Proceedings of the 2007 IEEE Symposium on Computational
Intelligence in Bioinformatics and Computational Biology (CIBCB 2007)

A Framework for Discrete Modeling of Juxtacrine Signaling Systems

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***Abstract*— Juxtacrine signaling is intercellular communication, in which the receptor of the signal (typically a protein) as well as the ligand (also typically a protein, responsible for the activation of the receptor) are anchored in the plasma membranes, so that in this type of signaling the activation of the receptor depends on direct contact between the membranes of the cells involved. Juxtacrine signaling is present in many important cellular events of several organisms, especially in the development process. We propose a generic formal model (a modeling framework) for juxtacrine signaling systems that is a class of dynamic discrete systems. It possesses desirable characteristics in a good modeling framework, such as: a) structural similarity with biological models, b) capacity of operating in different scales of time and c) capacity of explicitly treating both the events and molecular elements that occur in the membrane, and those that occur in the intracellular environment and are involved in the juxtacrine signaling process. We implemented this framework and used to develop a new discrete model for the neurogenic network and its participation in neuroblast segregation.**

I. Introduction

Juxtacrine signaling is an intercellular communication based on transmembrane proteins, "anchored" in the plasma membrane. The two principal types of transmembrane proteins participating in this system are *ligands* and *receptors*: a receptor can discharge a series of molecular reactions inside of a cell if it is activated (through a ligand/receptor binding) by a ligand positioned in the membrane of a neighboring cell.

As in juxtacrine signaling, both the receptor and the ligand are anchored in the membranes, the activation of the receptor depends on direct contact between the membranes (juxtaposed) of the involved cells. This implies that the ligands act in a more restricted way, only operating on the adjacent cells (immediate neighbors). There is a variety of signaling pathways that are discharged by ligands anchored in the membrane [1].

Juxtacrine signaling is vital in several phases of development and maintenance of the tissues, for instance, in the neurogenesis in *Drosophila melanogaster* [2], in the generation of cellular polarity in ommatidia [3], and in the previous development of vertebrates [4], among others. It actively participates in the processes of cellular patterning, particularly in the "fine" patterning. The two main mechanisms that operate in systems of juxtacrine signaling for the formation of patterns are the lateral inhibition and lateral induction.

A. Previous Work: Mathematical Models

The first, and well-referred, formal model for juxtacrine signaling [5] was formulated in terms of the activity of the ligand Delta and its receptor Notch. In this model the mechanism of lateral inhibition was described through a feedback loop through which small differences among neighboring cells are amplified and consolidated. In this work, Collier *et al.* proposed a set of differential equations to control the rate of production of these proteins.

The works of Owen and Sherratt [6], [7] and Wearing *et al.* [8], [9] improve on previous models: instead of adopting an "arbitrary" measure of the activity of the proteins as parameter, they suppose that the variables of the model are: a) the amount of free ligand molecules; b) the amount of free receptor molecules and c) the amount of complex ligands/receptor formed on the surface of the cell.

There are works [10], [9], [11], [12] that analyze the behavior of these models and the pattern type that are capable of generating in several geometries of cells (square, hexagonal, etc).

Most models proposed for juxtacrine signaling are essentially continuous. Some are continuous in both space and time [6], others are continuous in time and discrete in space [13], [7], [8]. Luthi *et al.* [14] proposed a model for juxtacrine signaling that is discrete in both space and time which consists primarily of a cellular automata with continuous state variables.

The most recent model for juxtacrine signaling [15] incorporated the treatment of distribution inhomogeneous receptors in the cell membrane. It is an extension of the model proposed by Owen, Sherratt [6], [7] and Wearing *et al.* [8], [9] adding relative terms to the diffusible transport of proteins between segments of membrane of the same cell as well as modifying the feedback functions so that considered the localised production of ligands and receptors.

B. Motivation

We defined a *juxtacrine signaling system* as the set formed by the molecular elements, including its interactions and the molecular mechanisms that participate in the process of juxtacrine signaling. These elements and mechanisms can be intracellular — for instance signal transduction pathways or

gene regulatory networks — or associated with the membranes of the cells in the communication process, for instance binding events between ligands and receptors positioned in the membrane.

The principal existing models for juxtacrine signaling systems (henceforth referred to as JSS) can be divided into three groups: a) the Models of Activity, for instance the model of Collier *et al.* [5]; b) the Ligand-Receptor Models, for instance the model of Owen and Serratt [6], [7] and c) the Segmental Models, for example the model of Webb *et al.* [15]. All of which:

(i) are based on differential equations, which makes them difficult to analyze if the number of dependent variables grows, since they demand the knowledge of many experimental parameters, which are typically not available;
(ii) except for the model type proposed by von Dassow *et al.* [16], [17], "hide" the participation of the components and intracellular mechanisms involved in the process of juxtacrine signaling, for instance the participation of certain critical genes and their corresponding regulation mechanisms; that is, these models do not describe in a detailed manner how these intracellular components operate in the signaling process. This is done by "encapsulating" the influence of the intracellular components in feedback functions, which makes these models focuses on the binding events that which in the membrane.

