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Abstracts

A DYNAMICAL MODEL OF CELL DIFFERENTIATION

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ABSTRACT

One of the major challenges in complex systems biology is that of providing a general theoretical framework to describe the phenomena involved in cell differentiation, i.e. the process whereby stem cells, which can develop into different types, become progressively more specialized. The aim of this work is that of describing a dynamical model of cell differentiation which is able to cover a broad spectrum of experimentally observed phenomena.

1. INTRODUCTION

Our aim is that of proposing a dynamical model of cell differentiation, i.e. the process whereby stem cells, which can develop into different types, become more and more specialized. The model is an abstract one (it does not refer to a specific organism or cell type) and it aims at reproducing the most relevant features of the process: (*i*) different degrees of differentiation, that span from totipotent stem cells to fully differentiated cells; (*ii*) stochastic differentiation, where populations of identical multipotent cells stochastically generate different cell types; (*iii*) deterministic differentiation, where signals trigger the progress of multipotent cells into more differentiated types, in well defined lineages; (*iv*) limited reversibility: differentiation is almost always irreversible, but there are limited exceptions under the action of appropriate signals; (*v*) induced pluripotency: fully differentiated cells can come back to a pluripotent state by modifying the expression of some genes and (*vi*) induced change of cell type: modification of the expression of few genes can directly convert one differentiated cell type into another.

This work is a part of a series of articles [2][12][22] aiming to develop a single model able to describe all these phenomena, whereas till now specialized model of some specific processes have been proposed. Typically these models make use of continuous descriptions and take into account the contributions of only few genes [19][21][8].

Here we hypothesize that the differentiation process is rather an emerging property due to the interactions of very many genes: its main features therefore should be shared by a variety of different organisms. To check this hypothesis we make use of a noisy version of a well-know model of gene networks, that is, the Random Boolean Network (RBN) model. RBNs in fact, in spite of their discrete approach, have been proven to describe important experimental facts concerning gene expression [3][4][5], allowing at the same time simulations of large networks [5]. We find that the introduction of noise in this framework (noisy RBN, or briefly NRBN) [1][2] allows one to effectively describe all the just listed issues.

In this work we present the main features of the model (section 2), and we will focus on some stochastic (section 3) and deterministic (section 4) aspects; finally we derive some conclusions (section 5).

2. THE MODEL

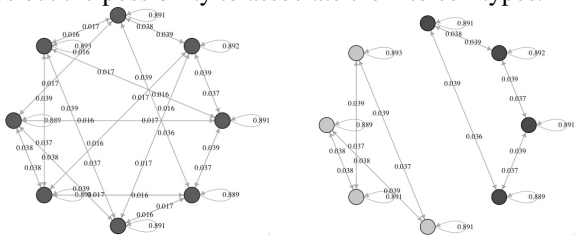
2.1. Noisy random Boolean network

A classical RBN is a dynamical system, based on a directed graph with N nodes (genes), which can assume binary values 0 or 1 (inactive/active); time is discrete, with synchronous updating of all the node values. Each node has exactly k input connections chosen randomly with uniform probability among the remaining N-1 nodes. To each node a Boolean function is associated, which determines its value at time t from the values of its inputs at the previous time step. The Boolean functions are chosen at random for every node, by assigning to each set of input values the outcome 1 with probability $\boldsymbol{p}$. Within the *quenched* strategy, both the topology and the Boolean function associated to each node do not change in time. We concentrate our study on so-called critical networks with $\boldsymbol{k}$=2 and $\boldsymbol{p}$=1/2 [6].

The network dynamics is discrete and synchronous, so fixed points and cycles are the only possible asymptotic states in finite networks; typically a single RBN owns more than one attractors. Note nevertheless that attractors of RBNs are unstable with respect to noise even at low levels, as for example transient flips of randomly chosen nodes. In fact, even if the flips last for a single time step one sometimes observes transitions from that attractor to another one. Therefore, by flipping all the states belonging to the attractors of a RBN, it is possible to create a complete map of the transitions among the RBNs' attractors (the attractors' landscape shown in Figure 1a)[1]. In these conditions, and because noise is known to play a role in key cellular processes[7][12], single attractors can no longer be associated to cell types, as proposed in the past[10][11]. Ribeiro and Kauffman [1]

[1] We assume that the noise level is small enough to allow the system to relax to an attractor before a new flip occurs; also, we are not considering multiple flips [1]

observed that it is possible to identify in the attractors' landscape subsets of attractors, which they called Ergodic Sets, which entrap the system in the long time limit, so the system continues to jump between attractors which belong to the set. Unfortunately it turns out that most NRBNs have just one such set: this observation rules out the possibility to associate them to cell types.



