Dynamics of a two-dimensional model of cell tissues with coupled stochastic gene networks

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(Received 13 March 2007; revised manuscript received 21 August 2007; published 20 November 2007)

Gene expression and differentiation were shown to be stochastic processes. However, cells in a tissue can coordinate their behavior, including gene expression and differentiation pathways choice. A tissue of coupled cells is modeled as a two-dimensional regular square lattice of identical cells, each a three-dimensional compartment with a gene regulatory network (GRN) and a toggle switch (TS). The dynamics is driven by a delayed stochastic simulation algorithm, nearest neighbor cells are coupled by normally distributed time delayed reactions allowing interchange of proteins, and gene expression is a multiple time delayed reaction. It is defined the coupling strength (C), to characterize the lattice structure as a function of the rate constants of the reactions coupling nearest neighbor cells. Conditions are investigated for the emergence of synchronization and stable differentiation of cells within a tissue. From the time series of the cells GRNs, the tissue dynamical stability (S) is computed from the average toggling period of each GRN. The synchronization of cells' proteins expression levels is measured by their time series entropy (H). It is shown that the tissue goes through various dynamical regimes as C is increased, by measuring H and S. For null C, the cells GRNs toggle asynchronously. For weak C, cells synchronize by regions of space. Increasing C, the tissue becomes homogeneously synchronous. As C is further increased, S goes through a phase transition, from synchronized toggling to stable, where all cells produce one and the same protein. Finally, increasing C even further, a new stable state emerges where both genes of all cells are expressed and bistability is lost. This state, resembling an infinitely long transient, is an emergent behavior not observable in a single TS. The results provide an explanation of how cells with bistable GRNs, inherently stochastic, can synchronize or uniformly differentiate into stable states, by interacting with nearest neighbors.

DOI: 10.1103/PhysRevE.76.051915 PACS number(s): 82.39.Rt, 82.20.−w, 05.10.Ln, 05.45.Xt

I. INTRODUCTION

It remains a challenge to understand how GRNs of cells can interact with the GRNs of neighboring cells when these cells are embedded in a tissue, and how and what emergent behaviors might arise from the interactions.

Cells in a tissue can interact and influence their neighboring cells by different mechanisms, such as via diffusible signaling molecules, direct contact through transmembrane proteins, and gap junctions that allow a direct interchange of signaling molecules [1–4].

Interactions among cells can lead to synchrony of internal cellular processes and formation of spatial patterns in colonies of cells [5–7]. For example, morphogenetic gradients and sequential induction have been proposed as possible mechanisms to explain pattern formation [1].

Many of these processes are controlled by the dynamics of the GRNs of the cells. Two features have been identified as non-negligible in the dynamics of GRNs: Stochasticity and time duration of transcription and translation.

Stochastic fluctuations of gene expression were proven to have a significant role at the single-cell level [8,9], causing stochastic pathway selection [10,11], and their relevance is enhanced by the discrete nature of the transcription factors and their binding sites, which exist in low copy numbers [12,13]. Stochasticity is further enhanced by the fact that many processes in cells, namely gene expression, involve a small number of molecules. For example, genes have only one or very few copies of its promoter region. In agreement, the more accurate modeling strategies at a detailed level [14,15], are based on the stochastic simulation algorithm (SSA) [16,17].

Time delays in gene expression have been shown to be an important regulating mechanism of GRNs [18]. Although models of nondelayed reactions accurately explain most experimental data regarding fluctuations in gene expression [10,19,20], these studies focused on steady state dynamics, where the delayed and nondelayed models have the same results after a transient. To accurately model GRNs in more complex conditions (e.g., involving feedback mechanisms), time delayed reactions are necessary for modeling transcription and translation [21–23]. For example, time delayed reactions were necessary to mimic recent measurements of single gene expression at the single molecules level [23,24].

Finally, in cellular tissue models, where the dynamics is constrained by spatial compartmentalization, diffusion and membrane crossing of signaling molecules should be modeled by reactions with delays following some normal distribution, accounting for their time duration [25,26].

Accordingly, the dynamics is here driven by SGN Sim [27], a simulator based on a modified version of the original SSA [17], the multiple time-delayed SSA [21], that can model gene expression as multitime delayed events [28] and cell-cell interactions as normally distributed delayed events.

Since it is studied how cells GRNs can synchronize and make collective choices, e.g., regarding differentiation pathways, the choice of what GRN to place in each cell is based on a long standing hypothesis that cell differentiation is con-

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trolled by bistable genetic subcircuits with many downstream genes [29]. In this process, a stem cell turns into a stable phenotype [30]. The GRN decision subcircuit for differentiating should be, at the same time, (at least) bistable, to allow branching into distinct cell types, and reliable, so that once a decision is made it is robust to internal noise and external perturbations, acting as a cellular memory unit [31].

It was experimentally shown that the toggle switch (TS), consisting of two genes mutually repressing each other, can be used by cells to adopt different stable states [10,31,32], phenotypically distinct. Models of differentiation pathways have been proposed using TS's as the decision subcircuits, at each bifurcation [33]. Interestingly, it was necessary to assume multi-step processes, to explain observations not accounted by the model used [33]. Such multistep processes can be correctly modeled, for the purpose of studying the global dynamics of the system, by time delayed reactions [22].

From the above, it was opted to model as GRN of each cell, a TS. Although most GRNs differentiation subcircuits in real cells are, most likely, more complex than the two gene network assumed here, there are experimentally confirmed gene circuits of differentiation that consist of very few genes and are bistable, whose choice of differentiation pathway is driven by stochastic fluctuations in gene expression [10,11,34,35].

Additionally, only sequential induction is considered between neighbor cells, by which a cellular GRN activity might affect its neighbor cells through its products of gene expression. Communication between nearest neighbor cells is done by the exchange of proteins. This is a simplification of the far more complex mechanisms used by real cells. For example, signaling proteins in the membrane have to be activated and produce signaling molecules. These can be detected by specific proteins on the membrane of neighbor cells, generating a cascade of reactions by which the signal arrives to its destination. However, the time duration of these mechanisms are accounted for, by modeling protein interchange between near neighbor cells via reactions with random normally distributed time delays, accounting for diffusion, membrane crossing, etc.

A recent work [36] focused on the dynamics of coupled nonidentical TSs within a single cell, and on the effects of varying binding affinities of proteins of a gene to another gene promoter region. The coupling mechanism between TSs differs significantly from the one assumed here, since spatial compartmentalization was not considered.

Here the focus is on characterizing the dynamics of bistable GRNs of individual cells within a tissue, with cell-cell communication between nearest neighbors. Since real GRNs dynamics are inherently stochastic and the time duration of processes such as gene expression are not negligible, each cell GRN dynamics is driven by the delayed SSA. The tissue is modeled as a two-dimensional regular square lattice with periodic boundary conditions, where each node of the lattice is a single compartmentalized cell. Each cell contains a single TS and, for simplicity, it is assumed that the TS dynamics is independent of the rest of the cell GRN dynamics (which thereby is not considered). It is studied the conditions necessary for the GRNs, in different cells, to synchronize their toggling and to attain "stability." As shown in the results section, from the interactions between cells, new states emerge, not possible for individual TSs, which can be seen as an evidence that cells within a tissue may behave differently than when isolated. The results are not reproducible by a delayed ODE model, shown for comparison.

Since these are stochastic models, by "stable states" it is meant "states where genes expression levels are, aside stochastic fluctuations, approximately constant in time," and where the toggling behavior is never observed (where a gene protein level "on" becomes "off," and vice versa).

The paper is organized as follows: First, a description is made of the "delayed SSA [21]." Next, the set of chemical reactions which constitute the GRN of each cell are introduced and, from this, the set of chemical reactions that model the tissue. After, definitions are given for stability, system entropy, and coupling strength between pairs of TSs and for the tissue of coupled TSs.

In the results section, first its measured the stability and system entropy of two coupled cells GRNs, as their coupling strength varies. Next, it is studied the dynamics of a tissue of 25 cells, organized as a two-dimensional square lattice of 5×5 cells with periodic boundary conditions.

Finally, conclusions are presented regarding the interpretation of results and possible biological implications.

II. MODELING STRATEGY OF TISSUES

A. Multiple delayed stochastic simulation algorithm

In the SSA [16], products of a reaction are released immediately after the reaction occurred. However, unlike simple bimolecular chemical reactions, gene expression is a highly complex chemical process that involves many reactants and sequential reactions. The set of reactions necessary for a gene to be transcribed by one RNA polymerase, spliced, translated by a Ribosome and folded, can be simplified [21], for our purposes, into a single step multidelayed reaction. This method of delaying the release of gene expression products by a time interval proved to be more accurate than those assuming instantaneous gene expression [21,23].

GRNs are modeled here by the methodology proposed in [28], with SGN Sim "stochastic gene networks simulator" [27] that allows modeling transcription and translation as single step multiple time delayed reactions [21].

The "delayed SSA [21]," unlike the nondelayed SSA, uses a waiting list to store delayed output events. The waitlist consists of a list of elements (e.g., proteins being produced), each to be released after a certain time interval (such time duration is set when they are placed on the waitlist). The algorithm proceeds as follows.