Models discrete in both time and space, for instance the model of Luthi *et al.* [14], also do not explicitly capture the intracellular molecular interactions that occur in the process of juxtacrine signaling.

In a "ideal" generic model (a framework) for JSS, we should observe the following characteristics:

- a similar structure to of the models typically used by biologists, so that it is intuitively strong to them;
- the capacity to represent several elements and their time evolution present in JSS;
- rich and sufficiently comprehensive to capture several types of molecular events (intra and extracellular) that occur in the process of juxtacrine signaling, for instance conformational modifications in membrane proteins, protein-protein interactions that can occur in the membrane or inside the cell, transcription, translation, dependence between genes and post-translational modifications;
- a capacity of modular representation of the signaling systems, because: a) the complexity of the signaling and regulation networks suggests that its analysis demands that the modeling methods are capable of treating parts of the network as modules and b) several signaling networks present groups of elements and mechanisms that present operation and modular organization [18];
- capable of working over several orders of magnitude in spatiotemporal scales, because signaling cellular networks operate with events whose answers vary from tenths or hundredths of seconds (for instance, protein modifications) to several minutes (transcriptional and translational regulation, for example) [19];
- allow for integrating experimental data of different types and sources.

Although the models of continuous time allows for a more detailed description of the variation rates involved (for instance of mRNA concentration and proteins), as already mentioned, they demand the knowledge of experimental data not always available (for instance values of kinetic constants). In addition, the discrete modeling of signaling systems [20] and of regulation [21], [22] is already a relatively well established activity.

In the following sections we describe a framework of discrete modeling of JSS that, at the same time, contemplates some of the characteristics mentioned above and can be used in several situations and applications, in the context of juxtacrine signaling process.

II. Description of Framework (Metamodel $\mathfrak{J}$)

We propose a general formal model for juxtacrine signaling systems named *Metamodel* $\mathfrak{J}$, as a class of dynamical systems with discrete time and space and state variables which may be discrete or continuous.

A. An overview of $\mathfrak{J}$

In $\mathfrak{J}$ a tissue structure is represented by a regular lattice, whose elements, denominated cells, have a $1:1$ correspondence with alive cells, as shown in Fig. 1. A lattice cell is an autonomous entity whose state is defined by the state of its intracellular components and membrane components.

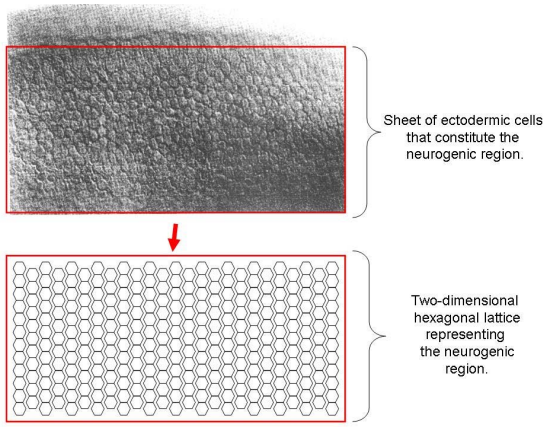

Fig. 1. A two-dimensional hexagonal lattice example representing cells of the neurogenic region, a group of ectodermic cells present during the neurogenesis in *D. melanogaster*.

The intracellular components of a lattice cell are a class of state variables that represent the states of the intracellular molecular elements of the living cell. The components of lattice cell membrane are another class of state variables, which are divided in subclasses, and each subclass is associated with a side (membrane segment) of the cell. The membrane components represent the states of the molecular elements

which are present in the segments of plasma membrane of the living cell, as shown in Fig. 2.

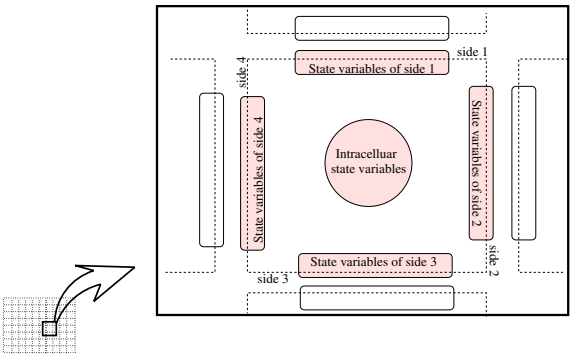

Fig. 2. All the lattice cells contain the same components: a class of state variables associated with the intracellular molecular elements and a class of state variables associated with the present molecular elements in each one of the sides (membrane segments) of the cell. The number of sides depends on the geometry and dimensions adopted for the lattice.

