Effects of coupling strength and space on the dynamics of coupled toggle switches in stochastic gene networks with multiple-delayed reactions

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Genetic toggle switches (TSs) are one of the best studied small gene regulatory networks (GRNs), due to their simplicity and relevant role. They have been interpreted as decision circuits in cell differentiation, a process long hypothesized to be bistable [1], or as cellular memory units [2]. In these contexts, they must be reliable. Once a "decision" is made, the system must remain stable. One way to gain stability is by duplicating the genes of a TS and coupling the two TSs. Using a recent modeling strategy of GRNs, driven by a delayed stochastic simulation algorithm (delayed SSA) that allows modeling transcription and translation as multidelayed reactions, we analyze the stability of systems of coupled TSs. For this, we introduce the coupling strength (C), a parameter to characterize the GRN structure, against which we compare the GRN stability (S). We first show that time delays in transcription, associated to the promoter region release, ensure bistability of a TS, given no cooperative binding or self-activation reactions. Next, we couple two TSs and measure their toggling frequencies as C varies. Three dynamical regimes are observed: (i) for weak coupling, high frequency synchronized oscillations, (ii) for average coupling, low frequency synchronized oscillations, and (iii) for strong coupling the system becomes stable after a transient, in one of two steady states. The system stability, S, goes through a first order phase transition as C increases, in the average coupling regime. After, we study the effects of spatial separation in two compartments on the dynamics of two coupled TSs, where spatial separation is modeled as normally distributed random time delayed reactions. The phase transition of S, as C increases, occurs for lower values of C than when the two TSs are in the same compartment. Finally, we couple weakly and homogeneously several TSs within a single compartment and observe that as the number of coupled TSs increases, the system goes through the phase transition in S, from oscillatory to stable and for C values lower than in the two previous cases.

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I. INTRODUCTION

It has long been hypothesized that cell differentiation could be based on bistable genetic subcircuits that control many downstream genes [1]. In this process, a stem cell becomes a stable phenotype [3], in agreement with the hypotheses that stable states of the gene regulatory networks (GRN) correspond to cell types [4].

The decision subcircuit of the GRN for differentiating must be, at the same time (at least), bistable, to allow branching into distinct cell types, and reliable, i.e., once a decision is made, remain stably in such "state," acting as a cellular memory unit [2].

It was observed experimentally that the TS (two genes which mutually repress each other) can be used by a cell to adopt different stable states [5,6]. Also, models based on TSs have been used to simulate differentiation pathways of hematopoietic cell lines [7], where the TSs act as the decision subcircuits at each bifurcation. The findings in [7] interestingly pointed out the existence of unknown multiple steps processes not included in their model, to explain the differences between model and experimental observations. Multiple step processes can be correctly modeled, from the system dynamics point of view, by time delayed reactions [8].

Stochastic fluctuations of gene expression were proven to have a significant role at the single-cell level [9,10], e.g., causing probabilistic pathway selection [5]. The relevance of having a model that correctly accounts for noise in the dynamics is enhanced by the discrete nature of the transcription factors and their binding sites, genes' promoter regions, which exist in low copy numbers [11,12].

GRNs have been modeled by random Boolean networks (RBN) [13], systems of ordinary differential equations (ODE) [14] and stochastic equations [15], among other methods.

In the RBN model, gene states are represented by binary variables with value 1, when a gene is being expressed, and 0 if not. In its original formulation [13], genes states are synchronously updated and regulated by other genes directly connected to it. A random Boolean function is assigned to each gene to define its state at each time step from the inputs' states at the previous time step. Noise is usually modeled by introducing a non-null probability p that each gene "misbehaves," i.e., does the contrary of what is determined by its boolean function and inputs states. Due to its simplicity, the RBN model allows studying the dynamical behavior of large networks [16]. However, real GRNs are not synchronous and genes' level of expression are not binary quantities. Also, in RBN models, a correct simulation of molecular noise is not possible. RBN models with asynchronous update have also been proposed [17,18]. Yet, these models do not assume any probability of genes "misbehaving." Only the time at which nodes update is random.

When a chemical system has many molecules of all intervening chemical species, its dynamics, i.e., the variation of

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the concentrations of the chemical species present, can be, in simple cases, computed approximately using ODE models [14]. Models of GRNs have been proposed using this framework [19]. These models are either deterministic or include some noise term, white or colored noise (e.g., see [20]). In the GRN model here used, proposed in [21], genes are treated as chemical species since in real GRNs they exist only in very small quantities (usually one to a few copies of the same gene). Since gene promoter regions are also subject to time delays [22], combined to their very small quantity, stochastic effects cannot be ignored as must be accounted for using the stochastic simulation algorithm (SSA) [23]. Therefore, a "mean field" approach is not accurate. Additionally, until now, attempts to simulate noise using Langevin equations were not very successful [24]. Finally, it remains to be shown that white or colored noise terms in ODE models capture the true nature of noise in real gene expression.

Recent works reporting experimental results showed that noise cannot be neglected in GRNs dynamics. Quantitative fluorescence measurements of gene expression products [25,26] show that GRN dynamics and cells differentiation depend highly on the noise. The results established that certain types of cellular differentiation are probabilistic and transient. Other experiments [2,12,27–33] showed that a population of genetically identical cells, exposed to the same environmental conditions, has phenotypically distinct individuals implying that GRNs are intrinsically stochastic. Accordingly, the modeling strategy for GRNs should be based on stochastic reactions kinetics.

Gene expression has, aside its stochastic nature, another important feature. Transcription and translation are complex chemical processes involving many steps and chemical species. In the first stochastic models of GRNs, gene expression was assumed to be an instantaneous process [5]. Yet, in transcription and/or translation, it may take a considerable time for a $\mathcal{R}$ polymerase (represented here by $\mathcal{R}p$) and/or ribosome to generate an $\mathcal{R}$ (where $\mathcal{R}$ stands for RNA) and/or polypeptide depending on the gene length, which varies significantly from gene to gene. An improvement when modeling translation and transcription reactions consists in introducing time delays in the appearance of the products, each time one of these reactions occur [22].

Although models using only nondelayed reactions can explain experimental data regarding gene expression fluctuations (see, e.g., [29]), these studies focused on steady state dynamics, where "delayed" and "nondelayed" models have the same results after a transient. However, to accurately model GRNs in more complex conditions (e.g., when involving feedback mechanisms), delayed reaction are necessary [22]. Namely, transcription and translation time delays [34–36] should be included to capture the features of transients [37,38]. For example, it was found in [21], when studying the dynamics of a single toggle switch (TS), that proteins production delays can cause a long transient, such that the two genes synchronize before the system reaches a "stable" state.

Recently, the real-time production of single protein molecules under the control of a repressed lac promoter in individual E. coli cells was directly monitored through an epifluorescence microscope [31]. It was found that the proteins are produced in bursts, with the distribution of the bursts per cell cycle fitting well a Poisson distribution, and that protein numbers in the bursts follow a geometric distribution. The bursts also display particular temporal spreads [31]. The model of gene expression proposed in [22], that includes multiple time delayed reactions, reproduces the observed kinetics.