(1) Set $t \leftarrow 0$, $t_{\text{stop}} \leftarrow$ stop time, read initial number of molecules and reactions, create empty wait list L for delayed generating events.

(2) Do an SSA step for reacting events to get next reacting event R_1 and corresponding occurrence time $t+t_1$.

(3) Compare t_1 with least time in L, t_{min}. If $t_1 < t_{\text{min}}$ or L is empty, set $t \leftarrow t+t_1$. Update number of molecules by performing R_1, adding to L delayed products (if existing) and the time they have to stay in L from appropriate distribution.

(4) If L is not empty and if $t_1 \geqslant t_{\min}$, set $t \leftarrow t+t_{\min}$. Update number of molecules and L, by releasing the first element in L; otherwise go to step (5).

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

The use of a delayed reactions model to drive the dynamics of GRNs models has been proven necessary at a detailed level [23], specially under complex conditions, e.g., in GRNs with feedback loops [21]. Recently, a detailed comparison between modeling strategies was made, studying at a detailed level models of single gene expression, a single TS and the repressilator [23]. Many relevant differences were found, when using different modeling strategies such as Boolean networks, coupled ODEs, and nondelayed stochastic models. For example, the delayed SSA was the only that reproduced results of a recent experiment where gene expression was measured at the single proteins level [24].

When a chemical system has many molecules of all intervening chemical species its dynamics, i.e., the variation of the concentrations of the chemical species present, can be, in simple cases, computed approximately using ODE's. These models are either deterministic or include some noise term, white or colored. In the GRN model here used, proposed in [28], genes are treated as chemical species since in real GRNs they exist only in very small quantities. Since promoter regions are also subject to time delays [21], stochastic effects cannot be ignored. Therefore, a "mean field" approach is not accurate. Additionally, until now, attempts to simulate noise using Langevin equations were not very successful [37]. Finally, it remains to be shown that noise terms in ODE models capture the true nature of noise in gene expression.

Since the models simulated here have coupled GRNs and feedback loops, it is necessary to use the delayed SSA to drive the dynamics, to obtain solutions as realistic as possible. A similar conclusion about the necessity of using the SSA to model GRNs was obtained in another work, related to the study of the dynamics of TSs [38], even when the systems have large number of molecules.

B. Model of the toggle switch

A TS consists of two genes repressing each other. The model used here [Eqs. (1)–(4)] does not include cooperative binding, where repression of promoters would be by dimmers of proteins, or self-activation reactions, where a gene resulting proteins would act as activators of its own expression. These two reactions are usually introduced in models to obtain toggling dynamics [23,33,38], however it was recently shown that introducing realistic time delays in transcription-translation [39] is sufficient to obtain robust flipping between two states for a wide range of parameters values [36], in agreement with experimental observations [31]. Also, this model is less demanding computationally than models requiring dimmers, by reducing the number of reactions necessary, allowing the simulation of larger lattices.

The set of reactions that define a TS are the following. Let z, z_1, $z_2=1,2$ and $z_1 \neq z_2$:

$$\text{RNAp} + \text{PRO}_z \xrightarrow{kt_z} \text{PRO}_z(\tau_z^1) + \text{RNAp}(\tau_z^2) + p_z(\tau_z^3), \tag{1}$$

$$\text{PRO}_{z_1} + p_{z_2} \underset{k_u(z_1,z_2)}{\overset{k_c(z_1,z_2)}{\rightleftarrows}} \text{PRO}_{z_1}p_{z_2}, \tag{2}$$

$$\text{PRO}_{z_1}p_{z_2} \xrightarrow{k_{dpz_1}} \text{PRO}_{z_1}, \tag{3}$$

$$p_z \xrightarrow{k_{dz}} \varnothing\,. \tag{4}$$

Reaction (1) models the transcription-translation process in a single step, accounting for the time each of the products takes on average [21] to be released, once the reaction is initiated. Such delays can be constant or drawn from distributions each time the reaction occurs [27]. The τ's represent the extent of time for a product to be released in the system after the reaction occurred. The τ's superscripts distinguish delays between products of the same reaction, while subscripts distinguish the delays of products of reactions associated to different genes.

For example, if reaction (1) occurs for gene 1, at time t, promoter of gene 1 (PRO_1) and one RNA polymerase (RNAp) are removed from the system and placed in a waiting list (along with the τ of each of them). PRO_1 is released at $t+\tau_1^1$, the RNAp is released (unchanged) at $t+\tau_1^2$ and protein p_1 is released at $t+\tau_1^3$ from the waiting list, becoming available for future reactions [21,28]. Unless time delays τ's are explicitly represented in the products, all events, reactants depletion and products appearance, occur instantaneously at t.

The time delay for the promoter clearance affects the system dynamics significantly, by limiting the number of RNAp's that can transcribe the gene simultaneously (in agreement with experimental observations [39]) and it cannot be repressed while on the waiting list. This delay is necessary for a TS without cooperative binding or a self-activation mechanism to toggle [36]. Also, given the nature of transcription-translation processes to model it as a single step event, one always sets $\tau_z^1 < \tau_z^2 < \tau_z^3$.

Reaction (2) controls the repression strength between the two genes of the TS, by setting the propensity [16] for repressors to bind and unbind to the promoters. When bound, they form a complex ($\text{PRO}_{z_1}p_{z_2}$) that is unable to express, since the RNAp cannot bind to it.

Reactions (3) and (4) are responsible for protein decay. Reaction (3) allows the protein to decay when bound to the promoter. Without this reaction, binding to the promoter would act as a "protection" against decay and affect the dynamics significantly, specially in regimes with small number of molecules. This reaction also affects, indirectly, the repression "strength." For example, given high decay, repression becomes weak, since the repressor protein only remains repressing for very short time intervals.

As an example, the dynamics of a TS was simulated for 5×10^6 s, with a sampling frequency of 50 s. The system was initialized with 100 RNAp's, one promoter of each gene and no proteins. The rate constants are set at $k_t=0.05\ \text{s}^{-1}$, $k_d=k_{dp}=0.001\ \text{s}^{-1}$, $k_c=0.1\ \text{s}^{-1}$, and $k_u=0.001\ \text{s}^{-1}$. Time delays depend mostly on gene length and RNAp transcription speed, which can vary significantly from gene to gene and

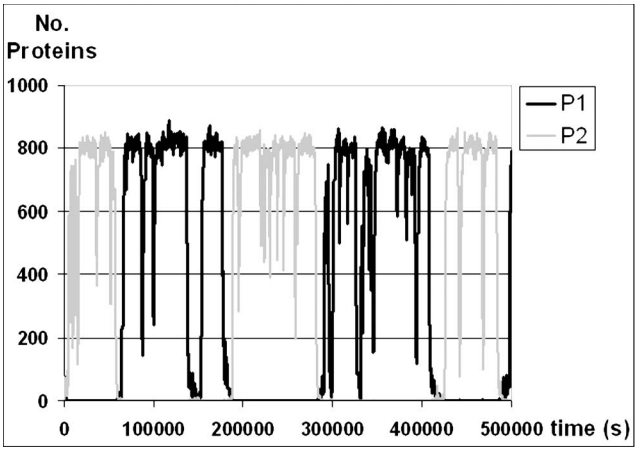

FIG. 1. Time series of the number of proteins of each gene of one TS with multiple time delayed transcription-translation, no cooperative binding, and no self-activation reactions.

also vary from one event to the next [39]. We set $\tau_1=1$ s, $\tau_2=20$ s, and $\tau_3=100$ s. The time duration of the simulation was set to allow observing a few toggles.

The resulting time series in Fig. 1 shows that the TS toggles from a state where $p_1 \gg p_2$ to the opposite, and vice versa. The delay of the promoters, limiting production, combined to the decay rate, causes no protein increasing in quantity above 1000.

C. Model of a square lattice tissue

Cells in tissues can spatially organize themselves in a variety of ways. Two extreme spatial structures are "connective tissues," with sparsely distributed cells and most space is occupied by extracellular matrix, and "epithelial tissues," with tightly bound cells by cell-cell adhesion, forming compact structures, and only a small fraction of the space is occupied by the extracellular matrix [1].

Here, its assumed a compartmentalized space organized in a 2D. regular square lattice structure of compartments with periodic boundary conditions. Each compartment contains one cell and has four neighbor cells. All cells of the tissue are identical. Due to the spatial structure imposed, the model is more similar to epithelial tissues rather than connective ones.

The coupling between GRNs is obtained by proteins in one cell diffusing to near neighbor cells. In real cells, this is very unlikely to occur in such a simple way. Usually, proteins do not diffuse through the cell membrane. The way proteins in one cell affect another cell is by binding to some membrane receptor which reacts, producing corresponding signaling molecules that will be detected by membrane receptors of another cell. This receptor will then produce signaling molecules which either interact directly with this cell GRN or activate a signaling pathway that can eventually interact with the GRN [1].

In the present work, the relevant feature of this complex chemical pathway is the time it takes for a signal to go from one cell to the next one. This time duration varies from one signal to the next one, following some distribution, here assumed to be a normal distribution. Thereby, to make the model as computationally simple as possible, it is here assumed that proteins can go from one cell to a next near neighbor one, via uni-molecular reactions with normally distributed time delays.