All the lattice cells contain the same components (intracellular and membrane) and their states, for each discrete time step, are determined by the application of the same transition rules valid for every lattice cell. The transition rules can be classified in intracellular (those which update the intracellular components) and membrane (those which update the membrane components).

The intracellular components along with their transition rules represent the signaling nets and present intracellular regulation in the living cell. The membrane components along with their transition rules represent the signaling mechanisms which operate in the plasma membrane of the living cell.

B. A more detail view of $\mathfrak{J}$

An M model in $\mathfrak{J}$ corresponds to a dynamical system whose general form is

$$M = (\mathcal{R}, \mathcal{V}, \mathcal{I}, \mathcal{T}),$$

where $\mathcal{R}$ is a regular lattice, $\mathcal{V}$ is a finite set of variables associated with each element of $\mathcal{R}$, $\mathcal{I}$ is a set of initial conditions associated with $\mathcal{V}$ and $\mathcal{T}$ is a set of transition rules. In the following sections, the properties and restrictions that define these components are better described.

1) Lattice characterization: A regular lattice $\mathcal{R}$ is a finite periodic net of elements, denominated *cells*, in a (finite) space of dimension d that is completely filled out by the cells. The elements that characterize a lattice are: a) its dimensions ($1D$, $2D$ or $3D$); b) its size, i.e., its number of cells; c) its topology; d) the boundary conditions, namely the number of neighbors of the cells that are located in the extremities (borders) of the lattice (periodic or linear boundary).

2) Characterization of the cells and of $\mathcal{V}$ *set:* Given a lattice $\mathcal{R}$, a cell in $\mathcal{R}$ is identified by its relative position p, represented by a point in the coordinated axes: $p = (x) \in \mathbb{N}$ (for one-dimensional lattice), $p = (x, y) \in \mathbb{N}^2$ (for two-dimensional lattice) or $p = (x, y, z) \in \mathbb{N}^3$ (for three-dimensional lattice). In addition to its identifier, each cell p in $\mathcal{R}$ contains a set of variables $\mathcal{V} = \{\mathcal{E}, \mathcal{G}, \mathcal{S}, \mathcal{B}, \mathcal{A}\}$, whose elements are described as follows:

- A finite set $\mathcal{E}$ of variables denominated *intracellular signaling and gene regulatory network input*, (*intracellular network input* for short). An element $e^k \in \mathcal{E}$ is called *input* and $e^k(t) \in \mathbb{E}$ is called *input value of* e^k *in the time* t, $t \in \mathbb{T}$, where $\mathbb{T} \subset \mathbb{N}$ denotes the domain of the discrete time and $\mathbb{E} \subset \mathbb{Z}$ is the finite set of the possible values that an input can assume. We represented the several values of input $e^k(t)$ by a two-dimensional vector E.
- A finite set $\mathcal{G}$ of state variables denominated *intracellular metabolites and gene state in the intracellular signaling and gene regulatory network* (*intracellular network state*). An element $g^k \in \mathcal{G}$ is called *state* and $g^k(t) \in \mathbb{G}$ is called *state of* g^k *at time* t, $t \in \mathbb{T}$, where $\mathbb{G} \subset \mathbb{Z}$ denotes the finite set of the possible states that a gene or intracellular metabolite can assume. We represented the states $g^k(t)$ by a two-dimensional vector G.
- A finite set $\mathcal{S}$ of variables denominated *intracellular signaling and gene regulatory network output* in short we will call it *intracellular network output*. An element $s^k \in \mathcal{S}$ is called *output* and $s^k(t) \in \mathbb{S}$ is called *output value of* s^k *at time* t, $t \in \mathbb{T}$, where $\mathbb{S} \subset \mathbb{Z}$ denotes the set of the possible values that an output can assume. We represented the various output values $s^k(t)$ by a two-dimensional vector S.
- Associated with each cell in $\mathcal{R}$, there is a finite set $\mathcal{F} \subset \mathbb{N}^+$ of segments representing the sides of the cell. The number $|\mathcal{F}|$ of sides of a cell depends on the geometry and the dimensions of the lattice.
  To each side $f \in \mathcal{F}$ of each lattice cell two sets of variables are associated:
  - A finite set of state variables $\mathcal{B}_f$, named *state of transmembrane signaling*. An element $b_f^k \in \mathcal{B}_f$ is called *signaler* and $b_f^k(t) \in \mathbb{B}_f$ is called *signaler state* b_f^k *at instant* t, where $\mathbb{B}_f \subset \mathbb{Z}$ is the set of the possible states that a signaler can assume. The states of the signalers are represented by a two-dimensional vector B_f.
  - A finite set $\mathcal{A}_f$ of variables called *state of environmental signaling*. An element $a_f^k \in \mathcal{A}_f$ is called *environmental signal* and $a_f^k(t) \in \mathbb{A}_f$ is called *value of the signal environmental* a_f^k *at instant* t, where $\mathbb{A}_f \subset \mathbb{Z}$ is the set of the possible values that an environmental signal can assume. The environmental signals represent not explicitly modeled external events and may alter the state of the membrane signalers. The states of the environmental signals are represented by a two-dimensional vector $A_{p,f}$.