Finally, recent studies on the TS dynamics [39] using ODE models and models based on the SSA, showed that bistability is attainable with noncooperative binding (i.e., the proteins do not combine into dimers) given careful parameters tuning. Importantly, this work [39] stresses the necessity of stochastic methods for modeling GRNs, even in the regime of high concentrations.

Here we investigate the conditions under which a TS becomes "stable," using a recent model of GRNs [21] driven by the delayed SSA.

Given that genes can be copied and in some cases, several copies of a gene exist in a genome [40], we investigate how such mechanism, applied to a TS, affects its dynamics, namely, its stability. Also, since cells in populations behave quite differently than isolated cells (see, e.g., [41]), we additionally study the dynamics of coupled TSs located in different compartments, with intermediate reactions representing the movement of transcription factors or their downstream products between cells.

We investigate the consequences of two possibilities of attaining more stability: the first is copying of a TS within a single cell, and the second is coupling TSs of neighbor cells. The latter case requires considering the time it takes for a transcription factor or a downstream product of it, to diffuse and cross cells' membranes. This event is modeled by normally distributed single time delayed reactions. We assume all TSs similar, i.e., their genes express proteins similar to the other TSs' proteins.

Previous work on spatial diffusion effects modeled diffusion as a delayed event [42,43] and showed how varying the delays can cause bifurcation of oscillatory solutions. However, the model is one-dimensional and does not consider noise of chemical reactions. In another work, a mechanism for coupling TSs was proposed [44], using intercell signaling to couple the TSs and induce synchronous oscillations. This model includes fast and slow reactions, but not time delayed reactions.

Here we use a simulator, *SGNSim* [45], so that the systems' dynamics are driven by the delayed SSA [22], based on the original SSA [23,46], but allowing to model transcription and translation as multiple delayed events, and diffusion through different compartments as normally distributed delayed reactions.

We investigate how coupling between TSs can force synchronized toggling and the conditions to attain stable states, using *SGNSim*. We focus on characterizing the stability S of these GRNs as a function of the coupling strength C and spatial compartmentalization. Since the models are stochastic, by stable states here we mean states where genes expression levels are, aside from stochastic fluctuations, approximately constant in time (in comparison with the oscillatory regime, where genes' expression levels toggle).

We organized the paper as follows: first we introduce the set of chemical reactions which constitute our model of

coupled TSs. Next, we define stability and coupling strength between two TSs and for networks of coupled TSs.

In the results section we first present the necessary conditions for a single TS to toggle, given multiple time delayed transcription and/or translation reactions and no cooperative binding. Next, we measure the stability of two coupled TSs within one compartment as their coupling strength varies. Also, we measure the stability of the same two TSs, but separated in two compartments, given that the proteins can diffuse between the two. Finally, we measure the stability of a system of coupled TSs, within a single compartment, as the number of TSs increases.

II. MODEL OF GENE NETWORKS OF COUPLED TOGGLE SWITCHES

In the SSA, products of a reaction are released immediately [23] when the reaction occurs. However, it was shown that gene expression is better modeled by delaying the release of gene expression products by a time interval. The set of complex reactions necessary for a gene to be transcribed by an $\mathcal{R}$ polymerase, spliced, translated by a ribosome, and folded, can be simplified, for most purposes, into a single step multiple-delayed reaction [22].

To model GRNs, we use the methodology proposed in [21], executed using SGN Sim"Stochastic Gene Networks Simulator" [45]. *SGNSim* allows modeling transcription and translation as single step multiple-delayed reactions and genes interact among each other via binding of their transcription factors to other gene promoter regions, resulting in a chemical complex either unable to express (total repression) or less able to express (partial repression) or more able to express (activated state) than by basic transcription (binding of the $\mathcal{R}p$ to the free promoter). Proteins can also bind to other proteins, forming multimers which will then be inputs to genes. Time delays can be constant or drawn from distributions, and reaction rates can be constants or computed from complex functions. SGN Sim output consists on a time series and corresponding fourier spectrum of all distinct chemical species involved in the system, given a preset sampling frequency.

SGNSim dynamics is based on the "delayed SSA" [22], that, unlike the nondelayed SSA, uses a waiting list to store delayed output events, proceeding as follows:

(1) Set $t \leftarrow 0$, $t_{stop} \leftarrow$ stop time, read initial number of molecules and reactions, create empty waiting list L.

(2) Do an SSA step for input events to obtain next reacting event R_1 and corresponding occurrence time t_1.

(3) If $t_1+t<t_{min}$ (the least time in L), set $t \leftarrow t+t_1$. Update number of molecules by performing R_1, adding delayed products into L as necessary.

(4) If $t_1+t \geqslant t_{min}$, set $t \leftarrow t_{min}$. Update number of molecules by releasing the first element in L.

(5) If $t<t_{stop}$, go to step 2.

Here we model networks of coupled TSs. A TS consists of two genes that repress each other and the model used here can be found in [21].

Suppose a system of N genes that is structured as $\frac{N}{2}$ TSs. Also, assume that those TSs are "identical," in the sense that the proteins of each gene "type 1" of each TS i represses all genes "type 2" of all TSs, and vice versa. Each chemical reaction responsible for the couplings has a rate constant, whose value determines how "strong" is such interaction. If such rate constant is null, the interaction does not exist. Thus, one can impose any topology between the elements (TSs) of the network by setting the rate constants of the "coupling reactions" as desired.

In general, the system of N genes, organized in $i=1,\ldots,\frac{N}{2}$ TSs, each composed of two genes ($j=1,2$), can be described by the following set of reactions: for all i,i_1,j,i_2,j_1,j_2, where $i,i_1,i_2=1,\ldots,\frac{N}{2}$, and $j,j_1,j_2=1,2$, such that $i_1 \neq i_2$ and $j_1 \neq j_2$:

$$\mathcal{R}p + \mathcal{P}_{\mathrm{i,j}} \xrightarrow{\mathrm{k_t(i,j)}} \mathcal{P}_{\mathrm{i,j}}(\tau^1_{i,j}) + \mathcal{R}p(\tau^2_{i,j}) + p_{\mathrm{i,j}}(\tau^3_{i,j}), \quad (1)$$

$$\mathcal{P}_{\mathrm{i_1,j_1}} + p_{\mathrm{i_2,j_2}} \underset{\mathrm{k_u((i_1,j_1),(i_2,j_2))}}{\overset{\mathrm{k_c((i_1,j_1),(i_2,j_2))}}{\rightleftarrows}} \mathcal{P}_{\mathrm{i_1,j_1}} p_{\mathrm{i_2,j_2}}, \quad (2)$$

$$\mathcal{P}_{\mathrm{i_1,j_1}} + p_{\mathrm{i_2,j_2}} \underset{\mathrm{k_{uw}((i_1,j_1),(i_2,j_2))}}{\overset{\mathrm{k_{cw}((i_1,j_1),(i_2,j_2))}}{\rightleftarrows}} \mathcal{P}_{\mathrm{i_1,j_1}} p_{\mathrm{i_2,j_2}}, \quad (3)$$

$$\mathcal{P}_{\mathrm{i_1,j_1}} p_{\mathrm{i_2,j_2}} \xrightarrow{k_d p((i_1,j_1),(i_2,j_2))} \mathcal{P}_{\mathrm{i_1,j_1}}, \quad (4)$$