Thus, in the reactions responsible for the diffusion of proteins between cells, two parameters are relevant. The reaction rate constant defines the average time it takes for a protein to find a signaling molecule in the membrane. Once this event occurs, it is assumed that the signal will always reach the other cell. The time delay of the reactions modeling this process accounts for its time length and is randomly generated from a normal distribution. The mean and standard deviation of the distribution are set to small values, when compared to the delays of transcription-translation processes, assuming therefore that neighbor cells are spatially close to one another.

In general, since the reaction modeling the movement of a protein p_i, from a cell to a nearest neighbor cell, is unimolecular, under the formalism of the SSA [16,17], its propensity depends only on the number of p_i in the first cell and the rate constant of the reaction (k_{cross}).

To describe the tissue constituents and reactions, the following notation was adopted: each compartment in the lattice is identified with the indexes i,j (row and column). Given that the lattice has n^2 cells, then $i,j=1,\ldots,n$. These two indexes are assigned to each chemical element, i.e., proteins, promoters and so on, indicating in which compartment they are at any moment in time. Gene promoters (PRO) and proteins (p) need an extra index, $z=1,2$, depending on which gene of the TS they correspond to (either type "1" or "2"). Proteins of the same type are assumed indistinguishable within one compartment, independently of their origin. The chemical reactions occurring in the tissue are

$$\text{RNAp}_{i,j} + \text{PRO}^z_{i,j} \xrightarrow{kt^z_{i,j}} \text{PRO}^z_{i,j}(\tau^{z,1}_{i,j}) + \text{RNAp}_{i,j}(\tau^{z,2}_{i,j}) + p^z_{i,j}(\tau^{z,3}_{i,j}), \tag{5}$$

$$\text{PRO}^{z_1}_{i,j} + p^{z_2}_{i,j} \underset{k_u[(i,j,z_1),(i,j,z_2)]}{\overset{k_c[(i,j,z_1),(i,j,z_2)]}{\rightleftarrows}} \text{PRO}_{i,j^{z_1}p^{z_2}_{i,j}}, \tag{6}$$

$$\text{PRO}^{z_1}_{i,j}p^{z_2}_{i,j} \xrightarrow{k_d p[(i,j,z_1),(i,j,z_2)]} \text{PRO}^{z_1}_{i,j}, \tag{7}$$

$$p^z_{i,j} \xrightarrow{k_d(i,j,z)} \varnothing\,, \tag{8}$$

$$p^z_{i_1,j_1} \xrightarrow{k_{\text{cross}}(c_{i_1,j_1},c_{i_2,j_2})} p^z_{i_2,j_2}(N{:}\tau_{\text{cross}},\sigma). \tag{9}$$

Reaction (5) models genes transcription and translation processes in a single multiple delayed reaction [21]. Reactions (6) (two reversible reactions) represent the interactions between proteins and the two genes inside each cell and this particular topology is known as a TS. The only restriction on indexes is $z_1 \neq z_2$: $z_1,z_2=1,2$. Reactions (7) and (8) are responsible for protein decay, regardless of which compartment they are in. Reaction (9) regulates the coupling between nearest neighbor cells and it is a random delayed

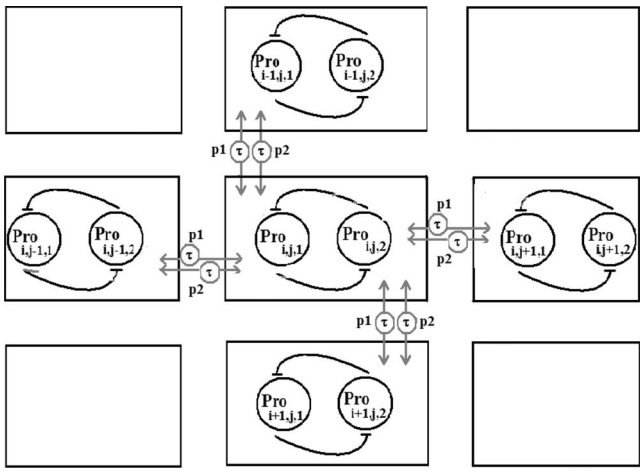

FIG. 2. A tissue modeled as a 2D square lattice of coupled of TS's. Only one cell and all its interactions with nearest neighbors are represented. Reactions allowing periodic boundary conditions are not represented.

reaction, whose delay, each time the reaction occurs, is drawn from a normal distribution with a mean of τ_{cross} and a standard deviation σ. This delay accounts for the time this event needs to be finished, *once* the reaction has been selected to occur by the SSA. Notice that the RNAp is also assigned a location in the cell. It is assumed that this molecule, like the promoters, cannot travel between cells. To define a 2D regular square lattice (such as the one partially represented in Fig. 2), the following restrictions are imposed the indexes values choice for reaction (9): $\forall i_1,i_2,j_1,j_2=1,\ldots,N$; $\forall z=1,2: z_1 \neq z_2$ and $i_1=i_2\pm 1 \wedge j_1=j_2$ or $i_1=i_2 \wedge j_1=j_2\pm 1$.

Next, the quantities used to characterize the system structure and dynamics are introduced.

D. Measure of coupling strength between cells

The coupling strength (C) between two cells intends to measure the expected fraction of time that the two cells will be interacting by the coupling reactions, during an experiment. Reaction (9) is one that directly affects C. The higher is this reaction rate constant (k_{cross}) the stronger is the coupling since, as it increases, a higher fraction of the existing proteins in the system will be, on average, moving between cells at any moment.

The rate constant of transcription-translation reaction (k_t) of the gene subject to repression also has to be considered, since this reaction competes with the repression reaction, due to the non-null delay of the promoter release after each trancription-translation reaction. Assuming that enough RNAp molecules exist, so that this molecule is not a limiting factor of transcription, the higher the rate constant of transcription-translation (or the longer is the delay on the promoter), the less time the promoter available for being repressed.

If two genes with promoter $\text{Pro}^{z1}_{i_1,j_1}$ and $\text{Pro}^{z2}_{i_2,j_2}$ are in two nearest neighbor cells, and $z_1 \neq z_2$. Given the rate constant of transcription-translation ($kt^{z1}_{i_1,j_1}$) of gene 1 of cell i_1, j_1, and $k_{\text{cross}}(c_{i_2,j_2},c_{i_1,j_1})$, the rate constant of the reaction by which proteins from cell (i_2,j_2) can go to cell (i_1,j_1), the C of (i_2,j_2) on (i_1,j_1) is defined as

$$C=\frac{k_{\text{cross}}(c_{i_2,j_2},c_{i_1,j_1})}{kt^{z1}_{i_1,j_1}}. \tag{10}$$

One can generalize this definition for a lattice with any topology, where any two nearest neighbor cells can have a unique C. Here, 2D square lattices with periodic boundary conditions are modeled such that all transcription-translation and crossing reactions are identical; thus C is the same for all pairs of nearest neighbor cells.

It should be noted that the time delays in transcription limit the gene production rate of proteins, not accounted by k_t alone. The reactions responsible for crossing also have delays not accounted for, in the formula defining C. These parameters also have be considered when characterizing the lattice structure. Nevertheless, as shown in the results section, this quantity C, as defined, can be directly related to the dynamical behaviors observed, here characterized by the tissue stability and entropy.

E. Measure of stability

To measure the ability of a GRN to "hold state" after a transient (a single state out of the possible ones), a stability measure (S) is introduced. It is here interpreted that the less a TS toggles between its two 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 be characterized, for the present purposes and under the constraints of GRNs topology, by the following relations 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 a TS in cell (i,j) that toggled $t_{i,j}$ times during that time interval, is defined by

$$S_{i,j}=\frac{\Delta t}{t_{i,j}+1}. \tag{11}$$

The dependence on Δt allows recognizing differences in S, in experiments with different durations. The only difference between S and the period of toggling is that a system that does not toggle has infinite period, while S will be equal to the total time Δt. The stability of the tissue is the average of the $S_{i,j}$ for each TS. Supposing a lattice of n^2 cells, where in a given a time interval Δt, each TS toggled $t_{1,1},\ldots t_{n,n}$ times, respectively, one has

$$S=\frac{n^2\Delta t}{\sum_{i,j=1}^{n}(t_{i,j})+1}. \tag{12}$$

Using this definition prevents having infinite S for a set of TS's when one or more TSs do not toggle. Notice that uncoupled TSs will not have null S. Each TS has an inherent stability, since it does not toggle infinitely fast.

This quantity can be used to compare the stability of single TS's within a tissue or, using Eq. (12), to compare the

average S of distinct tissues composed of TS's coupled with different strengths. Unfortunately, knowing S does not distinguish in what stable state the system is, and thus, it cannot detect transitions from one stable state to another. To do that, one must observe directly the time series of gene expression.

The measure of S, as shown in the results section, detects transitions from regimes where the TSs toggle, to regimes where they are stable, and vice versa.

Another measure must be introduced, to detect transitions between other dynamical regimes, also observed when varying C. Next, we introduce the measure used to detect synchrony between TSs within the same tissue.