3) Definition of the Initial Conditions: The third component of an instance of $\mathfrak{J}$ corresponds to the set $\mathcal{I}$ of initial conditions, from which the evolution of the system occurs. The set $\mathcal{I}$ is defined as being the value that each variable, in

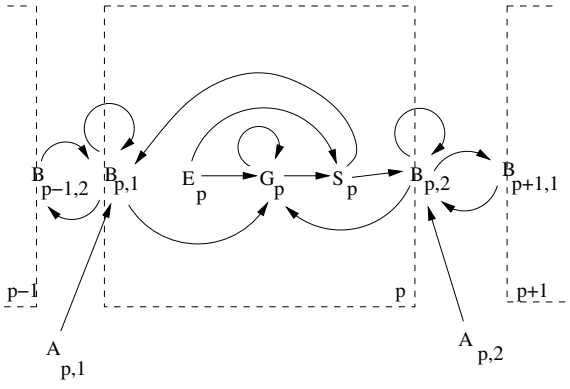

Fig. 3. Three cut-out cells ($p-1$, p and $p+1$) of a one-dimensional rectangular lattice. The dotted line represents the membrane of the cells. The cell p in this lattice has two neighbors: one to the left ($p-1$) and one to the right ($p+1$). $B_{p,i}$ denotes the vector of signalers B of the cell p in side i, and, similarly,$A_{p,i}$ denotes the vector of environmental signals A of cell p in side i. E_p, G_p and S_p, denote, respectively, the inputs, the state of the genes and/or intracellular metabolites, and the outputs of intracellular network of the cell p. The arrows represent the influence relations among the state variables.

each cell of $\mathcal{R}$ and on each side, assumes in the initial instant.

4) State Transitions: The state transition rules $\mathcal{T}$, valid for every cell in M, can be divided in the following classes:

Class Ψ

The updating of the state vector G, for every cell $p \in \mathcal{R}$, at each timestep, is made by a finite vector of functions

$$\Psi = [\psi_1, \psi_2, \ldots, \psi_{|\mathcal{G}|}],$$

where ψ_k denotes how the gene and/or intracellular metabolite g^k, $1 \leq k \leq |\mathcal{G}|$, is updated in time, in other words, it describes how the variable of state g^k (for every $p \in \mathcal{R}$) evolves in discrete time steps.

The functions $\psi_k : \mathbb{G}^{\tau+1} \times \mathbb{E}^{\tau+1} \rightarrow \mathbb{G}$ are called *functions of transition of the intracellular network* and are of the form

$$g_p^k(t+1) = \psi_k(g_p^u(t-x), \cdots, g_p^v(t-y), e_p^q(t-x), \cdots, e_p^r(t-y)),$$

where $1 \leq u, v \leq |\mathcal{G}|$, $1 \leq q, r \leq |\mathcal{E}|$, $g^k(t) \in \mathbb{G}$, $e^k(t) \in \mathbb{E}$ and $0 \leq x, y \leq \tau$, where τ corresponds to the earliest time used by ψ_k.

Class Φ

Similarly, the updating of the vector of outputs S, is made by a finite vector

$$\Phi = [\phi_1, \phi_2, \ldots, \phi_{|\mathcal{S}|}],$$

where ϕ_k denotes how the output k, $1 \leq k \leq |\mathcal{S}|$, of the intracellular network is updated in time, in other words, it describes how the variable of output s^k (for every cell $p \in \mathcal{R}$) evolves in discrete time steps.

The functions $\phi_k : \mathbb{G}^{\tau+1} \times \mathbb{E}^{\tau+1} \rightarrow \mathbb{S}$ are called *functions of output of the intracellular network* and are of the form

$$s_p^k(t+1) = \phi_k(g_p^u(t-x), \cdots, g_p^v(t-y), e_p^q(t-x), \cdots, e_p^r(t-y)),$$

where $1 \leq u, v \leq |\mathcal{G}|$, $1 \leq q, r \leq |\mathcal{E}|$, $g_p^k(t) \in \mathbb{G}$, $e_p^k(t) \in \mathbb{E}$, and, like in the previous case, $0 \leq x, y \leq \tau$.

