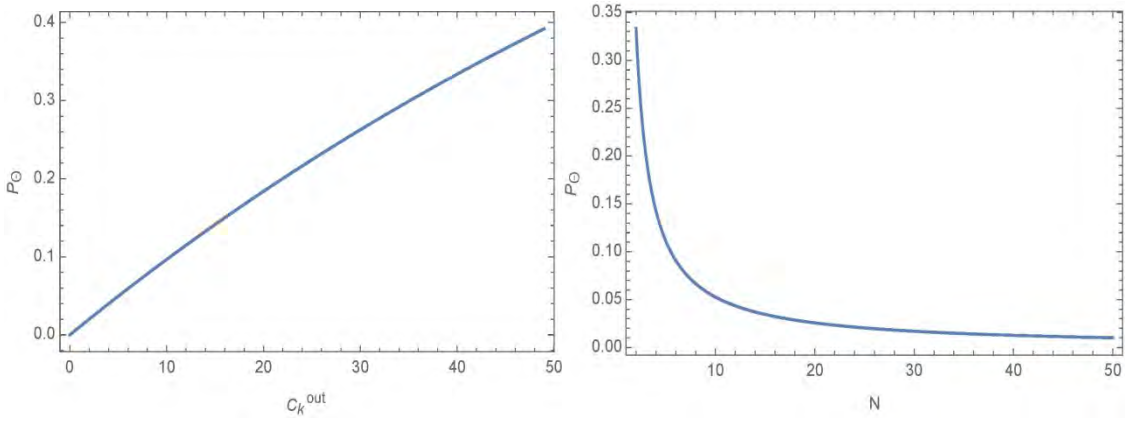
$$P_\Theta = \frac{(2N-1)^{C_k^{out}} - (2N-2)^{C_k^{out}}}{(2N-1)^{C_k^{out}}}$$

, which was found as the silenced gene's alteration probability on a cellular phenotype (at least one other gene than itself) for any random Boolean GRNs. C_k^{out} is the out-degree (Ebel et al., 2002) of the silenced gene and its maximum $N-1$. Note that we consider just one silenced gene in a phenotype to simplify the computation of its effects.

Real biological GRNs abide by the rules of randomly distributed networks, preventing us from having to write down any relation between the possible out-degree and network size, N. Thus, they need to be regarded as two different variables in the equation above (P_Θ). In the limit of maximum out-degree ($C_k^{out} = N-1$), the alteration probability approaches to its maximum (Fig. 2a). Probability function cannot go on since the maximum value of the out-degree is $N-1$. If we re-write P_Θ with C_k^{out} in its maximum value, and increase the network size to infinity

$$\lim_{N\to\infty}\left[1-\left(\frac{2N-2}{2N-1}\right)^{N-1}\right]$$

the limit converges to$1-(1/\sqrt{e})(\approx 0.393)$ which is found to be the maximum alteration probability limit for any Boolean network after silencing of a gene. As with influence of silenced gene on other genes, any changes in expression of affected genes do mean the same thing that is, contribution of silenced gene to phenotypic variation by attracting other genes to itself. Altered genes may affect others which also affect some others such as in a chain reaction so on. On the other hand, if we fix the silenced gene's out-degree to a constant and increase the network size then alteration probability decreases due to the growing number of accessible states ($2N-1$) and changes asymptotically (Fig. 2b).



a) b)

Fig. 2 a) Silenced gene's alteration probability with respect to the out-degree in a random GRN composed of 50 genes. The potential of silenced gene's influence rises curvilinear with its out-degree having a maximum on $C_k^{out} = 49,\ P_\Theta \approx 0.393$. **b)** Alteration probability with respect to the number of genes in network.C_k^{out} is fixed to 1, which turn out to be equation $P_{g_i} = 1/(2N-1)$, and horizontal asymptote at $P_\Theta = 0$.

Now we are here concerned with some real Boolean GRN models whose phenotypic gene expression dynamics start from all possible initial states $\Phi_s(t)$ with $s = 1,2,\dots,2^N$, and arrive at some stable states$\Phi_{\text{stable}}(t)$also called basin of attraction. All initial phenotypes attracted to the same basin are in the same class so that they are all associated with the same stable state (Krawitz and Shmulevich, 2007). Thus, number of attracted phenotypes is the basin size of related stable state. Now that stable phenotypes were initial conditions of cell-cycle, we studied effects of gene silencing on real data.