F. Measure of synchrony

Using the model of a single TS previously described, for certain values of C the TSs will be toggling (see results section). The toggling is caused by stochastic fluctuations in proteins concentrations and, for that reason, the moment at which any toggling occurs is random. However, as the coupling between next near neighbors increases from null, the toggling in one TS drives its neighbors to also toggle synchronously, within the sampling frequency of the system state.

The stability measure, S, detects if any TS of the tissue is toggling and quantifies how frequent is that toggling. Thus it can be used to distinguish if a system of coupled TSs is stable (fixed in one state) or toggling. However, this measure does not detect if the TSs of the tissue are toggling synchronously or not.

It has been shown that the measure of synchrony to be applied in a given case cannot be chosen according to fixed criteria, but rather it should be chosen in each case the measure yielding the most plausible results [40]. To measure synchrony between cells the following definition is assumed: Two cells time series are synchronous at a given moment t if they are in the same state.

Synchrony is measured here by the information-theoretical entropy of the 2-tuple (p_1, p_2) among the cellular population. The state of a cell i at moment t, $St_i(t)$, is defined as follows: If $p_1 > p_2$ the cell is in state "1," otherwise it is in state "0." The state of a tissue, St^{tissue}, is defined as the set of states of all its cells. Let P_0, at a given moment t, be the fraction of cells in state 0 and P_1 the fraction of cells at state 1. Then, the entropy of the tissue state, at any given moment t, is defined as [41]

$$H_{\text{tissue}}(t) = -P_0(t)\log_2(P_0(t)) - P_1(t)\log_2(P_1(t)). \quad (13)$$

Given the definition in Eq. (13), $H_{\text{tissue}}(t)$ is null if all cells are, at t, in the same state. The maximum $H_{\text{tissue}}(t)$ is equal to 1 and occurs when 50% of cells are in one state and the other 50% are in the opposite state.

Finally, the "tissue entropy" of one simulation is defined as the average entropy of all time moments:

$$H_{\text{tissue}} = \frac{\sum_{i=1}^{i=T} H_{\text{tissue}}(i)}{T}. \quad (14)$$

When computing the H of the time series of a given simulation, the initial transient is not discarded since its duration varies from simulation to simulation, and because for some values of C this transient is extremely long (and infinitely long for $C > 5$) and, thus, should be considered. Notice that this measure is not able to distinguish if the tissue is stable or fully synchronous (this distinction is done by observing the value of S).

III. RESULTS

The stability and synchronization of cells within a tissue are studied as a function of C between nearest neighbor cells. The tissue is modeled as a 2D square lattice structure with periodic boundary conditions and described by reactions (5)–(9).

In all cases, the following parameters values are used unless stated otherwise. At the beginning of each independent simulation, all proteins are initialized at 0 and all promoters are free to express. The RNAp's are assigned a location in the lattice and cannot travel between cells. The number of RNAp's is 50 per gene (thus, 100 for the two genes in each cell), in agreement with experimental observations [39]. Consequently, given the delays in the transcription-translation reaction, the average number of available RNAp's is ~30 per gene, thus not limiting transcription due to depletion. The volume of each cell is assumed to be equal to 1.

The delays in the transcription-translation reactions are set at $\tau^1 = 1$ s, $\tau^2 = 20$ s, and $\tau^3 = 100$ s. The delay in reaction (9) is randomly generated each time the reaction is chosen to occur, and follows a gaussian distribution of mean 10 s and standard deviation 5 s. The reactions rate constants are equal for all cells and set at $k_t = 0.1\ \text{s}^{-1}$, $k_d = k_{dp} = 0.001\ \text{s}^{-1}$, $k_u = 0.001\ \text{s}^{-1}$, and $k_c = 0.1\ \text{s}^{-1}$.

Only k_{cross} or k_t are varied to attain a desired C value. For each value of C, 100 independent experiments are made, with each experiment lasting $\Delta t = 10^7$ s. The system state is sampled uniformly each 50 s. The rate constants are in s^{-1} units since they are stochastic rate constants, i.e., frequencies independent of the concentrations [16]. A detailed justification of the values chosen for the rate constants and delays (except those related to diffusion reactions) can be found in Ref. [21].

In this section, figures of gene expression time series result from single runs and are shown as examples. In the graphs of H and S vs C, the average results of 100 independent experiments, for each data point, are shown.

A. Coupling two cells

A system of two coupled cells is modeled, driven by reactions 5 to 9, to observe in detail the effects of varying C in the dynamics. First, k_{cross} is varied while $k_t = 0.1\ \text{s}^{-1}$ and kept constant, to show that, the higher k_{cross} is, the more stable the system becomes. Next, the system dynamics is observed for the same C values as in the first case, but C is varied by decreasing k_t, for constant k_{cross}.

As the results show (Fig. 3), the two methods of varying C have an equivalent effect on S, for each C value.

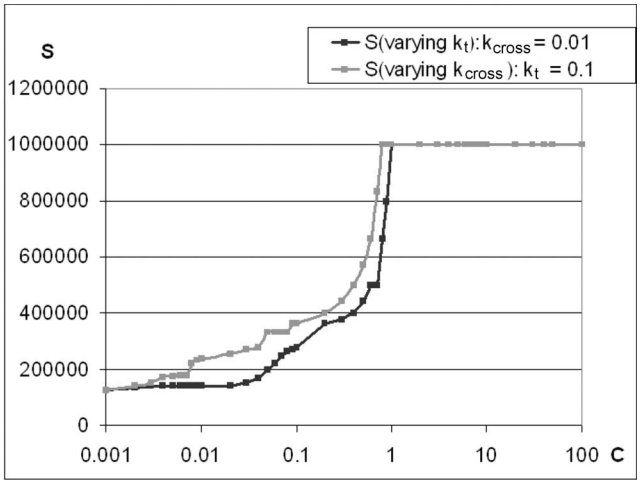

FIG. 3. Average stability S vs coupling strength C (in log scale). C is varied by varying k_{cross} with k_t fixed and vice versa. Each data point is an average of 100 experiments. A phase transition is observed in S as C increases, corresponding to the system dynamics changing from synchronous oscillations to stable.

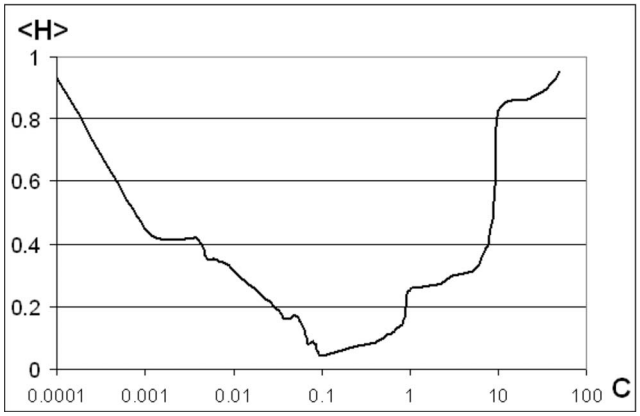

FIG. 4. Average H vs C (in log scale). C is varied by varying k_{cross} with k_t fixed. Each data point is an average of 100 experiments. H is minimized in the dynamical regimes of synchronization and stability, and the transitions from asynchronous to synchronous and from stable to infinite transient are easily detected from correspondent variations in H.

As seen in Fig. 3, the two curves are similar, validating the formula to compute C. Yet, one needs a slightly larger C when varying k_t to obtain the same results as when varying k_{cross}. The small difference is due to the delays in the transcription-translation reaction. While substances, such as RNAp and promoters, are on the waitlist, they do not contribute to reactions propensity. The formula for C assumes instantaneous reactions and thus, the more and larger delays this reaction has, the less accurate is the dynamics predictability by knowing the C value.

The system exhibits the following dynamical behaviors as C increases: (i) for $0<C<10^{-3}$ the cells toggle asynchronously; (ii) for $10^{-3}<C<0.5$ the two TSs toggle synchronously; (iii) for $0.5<C<5$ the two cells are stable in the same stable state; (iv) and for $C>5$ all genes of both cells are expressed (a state not attainable by a single TS without coupling to other TS).

Figure 4 shows, as C varies, the average H between the two cells. The value of H exhibits a good agreement with the mentioned dynamical regimes. Namely, it confirms the transitions between asynchronous to synchronous (seen by the significant decrease of H), and from the stable state to the "infinite transient" state (seen by the significant increase of H). For the C values at which the system is either synchronous or stable, H is very low (H is never absolutely null due to the initial transient). For the C values at which the system is asynchronous or when all genes are expressed, H is high as expected. The first case is due to the asynchrony between cells, while in the second case H is high due to all proteins being expressed more or less equally (and which is higher is always changing) never settling in one of the two states.

In transition regions between behaviors, the result varies for independent runs. For example, if $C=0.5$ one can observe in one experiment a stable state, while in the next a single synchronous toggling. Continuing the example, although in most experiments with $C=0.6$ one observes a stable behavior, in a few experiments one still observes one toggle, while for $C=0.8$ all experiments showed completely stable behavior. Thus the system behavior for a given C value is here characterized based on the most commonly observed behavior.