(a) (b)

Figure 1. Attractor transition graph in a RBN. Circles represent attractors; arrows represent transitions among attractors induced by single spin flips. The numbers on each arrow are the probability that, by flipping at random the state of a node in an attractor, that transition takes place. (a) the complete attractor transition graph; (b) the same graph, where links overpassing the threshold θ=0.02 are removed

A possible solution to this problem was proposed in [2][12], where the authors observe that flips are a kind of noise fairly intense, as they amount to silencing an expressed gene or to express a gene which would otherwise be inactive: this may well be an event too rare to happen with significant probability in the cell lifetime. It is possible therefore to introduce a threshold θ, and neglect all the transitions whose occurrence probability is lower than it (Figure 1b). In such a way, the notion of Ergodic Set has to be modified in that of Threshold Ergodic Set (briefly, TES or, when the value of the threshold is considered, TES_θ), a set of attractors linked only by jumps having a probability higher than θ, that entrap the system in the long time limit. A TES_θ is therefore a subset of attractors which are each other directly or indirectly θ-reachable (reachable by means of transition whose probability exceeds the threshold θ), and from which no transition can allow escaping. The threshold clearly is related to the level of noise in the cell, and scales with its reciprocal (the frequency of flips) [2].

An ergodic set can be described therefore as a TES_θ with θ=0; interesting is the fact that this structure, by increasing the threshold, breaks into more and more TESs, till all attractors are also independent TESs (i.e. they cannot be abandoned – see also Figure 2). Statistics on the increasing of the ratio between the total number of TESs and the total number of attractors vs. the increasing of the threshold are shown elsewhere [2]: in any case, when θ exceeds a network dependent value all the attractors become TESs.

In [2][12] we propose to associate cell types to TESs, that represent coherent stable ways of functioning of the same genome even in the presence of noise. According to this framework NRBNs can host more than one TESs, avoiding in such a way the problem that hampered the straightforward association of cell types to Ergodic Sets.

3. STOCHASTIC CELL DIFFERENTIATION

Several authors, on theoretical and experimental bases, associate different levels of noise to different levels of differentiation [9][14][15]; in particular the degree of differentiation is supposed to be related to the possibility for an undifferentiated cell to wander in a portion of phase space greater than the corresponding portions covered by more differentiated cells. This fact is reflected in the presence of higher noise levels in undifferentiated cells, with respect to more differentiated forms [16] [17][18].

In our framework, a convenient proxy for the available portion of phase space could be the number of attractors belonging to the TES. A TES_0 (i.e. the one found when θ=0), implying a wonder through a large number of attractors, could therefore be associated to a totipotent cell, while as the threshold is increased smaller TESs appear, corresponding to more differentiated biological forms (Figure 2). At high enough threshold values all the attractors are TESs (the fully differentiated cells). The increase of the threshold would correspond to a decrease of noise level: as other authors, we hypothesize that this effect could be related to an improvement in the mechanisms whereby fluctuations are kept under control [13][22]. This association of differentiation to changes in the noise level represents the most stringent outcome of the model, and could be amenable to experimental test.

This hypothesis explains in a straightforward way the fact that there are different degrees of differentiation (i.e. property *i*), corresponding to different threshold values in elaborating the attractors' landscape. It is then easy to describe stochastic differentiation [16] [19]: in this vision the fate of a cell depends on the particular attractor it is passing through when the systems experiences a change in the noise level and on the specific flip which occurs. The new cell type will be that corresponding to the new TES_θ to which the attractor belongs at the new threshold level (see Figure 2).

4. DETERMINISTIC CELL FATE

There exist indeed several processes, e.g. during the embryogenesis, in which cell differentiation is not stochastic but it is driven towards precise, repeatable types by specific chemical signals, which activate or silence some genes. In our model we can simulate these process by permanently fixing to 1 or 0 the state of some nodes. However, in our framework this single action doesn't influence the level of noise, and therefore doesn't enable differentiation processes: in order to have deterministic differentiation, we need the existence of particular genes, called switch genes, whose permanent perturbation, coupled with noise (which by itself would lead to the sto-

chastic differentiation - see Figure 2) always leads the system through the same differentiation pathway. In other words, nodes that uniquely determine to which TES the system will evolve.

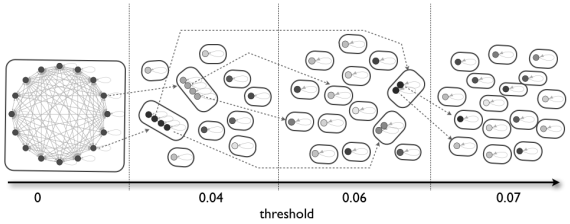


Figure 2. TESs and stochastic differentiation. As the threshold is increased the single TES_0 breaks into smaller disjoint TESs, corresponding to more differentiated cells, until eventually final cell types are reached. Examples of stochastic transitions are shown by dotted lines.

The existence of switch nodes has actually been verified to be a common property (found in about 1/3 of the nets), thereby proving the effectiveness of the model.

In Figure 3 one can see an example of differentiation, from a multi-TES_0 to a set of single-TESs. This case represents just one possible diagram obtained from simulations; the system shows indeed a very rich and complex landscape of possible behaviors, as in biological differentiation.

Please note that the model is actually able to describe also property *iv* concerning the existence of limited (in number and in completeness) exceptions to the irreversibility of cell differentiation (see figure 3 and [12] for a more detailed presentation).