Class Θ

The updating of the vectors of states $B_{p,f}$, $1 \leq f \leq |\mathcal{F}|$ and $p \in \mathcal{R}$, is made by the vector

$$\Theta = [\theta_1^1, \cdots, \theta_{|\mathcal{F}|}^1, \cdots, \theta_1^2, \cdots, \theta_{|\mathcal{F}|}^2, \cdots, \theta_1^{|\mathcal{B}_f|}, \cdots, \theta_{|\mathcal{F}|}^{|\mathcal{B}_f|}],$$

where θ_f^j denotes how the membrane signaler j, $1 \leq j \leq |\mathcal{B}_f|$, of the side f, is updated in time, in other words, it describes how the variable of state b_f^j (for every cell $p \in \mathcal{R}$) evolves in discrete time steps. We note that b^j may be updated independently at each side of the cell.

The functions $\theta_f^j : \mathbb{S}^{\tau+1} \times (\mathbb{B}_1^{\tau+1} \cup \cdots \cup \mathbb{B}_{|\mathcal{F}|}^{\tau+1}) \times \mathbb{B}_f^{\tau+1} \times \mathbb{A}_f^{\tau+1} \rightarrow \mathbb{B}$ are called *output and neighborhood functions of signaling* and they are used to update the signalers $b_{p,f}^j$ in the following manner:

$$\begin{aligned} b_{p,f}^j(t+1) = \theta_f^j \quad (\quad & s_p^k(t-x), \cdots, s_p^l(t-y), \\ & b_{z,u}^m(t-x), \cdots, b_{w,v}^n(t-y), \\ & b_{p,f}^m(t-x), \cdots, b_{p,f}^n(t-y), \\ & a_{p,f}^q(t-x), \cdots, a_{p,f}^r(t-y) \quad), \end{aligned}$$

where $1 \leq k, l \leq |\mathcal{S}|$, $1 \leq q, r \leq |\mathcal{A}_f|$, $1 \leq m, n \leq |\mathcal{B}_f|$, $z, w \in V(p) = \{v : v \text{ is neighboring } p \text{ in } \mathcal{R}\}$ (that is, z and w are neighboring cells to the cell p), $1 \leq u, v \leq |\mathcal{F}|$, and $0 \leq x, y \leq \tau$

E_p represents internal events — a signal of an alternative signaling pathway or the constitutive expression of genes, for instance — and $A_{p,f}$ represents external events — an environmental signal, for instance the temperature variation. They represent independent signals and are not explicitly modeled but may alter the state of the genes and/or intracellular metabolites. Their values are fixed in the model definition and are not altered by any system transition rule.

In Fig. 3 we present a schematic representation of the restrictions in the influence relationships — defined by the classes Ψ, Φ and Θ — for the case of the one-dimensional rectangular lattice.

C. Stochastic Models in $\mathfrak{J}$

In $\mathfrak{J}$ it is possible to define both deterministic and stochastic models. We denominate an instance of $\mathfrak{J}$ as being deterministic if only one transition function is associated to each variable of the model. If the model is stochastic, we define a list of functions per variable and we associate a probability to each of the functions. This implies that in the definition of a stochastic model it is necessary to define a finite set of functions F and a distribution of probabilities P_F in F.

III. Implementation

For building, simulating, analyzing and refining models in $\mathfrak{J}$ we have implemented a software, named $\mathfrak{J}$ *System* ($\mathfrak{J}S$), composed of three modules (see Fig. 4) :

a) A modeling setting; the main components of this module are an interface for edition and visualization of models and a system for verification of their consistency;

b) A simulator of models in $\mathfrak{J}$;
c) An interface for visualization and analysis of simulations and results, containing several components, such as a viewer of cellular patterns and reports and descriptive graph generators for the states behavior and evolution.

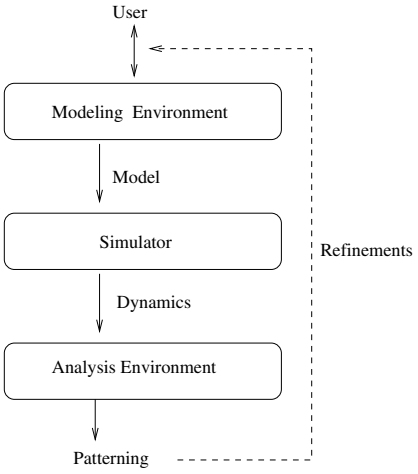

Fig. 4. The global architecture of software S$\mathfrak{J}$.

IV. Aplications

Due to its generality, the metamodel $\mathfrak{J}$ can have several applications in different contexts, for instance:

i) in the modeling and analysis of formation of patterns in juxtacrine signaling;
ii) in the study of methods of identification of units of signaling and metabolic pathways induced by environmental signals;
iii) in the simulation of cellular juxtacrine signaling and regulation networks;
iv) in the reconstruction and in the structural and dynamic analysis of networks of juxtacrine signaling.

In the following sections we showed the application of $\mathfrak{J}$ in the modeling of the elements and basic interactions (extracellular and intracellular) that participate of the neuroblast segregation in *D. melanogaster*.