The phase transition in both cells dynamics from synchronous oscillations to stable states as C increases is visible in Fig. 3, at $C\sim 0.5$. Not visible is the transition at $C\geqslant 5$, from stable with one gene per cell expressing, to the stable with both genes of both cells expressing.

The transient in the regimes of toggling behavior or stable states where only one gene of each cell is expressing (either both genes "1" or both genes "2" of each TS) is independent of the C value (for $C<1$) and has a mean of $\sim$10 000 s with a standard deviation of $\sim$5000 s.

For $C>1$ the transient increases rapidly with C increase and, for $C\geqslant 5$, becomes infinite. By infinite one means that in the 100 experiments for each C value, which for $C\geqslant 5$ it ran for 10^8 s, the system did not leave the state with all genes expressing.

This is caused by the diffusion reaction which is now very fast when compared to any other reaction rate of occurrence, due to its high rate constant, and especially in comparison with k_t. Therefore, most proteins are diffusing at any given moment. Since this reaction is time delayed, while diffusing between cells, proteins do not affect cells states.

The stochastic fluctuations, the mechanism by which differences between the two proteins quantities of a TS are created, are not sufficiently fast when compared to the rate of diffusion for this value of C. Small fluctuations in any of the cells are first "passed on" into the waitlist (here representing the inter cellular media). Since fluctuations equally likely increase either p_1 or p_2, on average, fluctuations will compensate one another and the amounts of p_1 and p_2 remain approximately equal in the waitlist.

Thus none of the genes in any cell is able to decisively "overcome" the other in protein concentration, since when a small difference between p_1 and p_2 quantities arises in a cell, it is "dissipated" by the fast diffusion to neighbor cells,

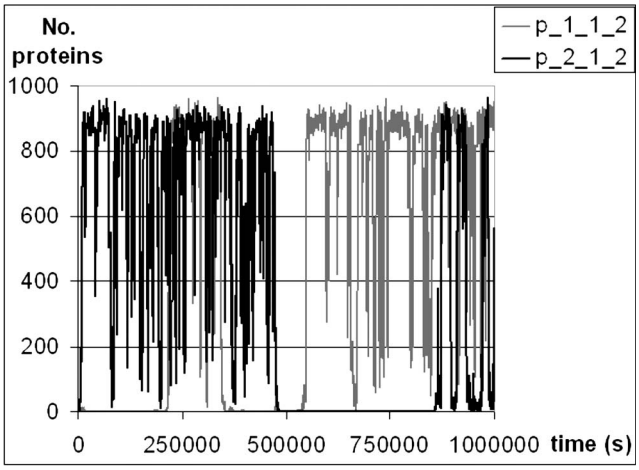

FIG. 5. Two coupled cells: $C=10^{-4}$. The two cells GRNs toggle asynchronously. Only the time series of p_2 of both cells are plotted, for easier visualization [$p\_1\_1\_2$ is p_2 of cell (1,1) and $p\_2\_1\_2$ is p_2 of cell (2,1)]. Notice, e.g., for $\sim 500\,000$ s $< t < \sim 900\,000$ s the two cells GRNs are in opposite states.

which works towards spreading evenly any differential in proteins quantities by the two cells.

Examples are shown of the dynamical regimes described, in plots of the proteins time series. The notation used in the legends is "Pi,j,z" such that (i,j) is the cell location in the lattice and z is either 1 or 2, depending on which of the two proteins, p_1 or p_2, is representing.

In Fig. 5, the time series of proteins p_2 of the two cells (1,1) and (2,1) are plotted. The rate constant k_{cross} is set so that $C=10^{-4}$, given $k_t=0.1$ s^{-1}. Accordingly, the two TS's toggle asynchronously. The speed at which the two cells exchange proteins is not sufficient to affect each other's states significantly.

In Fig. 6 a time series is plotted of proteins p_2 of the two cells (1,1) and (2,1), with $C=3\times 10^{-3}$. The two cells proteins

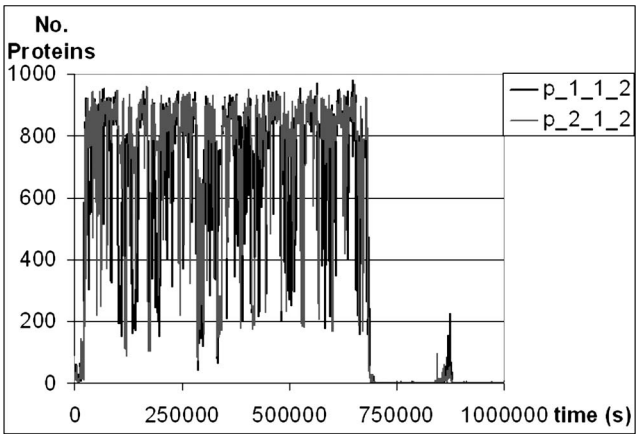

FIG. 6. Two coupled cells: $C=0.003$. The two cells GRNs toggle synchronously (almost identical time series), but less frequently then when not coupled. Only the time series of proteins p_2 of both cells are plotted for easier visualization [$p\_1\_1\_2$ is p_2 of cell (1,1) and $p\_2\_1\_2$ is p_2 of cell (2,1)]. Notice the synchronized toggling at $\sim 700\,000$ s of both cells from the state where $p_2 \gg p_1$ to the state $p_1 \gg p_2$.

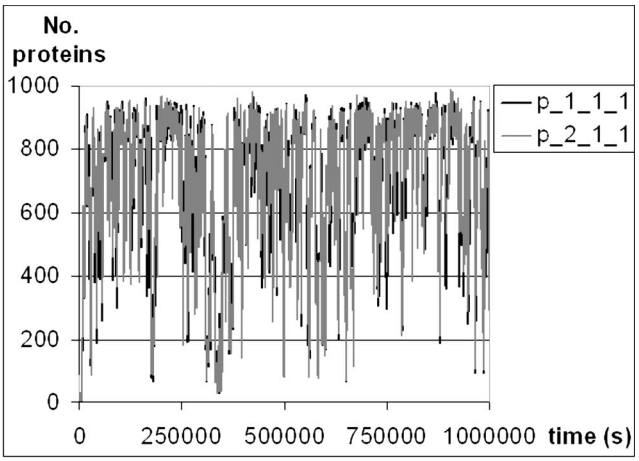

FIG. 7. Two coupled cells: $C=0.6$. Only the time series of proteins p_1 of both cells are plotted, for easier visualization [$p\_1\_1\_1$ is p_1 of cell (1,1) and $p\_2\_1\_1$ is p_1 of cell (2,1)]. The two cells GRNs attain a state that, due to the speed of proteins exchange between them, becomes stable. Once $p_1 \gg p_2$ in both cells, no toggling is observed.

time series are synchronized. The coupling reactions between them occur at a relative speed such that it gives enough time to any of the two TSs, to stochastically "decide" for one of the two possible states and, once that decision is made and "stabilized," propagate to the other cell, forcing it to adopt the same state. Since the change of state in one cell must be sufficiently strong to also impose a state change in the other cell, toggling is much less frequent than in uncoupled TSs.

Figure 7 shows the time series of proteins p_1 of the two cells, (1,1) and (2,1), of a single experiment with $C=0.6$. The coupling reactions between the two cells occur at a rate such that the two TSs, once stochastically "deciding" to settle in one of the two possible stable states, no longer can switch to the other state. The coupling is strong enough (i.e., occurs at high frequency) so that no TSs can stochastically toggle sufficiently fast enough to "escape" the coupling, and so the system remains stably in the same state, after the initial transient.

In Fig. 8 it is plotted, from one experiment, the time series of proteins p_1 and p_2 of the two cells, (1,1) and (2,1), with $C=10$. According to the SSA formulation, the system dynamics is driven towards a state of local maximum entropy (homogenous distribution of proteins by the two compartments in this case). The speed at which it does so is determined by the rate constants. In this case, the reactions responsible for diffusion between the two cells have such a high rate constant that the system never reaches a state where one protein overcomes the other. Most proteins will be on the waitlist, moving from one cell to other, and the two cells will have always almost the same amount of proteins p_1 and p_2 for an indefinite amount of time, for reasons previously described.

In the previous examples, with lower C, stochastic fluctuations created differences between the two proteins concentrations in one of the cells. Such differences were sufficient to allow one of the genes to become repressed and the other "active." The differential between the two proteins pro-

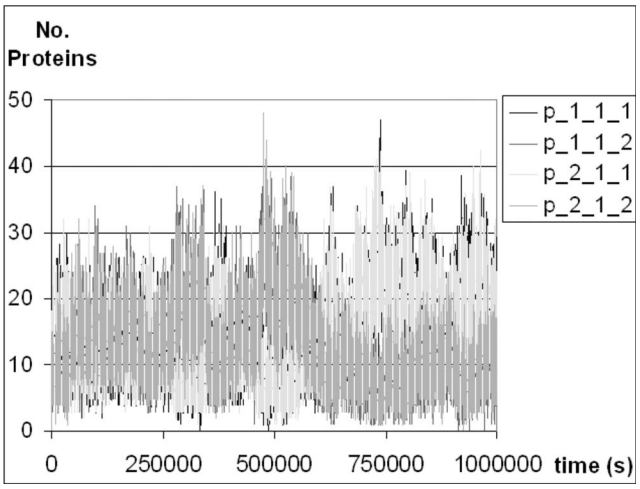

FIG. 8. Two coupled cells: $C=10$. Proteins p_1 and p_2 time series are plotted. The two cells exchange proteins so fast toward homogenous distribution by the two compartments that a stochastically created difference in proteins quantities in one cell is rapidly "dissipated" to the other cell. The system remains in a stable "infinite transient" where all genes of both cells express, and never settles in one of the stable states possible for lower C values. On average, 2000 proteins are on the waitlist, diffusing at each moment.

duction in that cell would then propagate to the neighbor cell and impose the same state there.