We set out with the cell cycle network of the model organism *S. cerevisiae* (Li et al., 2004) for computations (Fig. 3). It has a significant super state (largest basin size) indicating the G1 phase of the cell cycle, which is composed of two active and nine inactive genes. By exciting the state by activating Cln3 gene and under the regulation dynamics, one cell-cycle trajectory appears as in table (Table 1).

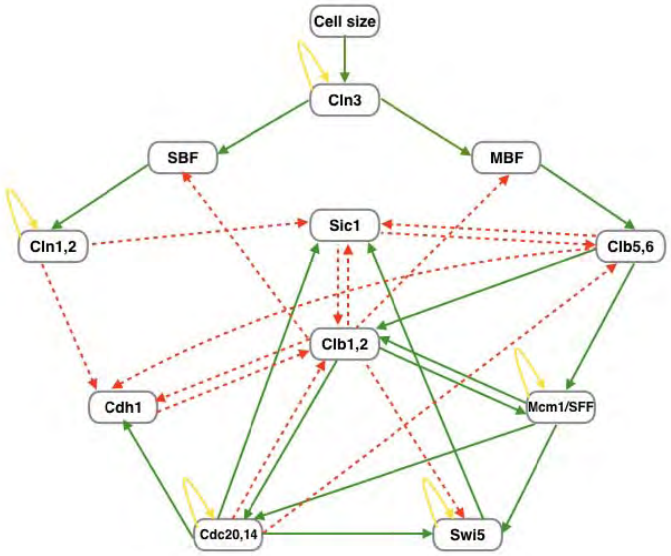


Fig. 3 Cell-cycle network of *S. cerevisiae*. Green links for an activating, dotted red links for an inhibiting interaction. And yellow loops are self-degredation processes which are also inhibitory.

Table 1 Cell-cycle trajectory by activation of Cln3 gene via start signal. After all other phases S, G2 and M, cell reaches to the stationary G1 state again.

	Cln3	MBF	SBF	Cln1,2	Cdh1	Swi5	Cdc20,14	Clb5,6	Sic1	Clb1,2	Mcm1/SFF
Start	1	0	0	0	1	0	0	0	1	0	0
G1	0	1	1	0	1	0	0	0	1	0	0
G1	0	1	1	1	1	0	0	0	1	0	0
G1	0	1	1	1	0	0	0	0	0	0	0
S	0	1	1	1	0	0	0	1	0	0	0
G2	0	1	1	1	0	0	0	1	0	1	1
M	0	0	0	1	0	0	1	1	0	1	1
M	0	0	0	0	0	1	1	0	0	1	1
M	0	0	0	0	0	1	1	0	1	1	1
M	0	0	0	0	0	1	1	0	1	0	1
M	0	0	0	0	1	1	1	0	1	0	0
G1	0	0	0	0	1	1	0	0	1	0	0
$G1_{stat}$	0	0	0	0	1	0	0	0	1	0	0

Since real networks show a certainty about the structure of the links, probabilistic calculations tend to be meaningless. We applied the gene silencing to observe likely phenotypic and trajectory variations. First, Sic1 gene which has an out-degree of two was silenced and the new trajectory is shown in Table 2, where we encountered a chain reaction. Having transcriptional attacks to two genes (Clb1,2 and Clb5,6), Sic1 altered the expression level of Clb5,6. More clearly, $\sum_j w_{\{j,Clb5,6\}} g_j(t) = 0$ with Sic1's $w_{\{Sic1,Clb5,6\}} g_{\{Sic1\}} = -1$ contribution to the sum of Clb5,6 whose expression was also zero in previous timestep, before silencing of Sic1. After silencing, -1contribution of Sic1 is gone and $I_{\{Clb5,6\}}(t) = 1$. Thus Clb5,6 is activated when needed to remain inactive. In next timesteps, altered Clb5,6 affects Mcm1/SFF and it affects Swi5, Cdc20, Clb1,2 so on. When the7^{th}timestep has reached the flow stopped, and only the silenced gene's expression level is comparatively different from the original cell-cycle loop. Other genes' expressions reached the metaphase such as in the non-silenced procedure, but from a different trajectory. It caused new cell cycle to stop that Sic1 was not active again, which implies an effect of silence on the system.