$$p_{i,j} \xrightarrow{k_d(i,j)} \varnothing, \quad (5)$$

where $\mathcal{R}$ represent RNA and $\mathcal{P}$ is the promoter. Reaction 1 represents the transcription translation of each gene of the network in a single step, accounting, given the multiple time delays on the products, for the time it takes on average for these two complex chemical processes to be finished once initiated [22]. In the same reaction, the superscripts on the τ's distinguish the delays between products, while subscripts distinguish the delays of products of reactions associated to different genes. E.g., if reaction 1 occurs for gene j of the TS i, at time t, $\mathcal{P}_{i,j}$ and one $\mathcal{R}p$ are removed from the system and placed in a waiting list of events. At $t+\tau^1_{i,j}$, $\mathcal{P}_{i,j}$ is released back into the system, at $t+\tau^2_{i,j}$ $\mathcal{R}p$ is released (unchanged), and at $t+\tau^3_{i,j}$ the protein $p_{i,j}$ is released from the waiting list, becoming available for reactions [21,22]. Unless time delays τ's are explicitly represented in products, all products appear in the system instantaneously at t.

The initiation frequency of the transcription process (reaction 1) is controlled by $k_t(i,j)$ and $\tau^1_{i,j}$ [47]. The rate constant $k_t(i,j)$ determines how frequently the binding event of $\mathcal{R}p$ to $\mathcal{P}$ happens on average, if $\mathcal{P}$ is available (assuming abundance of $\mathcal{R}p$), and the delay $\tau^1_{i,j}$ determines how long it takes for $\mathcal{P}$ to be available for another reaction. If the $\mathcal{R}p$ exists in large quantities such that the number of available $\mathcal{R}p$'s is approximately invariant, then $\tau^2_{i,j}$ can be neglected [47]. In addition, $\tau^2_{i,j}$ can be omitted as well if it is much smaller than $(\mathcal{R}P \cdot k_t)^{-1}$. The promoter clearance time is usually not considered in other models (to do so here one could set τ^1 null). One difference in the dynamics, caused by the delay on the promoter is the limitation on the number of $\mathcal{R}p$'s that can transcribe the gene simultaneously in agreement

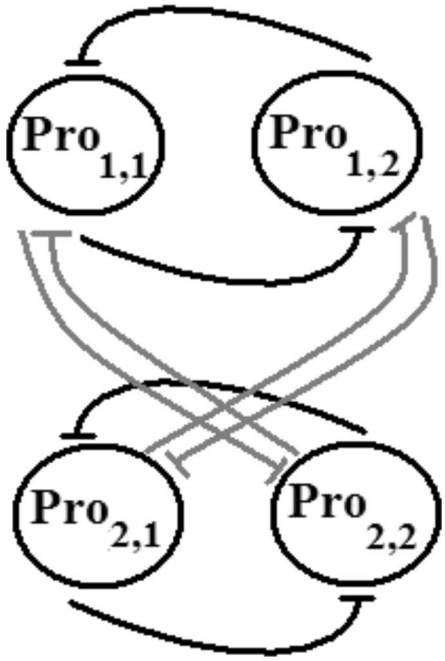

FIG. 1. Two coupled TSs in a single compartment.

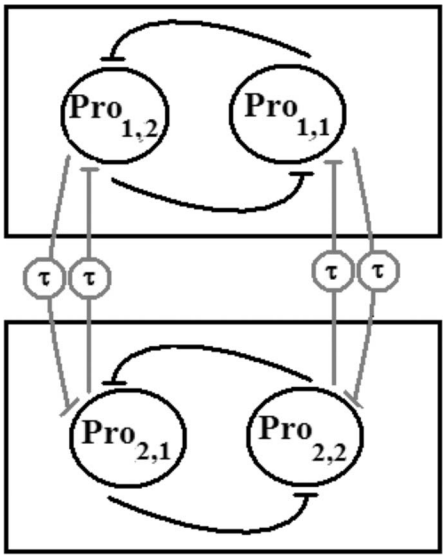

FIG. 2. Two coupled TSs spatially separated in two compartments. The τ's represent the time delay introduced in the coupling reactions to account for diffusion, membrane crossing, etc.

with observations [22]. Also, as we show here, this delay is necessary for a TS to toggle, given no cooperative binding or self-activation reactions. Due to the nature of transcription and/or translation processes, one always sets $\tau^1 < \tau^2 < \tau^3$. The delays can be random variables in a stochastic formulation.

Reaction 2 controls the coupling strength between genes of different TSs by setting the propensity for repressors to bind and unbind to the promoters. Additionally, one has reaction 3, in all equivalent to reaction 2, but coupling the two genes of each TS.

Reactions 4 and 5 are responsible for proteins decay. Reaction 4 allows the protein to decay when bound to the promoter at the same rate as when not bound. If absent, binding to the promoter would act as a "protection" against protein decay and affect the dynamics dramatically. This reaction also indirectly affects the repression "strength." Suppose we impose very high decay on the proteins. Because this causes proteins to have a very short lifetime, the effects of their repression are very weak since they can only remain for a short duration bound to the promoter, independently of the value assigned to the rate constants of the coupling-uncoupling reactions.

In Fig. 1 we show the interactions between genes of two coupled TSs within the same compartment. In Fig. 2, one also has two coupled TSs, but in different compartments. An extra delayed reaction is introduced latter on, to account for the delays due to diffusion and membrane crossing, indicated in the figure.

Given the set of chemical reactions to model systems of coupled TSs, we now introduce the quantities used to characterize the system's structure and dynamics.

A. Coupling strength

A system is a set of elements and interactions. Its structure is defined by the interactions [48]. The element of the networks here modeled is the TS. The interactions are the reactions by which proteins produced by the genes of one TS repress the promoters of other TSs.

These interactions via chemical reactions between genes of different TSs, besides defining which genes repress which genes, also have an associated rate constant that defines the average frequency at which they occur. Specifically, reaction 2 rate constant defines the propensity with which genes of different TSs interact [23], thereby defining the topology of the GRN, where TSs are the elements and repression reactions between the genes of different TSs are the interactions.

As said, these interactions can vary in "strength" depending on the rate constants values: the longer (within a time interval) gene j represses gene i, the more coupled these two genes are since their dynamics is more interdependent. Such fraction of time is controlled by the rate constants and consequent propensities [23,46] of the reactions of binding and unbinding of the repressors to the promoters. From our set of reactions (1 to 5), there is one reaction (reaction 2) responsible for binding (with rate constant k_c) and two reactions, (2 and 4), responsible for unbinding (with rate constants k_u and k_{dp}).

For example, suppose that the rate constant k_c of the binding reaction of the repressor (p_j) to the promoter ($\mathcal{P}_i$) is 100 times higher than the inverse unrepression reaction rate constant plus the decay of protein on promoter rate constant ($k_u + k_{dp}$). Assuming also that, on average, there are 100 p_j proteins in the system, then $\mathcal{P}_i$ will be repressed, on average, 50% of the time since the two reactions have, in these conditions, equal propensity. To increase the strength of the coupling between two genes, i and j, one can either increase k_c or decrease k_u and/or k_{dp}.