A. Delta-Notch na neurogenesis em D. melanogaster

The Notch pathway is a vital signaling pathway, present in several events in the development of several organisms, e.g., in the neurogenesis in *Drosophila* [2], in the previous development of vertebrates [23], [4], [24] and in the establishment of boundaries between veins and interveins in the *Drosophila* wing [25]. Signaling of the cell-cell type, mediated through the Notch pathway, it is a mechanism that operates in several situations where there are definitions of cellular fate.

One of the best known examples in which the Notch pathway operates, is in the formation of the nervous system in *Drosophila*, more specifically in the processes of neuroblasts segregation and determination of sensory organ precursor cells (that originate the sensorial bristles of the epidermis). Soon after gastrulation, the cells of the neurogenic region (an area of approximately 1,900 ectodermic cells formed by two longitudinal strips of cells along the anteroposterior axis of the embryo, in a position slightly dorsal in relation to the ventral mesoderm) assume a bipontential character: they can become neuroblasts — precursors of neural cells — or epidermoblasts, that are the precursors to the epidermis. The distribution of the neuroblasts and epidermoblasts in the neurogenic region, after each cell assumed its fate, depends on elements of intracellular regulation and cell-cell interaction.

The proneural genes (mainly of the achaete-scute complex) assign to the ectodermic cells the potential for becoming neural precursors. In the neurogenic region, cells expressing these genes become clusters of cells (called proneural clusters). Not all the cells of one neural cluster become neuroblast and the process that leads to its specification involves lateral inhibition: therefore after the formation of the clusters, all its cells have the potential for becoming neuroblast, until one of them (the future neuroblast) begins to express — through a random event — genes of the achaete-scute complex in higher levels than the others. This implies that this cell produces a signal, transmitted through juxtacrine interactions between Delta and Notch, which inhibits its neighbors from becoming neuroblasts, by making it a neural precursor and the remaining cells of the cluster become epidermic epithelial cells.

The architecture of this regulation network, its principal components and its interactions (which we will call "canonical network") are schematized in the Fig. 5. Its main characteristics ([26], [27], [28], [29]) can be summarized as follows:

- Delta is the ligand for the Notch receptor.
- When Delta activates Notch, the intracellular domain of Notch (Notch_intra) links to the Suppressor of Hairless (SU(H)) transcription factor, that is from the CSL family
- The dimer SU(H)/Notch_intra activates the transcription of the genes of the Enhancer of split (e(spl)) complex that codify for the transcriptional repressor E(SPL).
- E(SPL) represses the transcription of the proneural genes achaete (ac) and scute (sc), that are the primary determinant of the neural fate: cells with high concentrations of the products AC and SC become neuroblasts; AC and SC are transcription factors that contain a conserved motif of the basic helix-loop-helix (bHLH) type. They use the bHLH domain for dimerizing with other factors, becoming active as dimers.
- AC and SC link each one of them to the Daughterless (DA) cofactor and, in the heterodimers condition (AC/DA and SC/DA), they activate the transcription of both to themselves and each other (see Fig. 5).
- AC/DA and SC/DA also activate the transcription of the dl gene (that codifies for the Delta ligand) and of the e(spl) gene, whose product (E(SPL)), as previously mentioned, represses the ac and sc transcription .
- Thus, a loop is formed: a random event activates ac and/or sc in a cell of the proneural cluster. They activate dl, whose product (Delta) activates Notch in the neigh-

boring cells, in the ones which, through the complex SU(H)/N, e(spl) is activated that, consequently, represses ac and sc.
- Delta represses the activity of Notch in the cell itself.
- e(spl) also promotes autorepression.
- The genes for Notch and DA have constitutive expression.

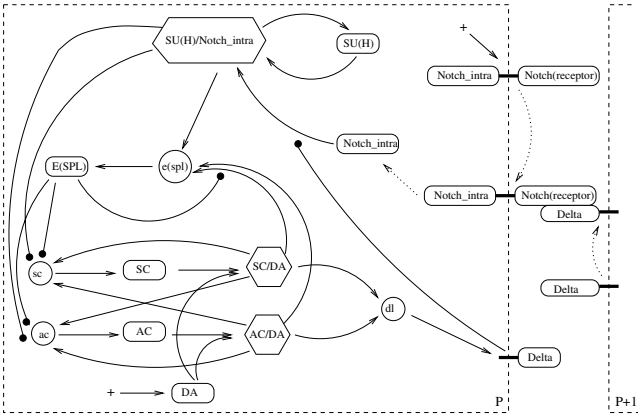

Fig. 5. Circles represents mRNAs, rectangles represents proteins, hexagons represents protein complexes. Notch and DA have constitutive expression, represented by ' + → Notch' and '+ → DA' , respectively. 'X → Y' denotes that X activates Y and 'X −• Y' denotes that X inhibits Y.