Table 2 New cell-cycle trajectory after the silencing of Sic1. Changes in the expression levels of other genes are shown in red and bold.

	Cln3	MBF	SBF	Cln1,2	Cdh1	Swi5	Cdc20,14	Clb5,6	Sic1	Clb1,2	Mcm1/SFF
Start	1	0	0	0	1	0	0	0	**0**	0	0
G1	0	1	1	0	1	0	0	0	**0**	0	0
G1	0	1	1	1	1	0	0	**1**	**0**	0	0
G1	0	1	1	1	0	0	0	**1**	**0**	0	**1**
S	0	1	1	1	0	**1**	**1**	1	**0**	**1**	**1**
G2	0	**0**	**0**	1	0	**1**	**1**	1	**0**	1	1
M	0	0	0	**0**	0	**1**	1	**0**	**0**	1	1
M_{stuck}	0	0	0	0	0	1	1	0	**0**	1	1

A similar event is observable in the silence of Cdh1. After Cdh1 whose out-degree is 1 was silenced, it did not lead to any expression levels on any genes without general disturbance of cell cycle (Table 3). However, when the system reached to fifth state of the M phase, cell-cycle cannot continue because silenced Cdh1 was not active again.

Table 3 New cell-cycle trajectory after the silencing of Cdh1. Cdh1 stopped the cell-cycle on its own.

	Cln3	MBF	SBF	Cln1,2	Cdh1	Swi5	Cdc20,14	Clb5,6	Sic1	Clb1,2	Mcm1/SFF
Start	1	0	0	0	**0**	0	0	0	1	0	0
G1	0	1	1	0	**0**	0	0	0	1	0	0
G1	0	1	1	1	**0**	0	0	0	1	0	0
G1	0	1	1	1	0	0	0	0	0	0	0
S	0	1	1	1	0	0	0	1	0	0	0
G2	0	1	1	1	0	0	0	1	0	1	1
M	0	0	0	1	0	0	1	1	0	1	1
M	0	0	0	0	0	1	1	0	0	1	1
M	0	0	0	0	0	1	1	0	1	1	1
M	0	0	0	0	0	1	1	0	1	0	1
M_{stuck}	0	0	0	0	**0**	1	1	0	1	0	0

We applied the gene silencing to super state caused by GRN of *S. pombe* model organism (Fig. 4) in the literature in 2008 (Davidich and Bornholdt, 2008) with somewhat similar results. The original cell-cycle trajectory is in Table 4. Ste9, Rum1 and Wee1 of the super state were silenced separately with the consequences in tables 5, 6, 7. First, both of which caused the phenotype fall into metaphase from a path like the original one except the level of the silenced gene, as also detected in the silenced Cdh1 gene of*S. cerevisiae*. When the trajectory completed and system reaches the 9^{th} time step, cell cycle imploded into a biologically invalid phase not the G1. The only reason for this is that the three silenced genes cannot be activated again.