Taking the above into consideration, to study the dynamics of coupled TSs as a function of the coupling between them, we define the coupling strength $C_{(i_1,j_1),(i_2,j_2)}$ between gene j_1 of TS i_1 and gene j_2 of TS i_2 as

$$C_{(i_1,j_1),(i_2,j_2)} = \frac{k_c(i_1,j_1,i_2,j_2)}{k_u(i_1,j_1,i_2,j_2) + k_{dp}(i_1,j_1,i_2,j_2)}. \quad (6)$$

In the models of the present work, the GRNs only have repression reactions between genes. However, this measure can account for activation reactions as well. Defining a separate measure for activation reactions provides an estimation of the fraction of time that a gene is expected to be "activated" (opposed to repressed and free). Combining all rate constants related to binding and unbinding to the promoter in a single coupling strength measure (rate constants of binding reactions summed in the numerator and rate constants of unbinding and decaying summed in the denominator), one would obtain the fraction of time that either an activator or repressor is bound to the promoter. Notice that this measure does not account for the number of proteins in the system at a given time t. It is merely defined from the rate constants. Thereby it is possible for two genes to be strongly coupled according to this measure, but not being dynamical related in a time series of gene expression because no proteins of the input gene are available in the system. On the other hand, this definition of C gives a measure to characterize the structure independently of the dynamics.

Given the definition of C between two genes, we define the average C between two TSs (i_1 and i_2) as the normalized sum of the two coupling strengths regarding the two pairs of genes of the two coupled TSs:

$$C_{i_1,i_2} = \frac{1}{2} \sum_{j_1,j_2=1;j_1\neq j_2}^{j_1,j_2=2} \frac{k_c(i_1,j_1;i_2,j_2)}{k_u(i_1,j_1;i_2,j_2) + k_{dp}(i_1,j_1;i_2,j_2)}. \quad (7)$$

Finally, given a GRN of N genes structured in $\frac{N}{2}$ TSs, suppose that each TS is coupled to all other TSs. Thus, the GRN has $\frac{N/2.(N/2-1)}{2}$ bidirectional connections between genes of different TSs. We define this GRN average C as the normalized sum of all C's of the pairs of genes of different TSs:

$$C = \langle C_{i_1,i_2} \rangle = \frac{\sum_{i_1,i_2=1,i_1\neq i_2}^{i_1,i_2=N} C_{i_1,i_2}}{\frac{N}{2}\left(\frac{N}{2}-1\right)} \quad (8)$$

Given this definition, C is directly proportional to the rate constant of the binding of repressors to promoters and indirectly proportional to the sum of the rate constants of the reactions responsible for unbinding of such repressor from the promoters. Therefore, C is independent of the number of proteins in the system at each moment and is solely dependent on the GRN topology, i.e., number of interactions and their rate constants.

Unfortunately, the definition accounts only for instantaneous reactions. That is, if a time delay is introduced in any of the reactions responsible by the coupling, it will affect the fraction of time the promoter is repressed, especially when the system is not in a stable state. In those cases, both delays and C must be considered to understand the system behavior.

For simplicity, we use only $k_c(i_1,j_1,i_2,j_2)$ as our control parameter of C, and maintain the other two parameters constant.

B. Measuring stability from the toggling period

To study the ability of the GRN of coupled TSs to "hold state" (a single state out of the possible ones) after a transient, we introduce a stability measure (S). The less a TS toggles between states in a time interval, the more stable it is, given that toggling corresponds to either p_1 becoming clearly larger in quantity than p_2 or vice versa. The state of the TS can, for the purposes of this work, be characterized by this relation between p_1 and p_2: both null or near null, both large or one much larger than the other. Given a time series Δt seconds long, the stability of TS i that during Δt toggled n_i times, is defined as

$$S_i = \frac{\Delta t}{n_i + 1}. \quad (9)$$

The dependence on Δt allows distinguishing S in experiments with different durations. The only difference between S and the period of toggling is that no toggling implies infinite period, while S will be equal to the total time interval Δt of the experiment. The stability S of the set of coupled TSs is the average of all S_i. Supposing a system of N genes, i.e., $\frac{N}{2}$ TSs, where in a given a time interval Δt, each TS toggled $n_1, \ldots n_{\frac{N}{2}}$ times, respectively, S is given by

$$S = \frac{\frac{N}{2}\Delta t}{\sum_{i=1}^{i=N/2} (n_i) + 1} \quad (10)$$

Thus, having a set of TSs where, for example, one of them does not toggle, will not cause the average S to be infinite. This quantity can be used to compare the stability of single TSs within a network and average stability of distinct GRNs composed of TSs more or less coupled.

III. METHODOLOGY

We intend to study the stability of GRNs as a function of their average coupling strength (C) and time delays of coupling reactions, when considering compartmentalization modeled by delayed coupling reactions.

To accomplish our purpose we use the following parameter values, unless stated otherwise. At the beginning of each independent experiment, all proteins are initialized at 0 and all promoters are free to express. The number of $\mathcal{R}p$'s is set to 50 per gene, in agreement with experimental observations [49]. Consequently, the average number of available $\mathcal{R}p$'s after a transient is $\sim$30 and, on average, it does not act as a limiting factor for transcription. The delays in the transcription and/or translation reactions are set at $\tau^1=2$ s, $\tau^2=20$ s, and $\tau^3=100$ s. The reactions rate constants are set at $k_t=0.5\ s^{-1}$, $k_d=0.001\ s^{-1}$, $k_c=0.1\ s^{-1}$, and $k_u=0.001\ s^{-1}$. We run 100 independent experiments for each set of parameters

values, each during $\Delta t = 10^6$ s and sampling at each 50 s. A detailed justification and references of the values set can be found in [22]. The rate constants are in s^{-1} units since they are stochastic rate constants, i.e., they are frequencies independent of the concentration [23].

All gene expression time series figures in the results section are shown as examples, and are taken from single runs. In the graphs, we present the average results of 100 independent experiments for each data point.

IV. RESULTS

A. Bistability as a result of time delays in transcription

In this section we model a single TS and analyze the influence in the system bistability, of time delays in the reaction responsible for transcription and translation. We simulate the following set of reactions:

$$\mathcal{R}p + \mathcal{P}_1 \xrightarrow{k_t} \mathcal{P}_1(\tau^1) + \mathcal{R}p(\tau^2) + p_1(\tau^3), \quad (11)$$

$$\mathcal{R}p + \mathcal{P}_2 \xrightarrow{k_t} \mathcal{P}_2(\tau^1) + \mathcal{R}p(\tau^2) + p_2(\tau^3), \quad (12)$$

$$\mathcal{P}_1 + p_2 \underset{k_u}{\overset{k_c}{\rightleftarrows}} \mathcal{P}_1 p_2, \quad (13)$$

$$\mathcal{P}_2 + p_1 \underset{k_u}{\overset{k_c}{\rightleftarrows}} \mathcal{P}_2 p_1, \quad (14)$$

$$\mathcal{P}_1 p_2 \xrightarrow{k_d p} \mathcal{P}_1, \quad (15)$$

$$\mathcal{P}_2 p_1 \xrightarrow{k_d p} \mathcal{P}_2, \quad (16)$$

$$p_1, p_2 \xrightarrow{k_d} \varnothing . \quad (17)$$

Given this set of reactions, we model four cases: (A) no time delays, all τ's are set to null (Fig. 3); (B) a time delay only on the protein production, namely, $\tau^3 = 100$ s, while $\tau^1 = \tau^2 = 0$ s (Fig. 4); (C) multiple delays, $\tau^1 = 2$ s, $\tau^2 = 20$ s, and $\tau^3 = 100$ s (Fig. 5); (D) same settings as case B but with a transcription rate constant, ($k_t = 0.005$ s^{-1}), 100 times smaller (Fig. 6).