In other works – [2][12] - we have shown that this model is able also to simulate induced pluripotency (property *v*), where the overexpression of few nodes (without changing the noise level) can allow the system to "come back" to a less differentiated state (see [20] for an experimental counterpart), and jumps between two completely differentiated cell types (property *vi* – [24] for the experimental counterpart) , in the last part of this work we'll focus our attention to the action of permanent perturbations on the attractors associated to the RBN system, in order to better characterize the TESs the system is reaching. What we wish to highlight is the fact that these TES are not simply subsets of the original and less differentiated one (as it happens in the case of stochastic differentiation, as for example in Figure 2): in fact, the permanent perturbation change in a enduring manner the original RBN, blocking the perturbed node, fixing to 1 (or to 0) the inputs of some other nodes (the nodes directly downstream the perturbed node) and congestioning and/or changing the information flow among a part of the other ones. This action could change the attractors expressed by the perturbed network, that under several aspects could be seen as a new RBN.

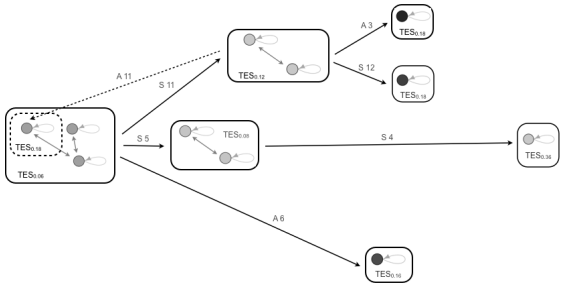


Figure 3. A case of deterministic differentiation. Box represent TESs and circles represent attractors. Arrows indicate possible different path differentiation and labels on arrow indicate the switch nodes are acting: it is reported the number of the node (A are switched-on nodes, and S switched-off nodes); every arrow implicates an increase of the threshold. Note that the switching-off of node 11 leads to a TES from which the subsequent switching-on of the same gene causes a return to (a subset of) the original TES_0. In other diagrams (not shown) the reversibility of the path could be induced by different genes, and could lead to ampler subsets of the original TESs

In order to observe these aspects we analyzed two groups of networks having N=10 and N=100 nodes, each composed by 100 networks. To find the RBNs' attractors we exhaustively checked all the possible initial conditions for the nets with N=10, and performed a random sampling for the nets with N=100. For the nets with N=10 we perturbed all the nodes by starting in all the phases, whereas for the nets with N=100 we perturbed the 20% of the total possibilities; the main results are shown in Figure 4.

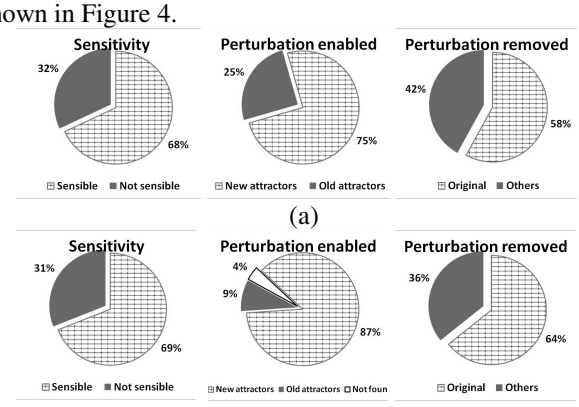


Figure 4. Consequences of permanent perturbation on RBNs' attractors. Graphs in row (a) are referring to nets with N=10 nodes, whereas graphs in row (b) are referring to nets with N=100 nodes. See text for a detailed explanation

The first column show the sensitivity, defined as the fraction of experiments where the RBN, initially on the attractor A, when a permanent perturbation is applied, goes toward an attractor A' not equivalent to A (we define equivalent two attractors that are equal in all the

nodes, with the exception of the perturbed one). The second column shows that, from all the cases where A' is not equivalent to A, the largest part of A' attractors are not equivalent to any attractor of the original RBN (they are totally new attractors). The third column refers only to the "new attractors" A', and describe what happens when the perturbation is released and the system is allowed to relax toward the attractors of the original not perturbed net. The graph shows how many times the final attractors B coincide with the original attractors A, and how many times B differs from A (B≠A). Note that in a limited number of cases (with N=100) is was not possible to individuate the attractors because of computational vincula. The main consideration we can derive from these simulations are:

1. the sensitivity (as before defined) seems to be not influenced by the net size
2. on the contrary, the bias toward already know A' attractors decreases with the net size
3. the perturbed nets can exhibit attractors different from those of the original nets, so allowing the formation of TES qualitatively different from these obtained from a mere change of threshold
4. the permanent perturbations have significant consequences also after the perturbation release, when in more than the 20% of the cases the final attractors B are different from the original ones A

5. CONCLUSIONS

We presented a single model, that can describe all the main features of differentiation; the explanation of differentiation makes use of the global properties of a generic dynamical system, without resorting to detailed hypotheses concerning very specific control circuits.

We think that the picture of a cell as a dynamical system and the idea that differentiated cells are more constrained in their wandering in phase space are general schemas, that could be applied also to other models of gene and cell dynamics [21].

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