In the case of Fig. 8, the relation between the time it takes for one cell to attain one of the two states ($p_1 \gg p_2$ or vice versa) and the time it takes for diffusion to neighbor cells to occur "inverts" comparing to the previous cases. Diffusion is now faster than the time it takes for stochastic fluctuations to impose one of the two states. The two cells exchange proteins so fast that any small difference between the two proteins quantities in one cell is rapidly "dissipated" to the other cell. The fluctuations in the two cells will be in both possible directions (p_1 larger 50% of the times and p_2 larger in the other 50% of the times) and thus, compensate each other. This leads to a stable "infinite transient," where all genes of both cells express and, for that reason, the system never settles in one of the stable states possible for lower C values. In this case it was observed on average 2000 proteins (p_1 and p_2) in the waiting list, diffusing at each moment, meaning that there are as many proteins in the system at any moment as for smaller C values, and they are only not "visible" in the time series plots because the majority is on the waitlist at all times. For this reason, genes states are considered "on," in this state.

B. 5×5 square lattice cells tissue with periodic boundary conditions

In this section, the results for a 5×5 square lattice with periodic boundary conditions are presented. Each cell has four nearest neighbors with whom exchange of proteins can occur.

The system has the following dynamical behaviors as C increased: (i) For $0<C<10^{-7}$ the cells toggle asynchronously; (ii) for $10^{-7}<C<10^{-6}$ nearest neighbor cells synchronize resulting in synchronization by regions of space; (iii) for $10^{-6}<C<10^{-5}$ all cells of the tissue toggle synchronously; (iv) for $10^{-5}<C<10^{-3}$ all cells becomes stable in the same stable state; (v) for $10^{-3}<C<10^{-2}$ all cells becomes stable, but only after a long transient whose duration increases with C increase; (vi) for $C>0.01$, all genes of all cells express, corresponding to an infinitely long transient.

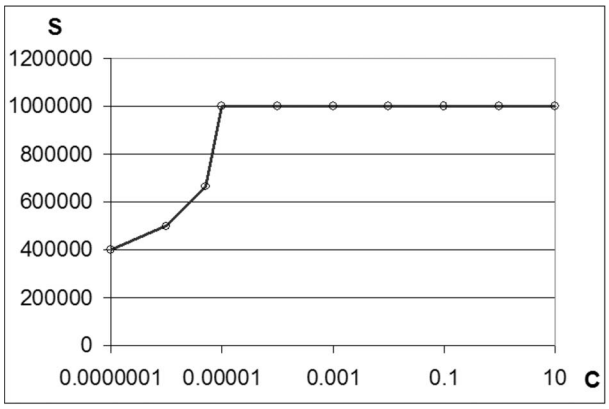

FIG. 9. S vs C (linear-log plot) of a 5×5 square lattice with periodic boundary conditions. S is the average stability of all cells and for 100 experiments per data point.

Figure 9 shows the measured stability S, averaged over all cells and 100 independent experiments per data point. S goes through a phase transition, from synchronized toggling to stable, at $C\sim5.10^{-6}$. Note that C is the C value between any two cells of the lattice. Since each cell has four nearest neighbors, the phase transition occurs for smaller C values than in the previous case (previously each cell had only one neighbor), shown in Fig. 2.

Figure 10 shows the average H of the tissue, as C varies. H varies according to the transitions from asynchronous to synchronous, and from stable state to infinite transient state (similar to those observed for the two cells' system). As before, H is very low when the cells are synchronous or stable.

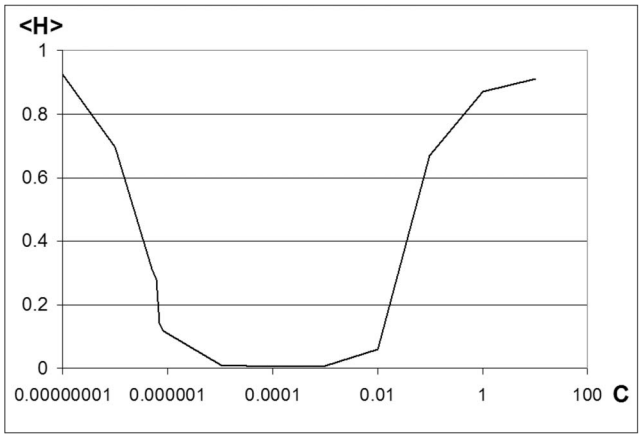

FIG. 10. Average H vs C (lin-log scale). C is varied by varying k_{cross} with k_t fixed. Each data point is an average of 100 experiments. H is smaller in the dynamical regimes of synchronization and stability. Transitions from asynchronous to synchronous and from stable to infinite transient are the cause of the large variations of H.

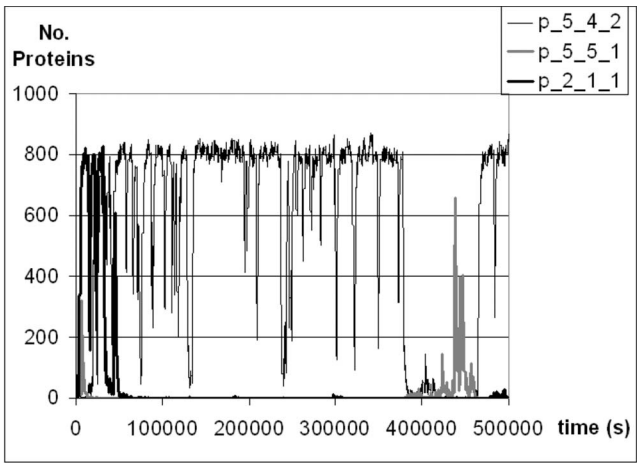

FIG. 11. Example of synchronization by regions in space, $C=8\times10^{-7}$, on a 5×5 square lattice. Time series of one protein of three cells. p_2 in cell (1,1) becomes "on" much before the other two proteins levels represented. The time series of these two other proteins [p_1 in (5,5) and p_2 in (5,4)], of nearest neighbor cells, are synchronous. Around 400 000 s, p_2 in cell (5,4) toggles to null and its nearest neighbor [p_1 in (5,5)] toggles accordingly to "on," but p_1 of cell (2,1) does not change in agreement, due to its distance in the lattice from the other two cells.

Notice that the values of H are lower, for the same C values, than in the two cells case. Synchronization and stability are "stronger" in the 5×5 lattice, since each cell has 4 neighbors forcing these states on each cell. H is never null, due to the initial transient.

Figure 11 show the system time series for a case where synchronization by regions occurred, for $C=8\times10^{-7}$. From the time series of one of the proteins within three cells of the lattice, it is visible that only two of those protein levels of different cells are synchronized. Namely, the time series of proteins p_2 of cells (5,4) and p_1 of (5,5), which are nearest neighbors, show that these two cells toggle synchronously. On the other hand, the time series of p_1 in cell (2,1) shows that this cell GRN is not toggling synchronously with the previous two. Yet, it is synchronized with some of its nearest neighbors (not show). This regime where synchronization by regions of space occurs is highly "unstable," i.e., occurs for a very small range of C values, and only in some experiments, due to the small size of the lattice.

Figure 12 shows a time series of proteins p_1 of cells (1,1) and (1,2), as examples, when coupling the cells strongly ($C=0.01$). All proteins p_1 on the lattice have similar time series and all p_2 levels are almost null most of the time, after the initial transient. The strong coupling makes toggling impossible for any of the cells, once a stable state is attained. Any stochastic fluctuation of the proteins concentration in one of the cells of the tissue, that could lead to such toggling, is "counterbalanced" by the neighbor cells (through diffusion of proteins between cells) faster than the necessary time to make its two genes toggle. All the cells are "acting" towards stabilizing all other cells in the same state. The long transient of ~150 000 s duration is dependent on C. It increases as C increases (within the interval $10^{-3}<C<10^{-2}$). Notice that a single TS cannot attain this stable state (with the model of TS here used). This dynamical regime emerges from the coupling between two or more TS's.