In case A (no time delays), the system does not toggle (Fig. 3) since we did not implement cooperative binding (dimers as inputs to the promoters) and also, unlike the TS model without cooperative binding studied in [39], there are no self-activation reactions.

After a long transient, the system settles into one of the two stable states (one gene on and the other off), each equally probably. The choice is purely driven by stochastic fluctuations. Once the choice is made, the system does not toggle. The average transient of the 100 independent experiments was ~14 000 s with a standard deviation of ~7000 s.

Decay and production equilibrate at ~50 000 proteins, in agreement with an ODE model of similar reactions [47].

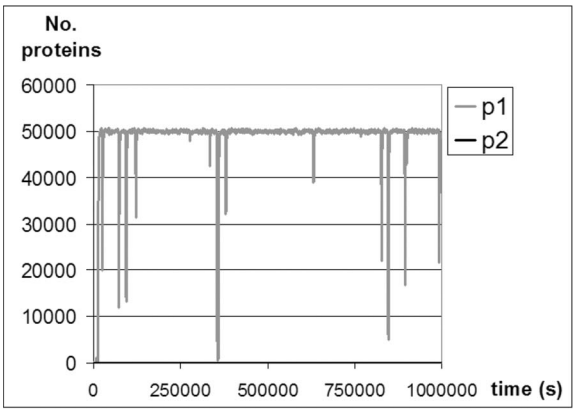

FIG. 3. Time series of a TS without delays or cooperative binding. After the transient ~14 000 s, the system remains stable. p_2 never grows beyond 20.

In case B, Fig. 4, time delays for the protein production are introduced, causing the system to be less stable than in the previous case (p_1 fluctuates more) but it still reaches a single steady state, rather than toggling. The average transient time and its standard deviation to attain the stable state increases in comparison with case A and is ~19 000 s with a standard deviation of ~12 500 s. Interestingly, due to the proteins initially being produced and still on the waiting list, there is a small burst of both genes' proteins at the beginning, i.e., both genes are "on" at this stage. Once this first set of proteins is produced and released in the system, they start repressing the genes promoters, while most of them decay. This lasts on average 4000 s and is only possible due to the existence of delays on the proteins release. After the transient, one of the genes becomes "on" again at the level of 50 000 proteins, and the other "off." That is, in this case, the delays only affect the initial transient and after that, the steady state solution is the same as if no delays existed.

In case C all delays assumed by the model are non-null (reaction 11). As seen in Fig. 5, the system dynamics changes

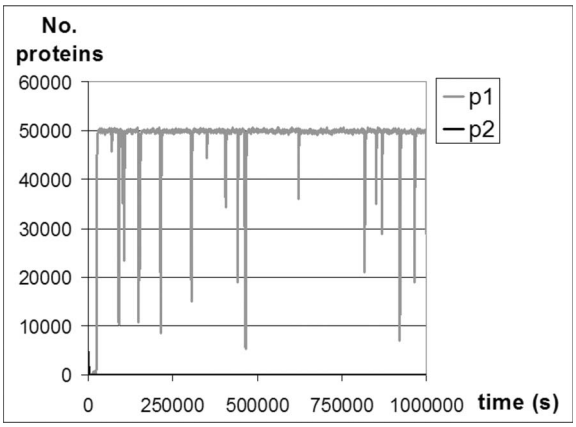

FIG. 4. Time series of a TS with 100 s delays on the proteins release and no cooperative binding. As in case A, after a transient, the system remains stable and the number of proteins of the repressed gene (p_2) never goes beyond 20, except during the initial transient (unlike in case A), due to the delay on the proteins release.

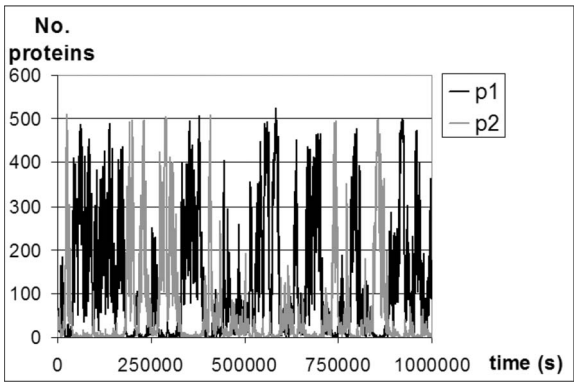

FIG. 5. Time series of a TS with multiple delayed transcription and/or translation and no cooperative binding. Delays: $\tau^1=2$ s (promoter), $\tau^2=20$ s ($\mathcal{R}p$), $\tau^3=100$ s (proteins). The system toggles due to the delay of the promoter region release, which also causes no protein increasing above 500.

drastically in comparison with the previous two cases. First, the maximum level that proteins reach is 500 (in comparison with 50 000), due to the delay on the promoter that limits the number of $\mathcal{R}p$'s that can be transcribing the gene at the same time. Since $\tau_1=2$ s, there can be at most 1 transcription per each 2 seconds. The delay on the $\mathcal{R}p$ release also diminishes the transcription reaction propensity (approximately by $\sim 20\%$) since a fraction of the $\mathcal{R}p$'s is not available at all times, while occupied transcribing a gene.

The system now toggles (from p_1 being in larger quantity to p_2 and vice versa), after an average transient for the first toggling to occur of 4900s with a standard deviation of 4050 s. The average number of toggles observed during Δt is 18.5 with a standard deviation of 3.75. Thus, the average toggling period is 50 000 s (which also corresponds approximately to its value of S).

Although the time series are more noisy than it is when using cooperative binding or self-activation reactions, the system clearly toggles even without these two conditions.

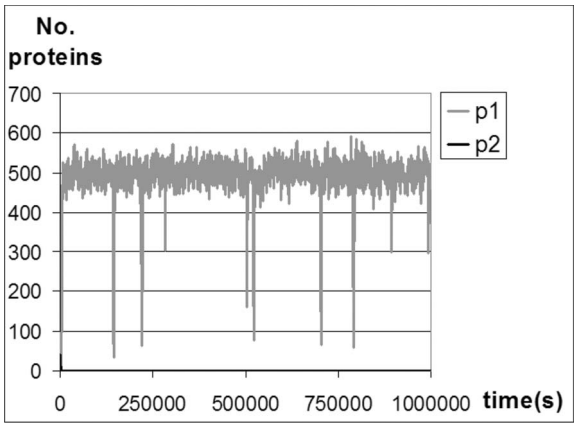

FIG. 6. Time series of a single TS with delays on the p's release only (100 s), and $k_t=0.005$ s^{-1}. The system does not toggle since there is no delay on the promoter, even though proteins never increase above 500, due to weaker rate of transcription and/or translation.