B. A Boolean Model in $\mathfrak{J}$ for Delta-Notch System

Based in the architecture suggested canonical network we have built a new deterministic $\mathfrak{J}$ model for neuronal precursor patterning. It is formulated in terms of the presence and/or absence of mRNAs, of the proteins and of the complexes involved.

The assumptions of the model are: 1) the state of each component is 0 or 1 (representing the absence or presence of the correspondent substance); 2) the transition rules are all logical functions that use the operators "AND", "OR" and "NOT"; 3) the transition rule associated with the transcription of a gene is represented by a Boolean function of the state of its activators and inhibitors; 4) the transition rule associated with translation of a protein is represented by a Boolen function of the state of the correspondent mRNA; 5) the transition rule associated with the binding of ligands to receptors in the membrane is represented by a Boolen function of the state of the free ligands and free receptors in the membrane; 6) transcription of a gene occurs if its activators are expressed and its inhibitors are not; 7) the effect of the transcriptional activators and inhibitors is not additive; 8) the effect of the transcriptional inhibitors is dominant in relation to the activators; 9) transcription and translation are state functions of an ON/OFF type; 10) if transcription is ON, mRNAs are transcribed in a one time step; 11) if translation is ON, proteins are translated in a one time step; 12) if binding is ON, ligand/receptor complex are formed in one time step; 13) mRNAs decay in a step of time if they are not transcribed; 14) transcription factors and other intracellar proteins undergoing some type of post-translational modification decay in one time step if its correspondent mRNAs are not present and 15) ligand/receptor complexes in the membranes decay in a time step if free ligands and free receptors are not present in the membrane.

The description of model is as follows:

(a) We have mapped the cells of the neurogenic region in a two-dimensional lattice $\mathcal{R}$ with periodic boundary conditions and containing 1936 hexagonal cells.

(b) In regards to the variables, to maintain adherence to the notation of Fig. 5 we have done the following:
- $\mathcal{G} = \{Notch\_intra, SUH/Notch\_intra, espl, ESPL, ac, sc, AC, SC, ACDA, SCDA, dl, DA, SUH\}$;
- we did not adopt discrimination in the sides of the cells, i.e., we assumed that each cell has only one side ($\mathcal{F} = \{1\}$), so that in each cell we have only one set of signalers $\mathcal{B}_1 = \{Delta, Notch, Notch/Delta\}$;

(c) We did not include environmental signals in the model;

(d) In relation to the transition rules, we have done the following:
- $Notch\_intra_p[t] = Notch/Delta_p[t-1]$.
- $DA_p[t] = 1$ (constant input representing the constitutive expression of DA).
- $SUH/Notch\_intra_p[t] = Notch\_intra_p[t-1] \wedge SUH_p[t-1] \wedge \overline{Delta_p[t-1]}$.
- $SUH_p[t] = SUH/Notch\_intra_p[t-1]$.
- $espl_p[t] = (SUH/Notch\_intra_p[t-1] \wedge ACDA_p[t-1] \wedge SCDA_p[t-1]) \wedge \overline{ESPL_p[t-1]}$.
- $ESPL_p[t] = espl_p[t-1]$.
- $ac_p[t] = \overline{ESPL_p[t-1]} \wedge \overline{SUH/Notch\_intra_p[t-1]} \wedge SCDA_p[t-1] \wedge ACDA_p[t-1]$.
- $sc_p[t] = \overline{ESPL_p[t-1]} \wedge \overline{SUH/Notch\_intra_p[t-1]} \wedge SCDA_p[t-1] \wedge ACDA_p[t-1]$.
- $AC_p[t] = ac_p[t-1]$.
- $SC_p[t] = sc_p[t-1]$.
- $ACDA_p[t] = AC_p[t-1] \wedge DA_p[t-1]$.
- $SCDA_p[t] = SC_p[t-1] \wedge DA_p[t-1]$.
- $dl_p[t] = ACDA_p[t-1] \wedge SCDA_p[t-1]$.
- $Delta_p[t] = dl_p[t-1]$.
- $Notch_p[t] = 1$ (constant input representing the constitutive expression of Notch).
- $Notch/Delta_p[t] = Notch_p[t-1] \wedge neighborhood$, where

$$neighborhood = \begin{cases} 1 & \text{if } \sum_{v \in V(p)} Delta_v[t-1]) \geq 4, \\ 0 & \text{otherwise,} \end{cases}$$

and $V(p)$ is the set of the neighboring cells to the cell p.

1) Simulations and Preliminary Results: We made some preliminary experiments simulating the process of neuronal precursors segregation. For this, we assumed that there are two interesting states in the model: the initial state, corresponding to the situation in which all the cells are bipotents, and the final, corresponding to the situation in which the neuroblasts are segregated.

To represent the initial state we assigned to every cell, at t

= 0, the logical value "1" for the Notch and DA variables and "0" for all the other state variables.