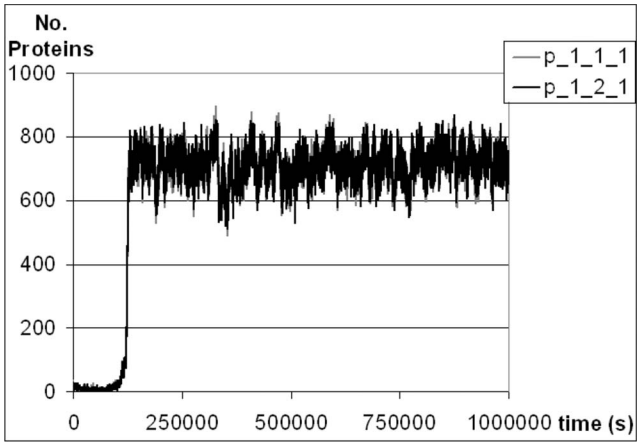

FIG. 12. Example, for $C=0.01$ s^{-1} on the 5×5 lattice, of a long synchronized transient until reaching one of two possible stable states. Proteins p_1 of cells (1,1) and (1,2) are shown as example. All cells behave similarly. The two time series are almost identical. The TS's do not toggle due to the strong interactions between cells.

Figure 13 shows the time series of both proteins (p_1 and p_2) of cells (5,5) and (3,3). In this case, $C=0.1$. The dynamics in all cells of the lattice is similar to these two cells, shown as examples. All proteins are being produced and no protein level becomes much higher than the other in any cell. Because C is so high, since the tissue has symmetric crossing reactions between any two cells and, since the SSA dynamics drives the system to a local maximum of entropy, the proteins are being constantly spread evenly through all cells (as in the example in Fig. 8). If in one cell one of the proteins, due to stochastic fluctuations, starts to overcome the other in quantity, the lack of balance on that cell is spread by distributing the proteins evenly trough all the cells in the tissue.

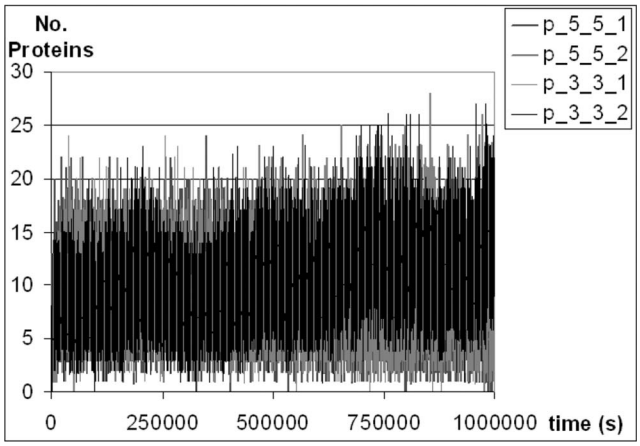

FIG. 13. Example, on a 5×5 square lattice, $C=0.1$ s^{-1}, of a stable state with both genes of each cell expressing. The infinite transient emerges from the delayed coupling reactions and is not a possible stable state for single TSs. Proteins p_1 and p_2 from cells (5,5) and (3,3) are shown as example. All proteins have similar time series. On average, ~25 000 proteins are on the waitlist (~500 proteins of each kind), diffusing at each moment.

Future fluctuations in the opposite direction compensate the small differentials and force, as well, a system state where both proteins exist in the same quantity.

As previously noted, while on the waitlist diffusing between cells, these proteins cannot cause gene repression, while stochastic fluctuations, which can occur equally likely towards increasing p_1 and p_2 quantities, do not create a sufficiently high difference between these two proteins concentrations to decisively settle into one the the two stable states. Therefore, the dynamics observed in the initial transient in the previous cases, i.e., all genes simultaneously expressing is, for $C>0.01$, kept indefinitely in the 5×5 square lattice. This dynamical behavior is not possible for a single uncoupled TS [36].

In the previous example (Fig. 12), exchange of proteins between cells is not fast enough to avoid the appearance of differences in the two proteins concentrations in some cells, caused by small fluctuations, that eventually result in reaching one stable stable. Once reached, the flux is sufficiently fast to sustain that state indefinitely (since change of state requires a "long lasting" fluctuation).

It is noted that, in Figs. 7 and 12, genes considered "on" have proteins' levels of ~800. In Fig. 13 such amount of proteins is not visible. The reason why all genes are also considered in a "on" state is the same mentioned for the case in Fig. 8. Most proteins are moving between cells at any given moment and, thus, are on the waitlist, since the reaction responsible for proteins diffusion between cells is a delayed reaction, not showing on the time series. For the specific case of the simulation shown in Fig. 13, after an initial transient of around ~5000 s, at any moment of the simulation, on average, there are ~25 000 proteins on the waitlist (~500 proteins of each kind are present in the waitlist).

Another simulation was made (data not shown) setting $C=0.1$, but with different initial concentrations, i.e., p_1 of cell (1,1) was initiated with non-null quantities (all other proteins are null at $t=0$ s). If $p_1(t=0)<1000$, the behavior does not change in comparison to starting with null quantity of proteins. However, starting with $p_1>1000$ in any given cell, these are immediately spread through all cells and, since this quantity is sufficient to have a relevant differential between p_1 and p_2 in all 25 cells, the system remains stable in the state where all cells only express p_1, with p_2 kept indefinitely repressed.

Finally, it was tested if increasing the number of cells causes changes in the observed dynamics. A 10×10 square lattice with periodic boundary conditions was simulated (data not shown). Aside from the fact that the behavior "synchronization by regions of space" becomes more easily attainable within a larger range of values of C than in the 5×5 tissue, no significant change was observed, compared to what has been described.

C. Comparing the dynamics with a delayed deterministic model

A study on the dynamics of TSs [38] showed that, without cooperative binding, a TS can only toggle if there are self-activation reactions for each gene and, importantly, if the system is inherently stochastic. Additionally, it was shown that ODE models could not reproduce the results obtained by stochastic methods, even in the regime of high proteins concentration [38]. The model used did not include time delays related to transcription-translation.

A subsequent work [36] showed, using a stochastic framework, that introducing realistic time delays in transcription-translation reactions, for proteins and promoters release is sufficient for the TS to toggle for a wide range of parameters values, without self activation and dimmerization reactions.

In this section, a ODE model of coupled TSs with time delays in gene expression is simulated with dde23 function in MatLab [42], to allow a direct comparison of deterministic and stochastic simulations. While the importance of time delays has been established, the comparison between delayed ODE and delayed SSA models allows determining the importance of noise in the dynamics.

The model of TS used is similar to the one used in Ref. [38]. The only addition to the latter is the introduction of delays in gene expression, shown to be non negligible in the dynamics of TSs [36]. The delayed ODE model of two coupled TSs can be described as follows [Eqs. (15)–(18)]:

$$\frac{dp_1(t)}{dt} = \frac{g}{1 + kp_2(t-\tau_1) + k_{\text{cross}}p_4(t-\tau_2)} - dp_1(t), \quad (15)$$

$$\frac{dp_2(t)}{dt} = \frac{g}{1 + kp_1(t-\tau_1) + k_{\text{cross}}p_3(t-\tau_2)} - dp_2(t), \quad (16)$$

$$\frac{dp_3(t)}{dt} = \frac{g}{1 + kp_4(t-\tau_1) + k_{\text{cross}}p_2(t-\tau_2)} - dp_3(t), \quad (17)$$

$$\frac{dp_4(t)}{dt} = \frac{g}{1 + kp_3(t-\tau_1) + k_{\text{cross}}p_1(t-\tau_2)} - dp_4(t). \quad (18)$$

The parameters are set to the values used in the delayed SSA model: $g=0.1\ \text{s}^{-1}$, $d=0.001\ \text{s}^{-1}$, $k=0.01\ \text{s}^{-1}$, and $k_{\text{cross}}=0\ \text{s}^{-1}$. Also, $\tau_1=100$ s, $\tau_2=\tau_1+10$ s (this delay includes gene expression delay and diffusion delay), and RNAp $=200$.

First, setting RNAp$=100$, a single TS time series was observed. Independently of the proteins initial quantities [including $p_1(t=0)\neq p_2(t=0)$], the system goes to a steady state ($p_1=p_2\sim1000$). This value corresponds approximately to the protein level of the gene "on" in the delayed SSA version, however the dynamics of the TS is completely different in the two simulation methods (toggling in the delayed SSA, stable in the delayed ODE for these parameters values). Thus, in agreement with previous results [38], without stochasticity the system is unable to have a switching behavior even considering the time delay in transcription-translation. In Fig. 14, a time series of a TS is shown as example.

Next, a delayed ODE model of two coupled TSs was simulated, with $k_{\text{cross}}=0.01$ and 200 RNAp's. In the delayed SSA, the two TSs would toggle synchronously since $C=0.1$. Shown in Fig. 15, the result is a stable state for both TSs, with all proteins equally expressed.

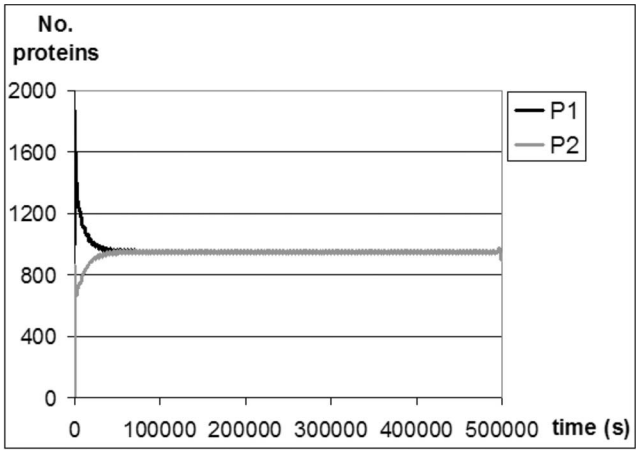

FIG. 14. Time series of p_1 and p_2 of a single TS (delayed ODE model). At $t=0$ s, $p_1=1000$ and $p_2=0$.