We now investigate the cause or causes for the toggling to occur. We observed that for our system of reactions describing the TS there is no toggling, when there are no delays or when there is a delay only on the protein release. Thus, the observed toggling in Fig. 5 either is due to the delay on the promoter or is a consequence of having a far smaller maximum number of proteins of each gene and thus stochastic fluctuations cause the system toggle. Notice that if it is the second case, then it is indirectly caused by the delay on the promoter. Additionally, we observed that the delay on $\mathcal{R}p$ release is not a cause for the toggling. When setting the $\mathcal{R}p$ delay to null in the previous case, oscillations persisted (data not shown).

Case D shows that the toggling is caused by the delay on the promoter and not by having a small maximum number of proteins of the gene "on." It consists in a TS with transcription and/or translation delays only on the protein release, as in case B, but with transcription rates 100 times smaller so that the level at which the protein is in highest quantity, is the same as in case C. With that goal, we set $k_t=0.01$ s^{-1}. Such decrease, as seen in Fig. 6, sets the maximum number of proteins observed in 500 as in case C, but unlike this case, no toggling was ever observed.

We conclude that given no cooperative binding or self-activation, the feature that allows toggling is the time delay on the promoter release.

B. Two coupled toggle switches: Varying the coupling strength

Using model C as our networks basic element, we now test what is the effect of coupling two of these multidelayed TSs. We analyze the dynamics of two coupled TSs as a function of their coupling strength. This model is a particular case of the more general network described by reactions 1 to 5. We have only two TSs and the rate constants are the same for the same kinds reactions (i.e., k's do not vary with the indexes i and j in this case). For that reason, in this section we do not refer to indexes of rate constants.

We assume that C within the genes of one TS is constant: $k_{cw}=0.1$ s^{-1}. Such interactions occur via reaction 3.

The repression constant (k_c) between genes of distinct TSs is varied, from 0 to 1 s^{-1}, in steps of +0.01, while k_u and k_{dc} are kept constant and equal to 0.001 s^{-1}), resulting in different values of C.

In Fig. 7, the time series of p_1 of TS 1 and p_3 (protein transcribed by gene "$j=1$" of TS $i=2$) show asynchronous toggling as expected, since they are not coupled (proteins p_2 and p_4 are not plotted to facilitate visualization).

We now couple the two TSs. In Fig. 8, the time series of p_1 of TS 1, and p_3 (protein of gene 1 of TS 2) are in almost perfect synchrony given the sampling time of 50 s. Only slight delays between the two "signals" exist, due to the time it takes for a change in one TS to affect the other (due to the delays on promoter release and protein production). Curiously, they are able to synchronize but the coupling is not strong enough to stop their toggling.

In Fig. 9 the time series of p_1 and p_3 are synchronous but the system toggles at a much slower frequency than in Fig. 8. This is caused by a stronger coupling than before. Although

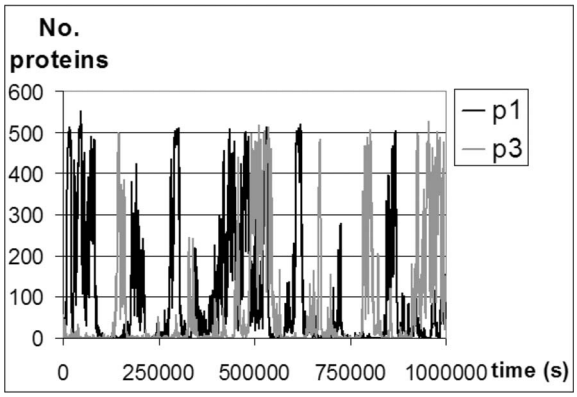

FIG. 7. Two TSs uncoupled (k_c=0 s^{-1}). Time series of proteins p_1 and p_3 (corresponding to genes "1" of each of the toggles). The oscillations of the two TSs are not correlated.

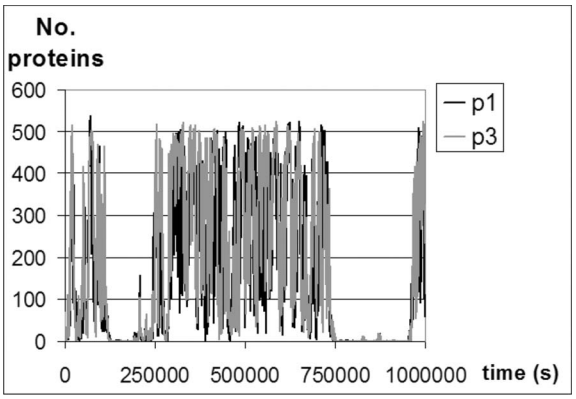

FIG. 9. Two coupled TSs: k_c=0.1 s^{-1}. Time series of p_1 and p_3. The two TSs oscillate synchronously, but at a lower frequency than in Fig. 8.

still allowing toggling is some of the cases where one of the two TSs changes its state fast enough to escape the influence of the other TS, this event is less likely.

As seen in Fig. 10, due to the strong coupling, the time series of p_1 and p_3 no longer oscillate (i.e., no toggling is occurring). The coupling is strong enough (i.e., occurs at high frequency) so that when one of the two switches "tries" stochastically to toggle, such change is not fast enough to "escape" the coupling, leading the system to a steady state.

To characterize the system behavior as a function of its structure properties, we plotted S versus C in Fig. 11. The system goes through a phase transition from oscillating to stable for a linear growth of C.

This result expresses that stability is obtainable by coupling two TSs. Also, it shows what are the necessary C values to attain synchronization and stability.

We investigated if the initial transient varied with the C value but it does not appear to be the case. The average time for the first oscillation was ~7000 s with a standard deviation of ~3500 s.

C. Effect of randomly distributed time delayed coupling reactions between two toggle switches

In this section we address the possibility that stability of a TS is gained by cell-cell interaction rather than by increasing the number of TSs within one cell compartment. Here we study the dynamics of two coupled TSs located in separate compartments.

To model the dynamics of two coupled TSs in separate compartments, one must consider that the proteins produced by each TS have to diffuse from one compartment to the other by crossing the membrane between them (which could be by diffusing through the membrane, or by transport through a protein channel or by endocytosis, etc.). We model this by introducing an extra reaction, with a normal distributed random time delay, to take in account the time for the proteins to cross between the two compartments.

We assume only 2 compartments, (c_1 and c_2) each with one TS. Proteins must now be represented in the form $p_{i,j}(c_1)$ to indicate in which compartment they are at each moment. For simplicity, we assume that all proteins diffuse by the same mechanism and have the same propensity to cross the membrane, and thus the random delays (τ_{cross}) of the reaction to move between compartments are generated from the same distribution and the rate constants of these reactions are the same for all proteins.