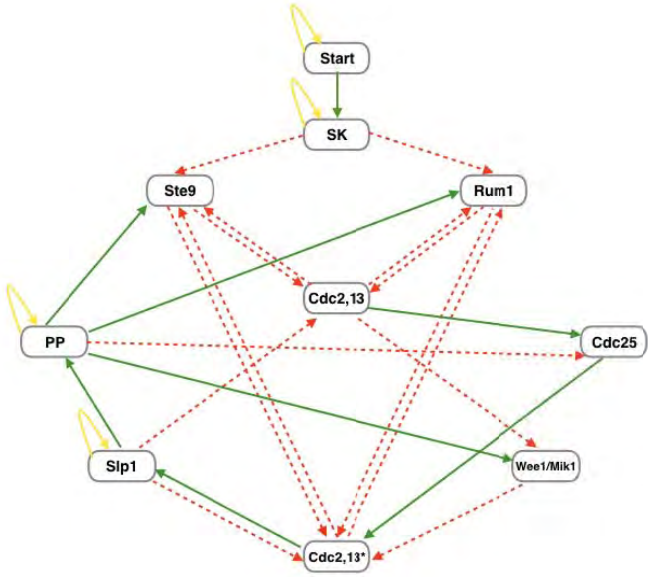


Fig. 4 Cell-cycle GRN of *S. pombe*. Dynamical differences between *S. Cerevisiae* GRN are Cdc2,13 and Cdc2,13* complexes have activation thresholds which are different than zero.

Table 4 Cell-cycle loop of *S. pombe*.

	Start	SK	Cdc2,13	Ste9	Rum1	Slp1	Cdc2,13*	Wee1	Cdc25	PP
Start	1	0	0	1	1	0	0	1	0	0
G1	0	1	0	1	1	0	0	1	0	0
G1/S	0	0	0	0	0	0	0	1	0	0
G2	0	0	1	0	0	0	0	1	0	0
G2	0	0	1	0	0	0	0	0	1	0
G2/M	0	0	1	0	0	0	1	0	1	0
G2/M	0	0	1	0	0	1	1	0	1	0
M	0	0	0	0	0	1	0	0	1	1
M	0	0	0	1	1	0	0	1	0	1
$G1_{stat}$	0	0	0	1	1	0	0	1	0	0

Table 5 Ste9 silenced.

Start	1	0	0	**0**	1	0	0	1	0	0
G1	0	1	0	**0**	1	0	0	1	0	0
G1/S	0	0	0	0	0	0	0	1	0	0
G2	0	0	1	0	0	0	0	1	0	0
G2	0	0	1	0	0	0	0	0	1	0
G2/M	0	0	1	0	0	0	1	0	1	0
G2/M	0	0	1	0	0	1	1	0	1	0
M	0	0	0	0	0	1	0	0	1	1
M	0	0	0	**0**	1	0	0	1	0	1
unk.	0	0	0	**0**	1	0	0	1	0	0

Table 6 Rum1 silenced.

1	0	0	1	**0**	0	0	1	0	0
0	1	0	1	**0**	0	0	1	0	0
0	0	0	0	0	0	0	1	0	0
0	0	1	0	0	0	0	1	0	0
0	0	1	0	0	0	0	0	1	0
0	0	1	0	0	0	1	0	1	0
0	0	1	0	0	1	1	0	1	0
0	0	0	0	0	1	0	0	1	1
0	0	0	1	**0**	0	0	1	0	1
0	0	0	1	**0**	0	0	1	0	0

Table 7 Wee1 silenced.

Start	1	0	0	1	1	0	0	**0**	0	0
G1	0	1	0	1	1	0	0	**0**	0	0
G1/S	0	0	0	0	0	0	0	**0**	0	0
G2	0	0	1	0	0	0	0	**0**	0	0
G2	0	0	1	0	0	0	0	0	1	0
G2/M	0	0	1	0	0	0	1	0	1	0
G2/M	0	0	1	0	0	1	1	0	1	0
M	0	0	0	0	0	1	0	0	1	1
M	0	0	0	1	1	0	0	**0**	0	1
unk.	0	0	0	1	1	0	0	**0**	0	0

4 Discussion

As for Boolean network formalism of GRNs, gene silencing applied to the systems was studied. What type of effects silenced gene had on other genes were algebraically and probabilistically explored first, then some numerical examinations were performed in real data. According to the obtained results, when gene silencing was applied one by one to active genes of the super states to which GRNs carried cellular phenotypes, there are if little likelihood for other genes to change expressions (max. $1-(1/\sqrt{e})$). However, the system in a whole cell cycle mostly tries to adjust itself to the original cycle again. And constantly inactivated expressions of silenced genes make the phenotype stuck in related phases causing the cell cycle trajectory to be stopped. Moreover, genes having been silenced also exist which carry the system to different cellular phases (possibility of apoptosis or invasion). This issue can be investigated by looking at the tasks of silenced genes in biological databases for related organism.

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