The model was simulated for all values of C previously tested (data not shown). For all C values, the result is always a stable state, similar to the one observed in Fig. 15. Once the two proteins quantities match, they can never separate again, since the two are equally likely to be expressed and repressed, thus the system remains in this unstable attractor. This shows that it is the noise in the dynamics that creates differences between p_1 and p_2 quantities that generate the toggling behavior [38].

Finally, we tested if adding noise terms to the delayed ODE's would allow reproducing the results obtained by the delayed SSA. A Gaussian noise term with a mean of 0 and standard deviation of 1 was added to the delayed ODE model of two coupled TSs. The result is shown in Fig. 16 and does not match in any way the results of the delayed SSA model. Adding a noise term creates temporary fluctuations away from the equilibrium point, but it does not generate a toggling behavior.

These results first confirm that without inherent stochasticity in the dynamics, the TS will not toggle [38]. Also, it is shown that introducing delays in the ODE model is not sufficient to reproduce the results attained in the delayed SSA model. Additionally adding white noise terms is also not sufficient [38]. The results stress the necessity of modelling GRNs dynamics using the delayed SSA to be able to, at the same time, correctly account for the noise in the dynamics and to be able to introduce time delays in reactions whose time duration is non negligible.

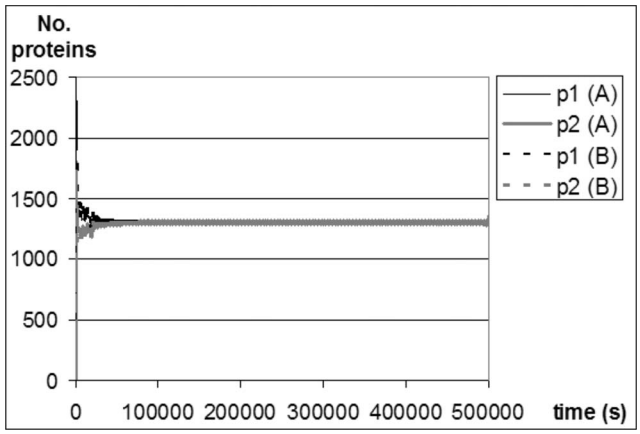

FIG. 15. Time series of proteins p_1 and p_2 of two coupled TSs (A and B), with $C=0.1$ (delayed ODE model). Initially, $p_1(A)=100$, $p_2(A)=0$, $p_1(B)=500$, and $p_2(B)=0$.

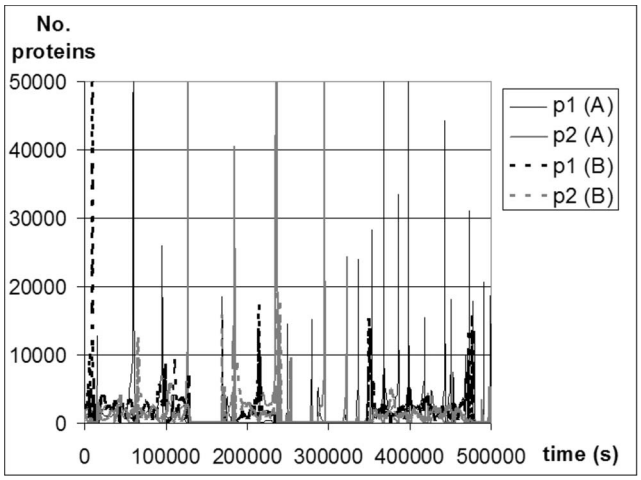

FIG. 16. Time series of p_1 and p_2 of two coupled TSs (A and B), with $C=0.1$ (noisy delayed ODE model). Initially, $p_1(A)=100$, $p_2(A)=0$, $p_1(B)=500$, and $p_2(B)=0$.

IV. CONCLUSIONS

Cell differentiation is a process hypothesized to be driven by small subcircuits of GRNs, which are, at least, bistable [29]. Following this, it is natural to assume that, once deciding into one of its possible final states, the circuit should remain stable, so that the cell becomes unable to reverse the differentiation process.

Recently, it was shown that gene expression and GRNs dynamics are inherently stochastic, and that time delays need to be accounted for, since transcription and translation take non negligible time duration [21].

Because GRNs dynamics are stochastic we asked what mechanisms can make a GRN remain "stable," once a differentiation pathway choice is made. Differentiation pathways choices are known to depend on the cells' spacial location within an organism. In some cases this is due to external gradients, while in other cases it could be due to interactions between neighbor cells. Here, only the second mechanism was investigated.

Modeling a tissue of cells, as a 2D square lattice with periodic boundary conditions, where cells can exchange products of gene expression with nearest neighbor cells, it was investigated if cells, which individually cannot hold state, might become stable or synchronized, due to the interactions. In each cell, the GRN consists of a single a TS, a circuit shown to be used in some cases by cells to choose between two possible differentiation pathways, acting as the "decision circuit" of differentiation [31,33].

Although this GRN is rather simple, with only two genes per cell, and most real genetic subcircuits controlling differentiation may involve many genes, several examples also

exist of differentiation controlled by a very small number of genes. For example, it was recently reported that the Drosophila dioxin receptor Spineless (SS) is both necessary and sufficient for the formation of the ommatidial mosaic [34], responsible for the fly color vision. The creation of the retinal mosaic is driven by the expression level of a single gene (spineless) during a specific stage of development. The stochastic expression of SS acts as a binary switch determining the cell's fate (R7 or R8 cell type), and is an example of cells' fate determined by a single stochastic variable [34,35].

This case, besides being an example of differentiation driven by a bistable GRN, shows how differentiation can, in some cases, be stochastically driven (and thus, should be modeled by the delayed SSA), as the model here studied. Similar examples have recently been reported [11].

Because the TS can stochastically toggle between two states, if those states correspond to different cells types, then single cells whose differentiation is based on such mechanism could, in some cases, exhibit transient and probabilistic differentiation, which has been recently observed [11]. However, in most cases, differentiation is an irreversible process in normal conditions and therefore, assuming the TS as a possible "basic" circuit for differentiation, it was studied here if this GRN could gain stability by interacting with neighbor cells. Additionally, it is also studied if this GRN can be used as well to synchronize cell's GRNs dynamics.

As the coupling strength, which controls the speed at which interactions between neighbor cells occur, was increased several, several observations were made.

First, it was shown that having exchange of proteins between nearest neighbors at a slow rate, when compared to the rate at which gene expression occurs, the cells' proteins time series synchronize. Biologically, the ability of cells within a tissue to synchronize their dynamics is known to be important to perform some functions. It was also observed that the toggling frequency diminished in comparison to the observed toggling frequency of individual TSs, and that the fluctuations in proteins concentrations, caused by the stochastic nature of the underlying dynamics, were far smaller than for uncoupled cells. Therefore, coupling between cells can also useful to reduce noise in GRNs dynamics. Importantly, the rate of proteins interchanged can tune the frequency at which the TSs toggle. Importantly, this shows how inherently stochastic GRNs can synchronize their dynamics via interchange of proteins (or via signals activated by these proteins), which allows cells GRNs within a tissue to carry out coordinated tasks.

Another interesting observation made is that, given the proper coupling strength, cells can synchronize and stabilize by regions of the space. This case shows that the coupling mechanism is able to create tissues with cells differentiated into different stable states (as in the experimentally observed case of the ommatidial mosaic of *Drosophila*) and spatially organized, starting with identical cells in the same initial state (importantly, this was achieved without imposing any external gradient).

As cells are coupled more strongly, a stable state emerges, where all cells GRNs stabilize in the same state. The interchange of proteins between cells creates a positive feedback mechanism (where cell A enhances cell B to remain in the same state as A is, and vice versa), between all pairs of cells in the lattice from which a robust stable state emerges in all cells, not observable in single cells. The normally distributed time delayed interchange of proteins between cells (a simplified model of interchange of signals between cells) is sufficient to provide the cells within a tissue the necessary stability, so that, for example, once they commit to differentiate into one of the two stable states, they indefinitely remain in that state, although each cell GRN dynamics is highly stochastic. Thus coupling can be used to obtain uniform stable differentiation in stochastic GRNs.

Finally, it was observed that the cells can be kept in an "undifferentiated state" for as long as desired. For that, one needs to impose (relatively) very strong coupling. In this condition, cells interchange proteins so fast that stochastic fluctuations are not fast enough to accumulate a difference between the quantities of the two proteins. This mechanism could be used to delay cells differentiation. Decreasing the coupling strength at a desired moment, would allow a stable state to emerge.

Although computationally expensive, the use of detailed models provides a better understanding of how GRNs of cells in a tissue interact, and what behaviors emerge from these interactions, providing clues to how to intervene to obtain desired behaviors.

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