To study the effect of τ_{cross} in the stability of the system, in comparison with the previous case, we set k_{cw}=0.1 s^{-1}

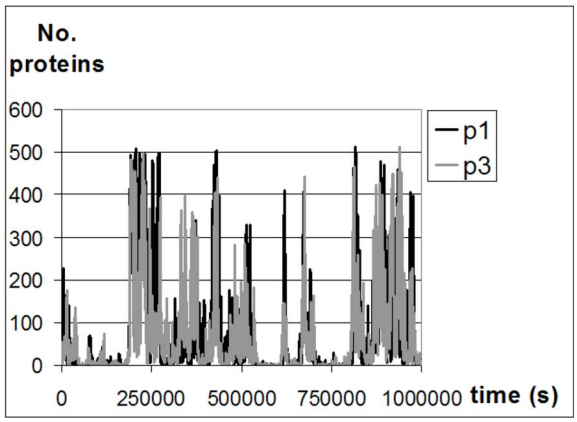

FIG. 8. (Color online) Two TSs coupled: k_c=0.01 s^{-1}. Time series of p_1 and p_3. The two TSs oscillate almost synchronously.

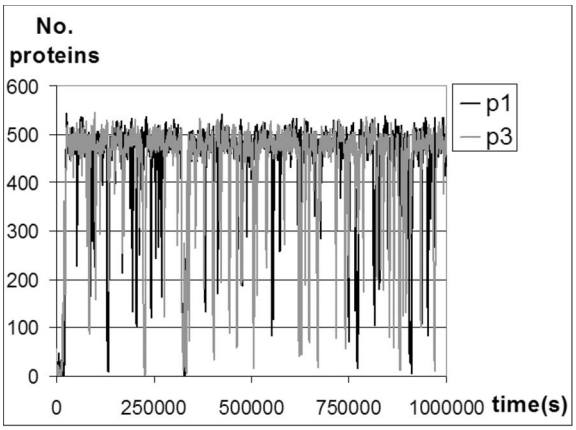

FIG. 10. Two coupled TSs, k_c=1. Time series of p_1 and p_3. Due to the strong coupling, the system is stable.

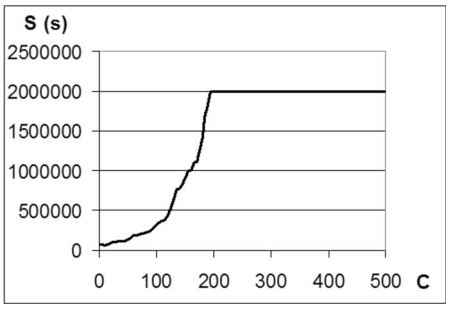

FIG. 11. Stability (S) versus coupling strength (C), averaged over 100 independent experiments for each data point. The system goes through a phase transition, from oscillating (independent oscillations for weak coupling and synchronized for stronger coupling) to stable (for strong coupling).

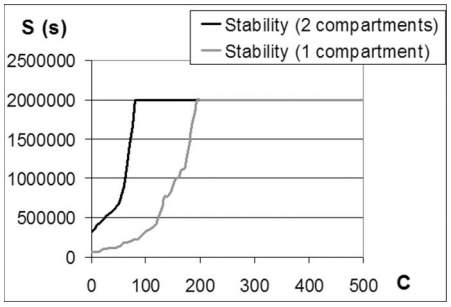

FIG. 12. Stability (S) versus coupling strength (C) of two TSs in different compartments, averaged over 100 independent experiments for each data point. The system goes through a phase transition, from oscillating to stable, for smaller values of C than when in a single compartment, also plotted for comparison.

and vary k_c. Also, we define a new rate constant, k_{cross} $=0.1\ s^{-1}$, associated to the reactions to move between compartments. The time delay for these reactions is randomly generated (each time such a reaction occurs) from a normal distribution with a mean of 100 s and standard deviation of 50 s. Given, $\forall i,j,z,w:i,j,z,w=1,2\wedge z\neq w$, such reactions to move between compartments can be described as [45]

$$p_{i,j(c_z)} \xrightarrow{k_{cross}(c_z,c_w)} p_{i,j}(c_w)(\text{normal:}\tau_{cross},\text{std}). \qquad (18)$$

The variable delay is inserted in reaction 18 using the notation X (normal: mean, std) to indicate that each time this reaction is selected as the next to occur by the SSA, the products (X) are placed on the waiting list with a time delay randomly generated from a Gaussian distribution with that mean and standard deviation. *SGNSim* ensures no negative delays by truncating the negative part of the distribution, when necessary [45].

We ran the system such that $k_c=0\ s^{-1}$ to $1\ s^{-1}$, with increments of +0.01, and measured the toggling frequencies. In Fig. 12 we plot the average S observed in 100 independent experiments, as C varies. For comparison, we also plot the previous results attained when the two TSs are in the same compartment (Fig. 11). The phase transition to stability occurs for smaller values of C than when in a single compartment. This is in agreement with another observation, that it also toggles far less frequently for equal values of C. We observed that the two TSs are able to synchronize for k_c $=0.1\ s^{-1}$ and become stable for $k_c>0.15\ s^{-1}$, whereas when they are in a single compartment they become stable only for $k_c>0.4\ s^{-1}$.

The only difference between this and the previous system are the extra reactions related to the delayed crossing between compartments. Attaining stable states for lower values of C is due to placing proteins in the waiting list as they go from one compartment to the other. When the two TSs are in the same compartment the system toggles when one of the TSs toggles reliably enough (for enough time) to force the other one to toggle also. Yet, here, when that happens, one must still take in account those proteins on the waiting list (that are traveling from one compartment to the other), whose quantities reflect not the current system state but the state τ_{cross} seconds before. They will act towards imposing to the system the previous state (supposing a stochastic toggle on the quantities of the proteins not on the waiting list), making toggling less likely, which results in higher stability.

We observed that in all cases of separate compartments the number of proteins of the genes "on" is far smaller than in previous cases of coupled TSs. The number of proteins in both compartments never goes beyond 200. This is due to the extra delayed reactions for crossing between compartments. On average, ~350 proteins are on such list and therefore do not appear in the concentrations observed. This is equivalent to assuming that they are not able to react while going from one compartment to the next. The slightly larger number of proteins (summing those available to react and those on the waiting list) is also due the large fraction of proteins on the waiting list and, during that time, not subject to decay.

Another consequence of these extra reactions and the resulting smaller number of proteins available to react at each moment is the large average transient and the very high standard deviation of this transient for the system to reach stability, or for a first oscillation to occur (depending on C value) in comparison with the single compartment case. The average initial transient is ~30 000 s with a standard deviation of ~15 000. Again, this transient does not exhibit dependency on C.

D. Several identical toggle switches, homogenous coupling: Stability as a function of the number of toggle switches

We now study the system dynamics, as we have many TSs, each weakly coupled to all TSs of the network.

Suppose that C cannot be changed and is weak (k_c $=0.01\ s^{-1}$). The system is not able to attain stability by coupling only two TSs. Another possibility for attaining stability is to couple several TSs, copies of an initial one.

We test that possibility and measure S as a function the number of TSs and correspondent total C on which TS (the sum of the C between a TS and each of the other TSs connected to him).

The coupling of many TSs will result in a stronger k_c for any given TS of the network, since it receives inputs from all

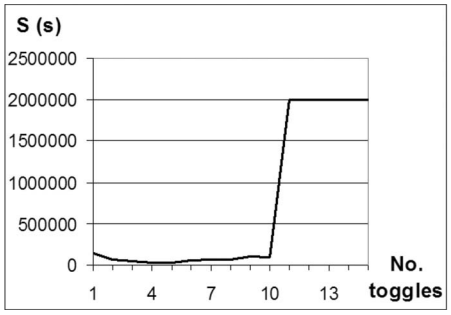

FIG. 13. Average S of each TS in systems of increasing number of TSs averaged over 100 independent experiments for each data point. The system goes through a phase transition, from oscillating to stable.

other TSs. Equation (8) can be used to compute such global k_c on a given TS.

We start with two TSs, than compare it to a weakly coupled set of 3,…, 15 TSs as these are added. In all cases, 100 independent experiments were done, and the coupling rate constant within TSs is set to $k_{cw}=0.1\ s^{-1}$.

Again, this a particular case of the general set of reactions described in the model section. Reactions rate constants do not need indexes since they are equal for all TSs and between pairs of TSs.

The average initial transient of the experiments of Fig. 13 is ~15 000 s with a standard deviation of ~9000. This initial transient fluctuates significantly from case to case although it is visible that, on average, it increases slightly with the number of TSs.

Figures 13 and 14 show the phase transition that occurs, as more TSs are coupled, for $N=20$ or, equivalently, for $C=50$.

From Fig. 14 it is visible that, given C definition for a network of TSs, the phase transition occurs for far smaller values of C than when the system has only two TSs.

The reason for this lies on the C value in each gene being now the result of many interactions, instead of a single one as before. The many genes, coupled to each single gene, produce via their transcription and/or translation multiple delayed reactions, the proteins responsible for such coupling. This results in a very "stochastic" C. This effect is similar to the one caused by the delayed diffusion mechanism, i.e., it gives the coupling a stochastic and delayed nature, resulting in a stronger coupling effect, for equal C values.

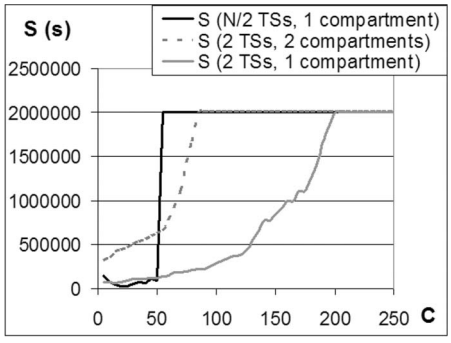

FIG. 14. Average S vs average C for a single TS. 100 experiments per data point. The system goes through a phase transition from oscillating to stable. For comparison, the two previous cases are plotted (2 TSs in one compartment and 2 TSs in two compartments)

Since these delays are not taken into consideration in the formula to compute C, and contribute to the system higher stability, the cases where the coupling is via these delayed and stochastic coupling mechanism, achieve stability for lower C values, as seen in Fig. 14.

V. CONCLUSIONS AND FUTURE WORK

Using a recent modeling strategy of GRNs, driven by a modified version of the SSA that allows multiple delayed reactions, we studied the dynamics of coupled TSs within the same compartment and when they are in separate compartments, by observing two parameters: the coupling strength, which characterizes the GRN structure, and stability, which characterizes its dynamical behavior.

The modeling strategy used here was chosen to be in agreement with recent experimental observations on the dynamical behavior of individual genes and small GRNs. Namely, our model accounts for the fact that the dynamics is stochastic and for the time delays involved in the two main processes involved in gene expression, transcription, and translation.

When not considering time delays, a TS requires cooperative binding of at least two proteins, or self-activation reactions for each gene, to exhibit bistability [39].

Here, we first showed that a stochastic TS with bistable state (toggling) TS can be attained without cooperative binding or self-activation reactions, when introducing a time delay on genes promoter region release after each transcription reaction occurrence.

After, we modeled a system of two coupled TSs. As we varied C, by varying the rate constant of binding of repressors to promoters, the system stability goes through a first order phase transition from oscillating to stable. For null or very weak coupling, the two TSs are independent and unsynchronized. For weak coupling, they toggle at the same frequency almost synchronously. The time lag between the oscillations is due to the existence of delays on promoter release and protein production. When "moderately" coupled, as C increases the system goes from synchronized toggling (with low frequency oscillations) to stable. If strongly coupled, the response time of the coupling is faster than the rate of change of any the TSs state, and, therefore, a stable state emerges after a transient, and no more toggling is observed. Such initial transient is highly stochastic and does not appear to depend on the coupling strength.

We note that, given the parameters of a single TS, i.e, the rate constants and time delays, this "stable" state is only possible with at least two coupled TSs, i.e., it is not observable in single TSs.

The results here presented show that the mechanism of gene copy can be used by a cell to attain a periodic oscillator with a desired period, or to obtain a mechanism able to make a stable decision, from two possible choices, in processes

such as differentiation, if the rate constants of the reactions coupling the genes of the two TSs can be properly tuned.

Next, we showed that two spatially separated TSs, coupled by reactions subject to normally distributed time delays, behave qualitatively the same as in the previous case. The phase transition of S with the increase of C occurs for lower C values than before. Another consequence of the extra delayed reactions is the large average transient, and very high standard deviation, for the system to reach stability or a first oscillation (depending on C value) in comparison with the single compartment case. Again, this initial transient did not exhibit dependency on the C value.

The only difference between spatially separated coupled toggle switches and those within the same compartment is the extra reactions related to the delayed crossing between compartments. The reason for attaining stability with lower values of C is that a large fraction of the existing proteins are on the waiting list as they go from one compartment to the other and are unavailable to react. These proteins, "protected against decay," provide more stability to the system.

The results are an example of a possible mechanism that make cells, within population of cells that can chemically communicate with their neighbors, behave very differently from isolated cells.

All the results of the compartmentalized space depend on the fact that proteins, while moving from one compartment to the other, are not available for reactions. For that reason, to correctly model this system one has to use a modeling strategy of the dynamics that can deal with small number of molecules available for reactions, that results in highly stochastic behavior. It is important to stress that reactions dynamics under these conditions should be modeled with the SSA.

Finally, we coupled weakly and homogeneously several TSs within a single cell. Starting with a single TS and adding TSs which are coupled to the existing ones, again a phase transition in stability was observed, due to the increase of coupling strength applied to each TS. If the rate constants of coupling reactions are weak and cannot be changed, we showed that one way to attain stability is to make several copies of identical TSs, until stability is attained.

Given the experimental results concerning gene expression, namely its stochastic nature and the time delays involves, the most accurate modeling strategy, which includes all these features, is, so far, the delayed SSA. Other strategies, such as ODE models with noise terms, can obtain to a certain extent, some of the results here described. However, we notice that important features of the dynamics of simple real gene networks, such as gene expression occurring in bursts, are not easily mimicked by other modeling strategies. As the systems to model become more complex, involving many genes and interactions, feedback loops, spatial compartmentalization, among other features, the differences between the results of different modeling strategies will increase, and the models that best capture real GRNs features will have to be used.

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