Once we simulated the model, we observed that the system remained in the steady state regarding the initial conditions (bipotent cells). In the sense used by Wensche [30], this state and its attraction basin correspond to a cell type. We then introduced (by independent signals) several flotations in the state of some model component, starting from the initial state. We used these flotations in order to reproduce *in silico* the best results in wet experiments well described in literature.

At first, we chose a random cell from the neurogenic region and we increased the expression levels of ac and sc. As expected this cell becomes a pro-neural precursor (defined by the DL state) and in time it inhibits its neighbors from changing in the same way. In these conditions, the system enters a stationary state, which suggests that this state and its attraction basin correspond to another cell type and that the trajectory followed by the model represents the related differentiation pathway.

Afterwards, we performed simulations varying the levels of ac and sc expression in random cells and at random timesteps. In these cases, we observed that:

- the cells in which the levels of ac and sc expressions were disturbed (augmented) behaved as in the previous simulation, became neuronal precursors.
- when the system enters in steady state we verified that: a) lateral inhibition is fully observed (there were not two neuronal precursors neighboring each other) and b) from the total of cells, 24% became neuronal precursors, which is compatible with the expected results. Fig. 6 shows the pattern obtained.

Finally we simulated the knock out of ac and sc in two ways: first we fixed $e(spl)$ in "0" and then we did the same for $SU(H)$. In both cases we obtained an equivalent pattern to the Notch mutant, with an excess of neuroblasts. We should note that these observations are compatible with experimental results [26], [2], [4].

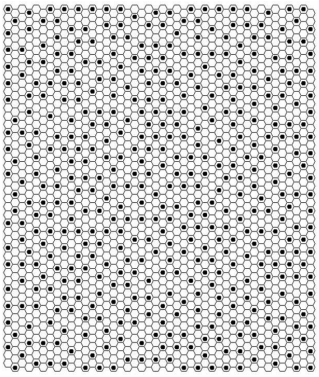

Fig. 6. Example of patterning of the neuronal precursors where we can verify the occurrence of lateral inhibition. The cells painted by white correspond to the epidermoblasts and by blank correspond to the neuroblasts.

V. Discussion and Conclusion

The organization adopted for the metamodel in $\mathfrak{J}$ was designed to possess a structure similar to the one of the models commonly used by the biologist community for JSS since we consider this a desirable characteristic. We expect that the structure of the lattice (where each cell is an autonomous entity with the same components) representing the tissues, the grouping of the state variables in intracellulars and of membrane and the imposed restrictions in the transition rules (distinguishing the representation of the events that occur in the membrane of those that occur in the intracellular medium), might have offered the similarity that we long for.

The formal models for JSS developed so far focus on the binding events between ligands and receptors that occur in membranes of communicating cells. $\mathfrak{J}$ is capable of representing the participation of the intracellular components, which extends the representation power of the current JSS models, allowing the most detailed representation of the several structures (membrane and intracellular ones) and present interactions in the transduction pathways, specially those related to gene regulation involved in the formation of patterns in juxtacrine signaling.

The capacity to operate with different scales of time is guaranteed in $\mathfrak{J}$ through the construction of delay cycles modeled in the system memories. For that it is necessary to define, for the specific model in subject, its unit of time, that corresponding to a discrete timestep. This unit can be defined as being, for instance, the interval of time of the fastest considered molecular event. The "amount of time" of the other considered events will always be proportional (larger than or equal to) the established unit.

Good models of juxtacrine signaling should be capable of treating the inhomogeneous distribution of ligands and receptors in the membranes, because it influences polarization events, for instance in dorsoventral polarization in the eyes of *Drosophila* [3]. In this sense, the division of the membrane in segments (sides) adopted in $\mathfrak{J}$ it is important to allow the representation of the located accumulation of proteins, which can occur by local protein synthesis, active transport from intracellular stores and selective degradation [31].

$\mathfrak{J}$ can "emulate" other formal models of juxtacrine signaling, as long as these are originally conceived with the discrete time and space or it can be converted into a discrete approach. We know that numeric methods of resolution of differential equations correspond to conversions of this type; in addition, many systems of differential equations can be approximated by a system of equations of differences. Then we conclude that $\mathfrak{J}$ can be applied in the emulation of a wide range of models, which reinforces its generality.

There is some similarity between $\mathfrak{J}$ and P systems [32], which are a class of distributed and parallel computational devices. These devices are strongly inspired in cellular organization of membranes. In particular, a P systems variant, called PBE systems [33], definitely present several similarities to $\mathfrak{J}$, for instance: a) present a structure composed of regions

delimited by several membranes, hierarchaly related; b) they have transition rules (intern to regions) and communication rules (sensitive to what occurs in the membrane boundaries) and c) allows the environment representation (elements placed outside the regions).

P systems are universal computational devices, that is, equivalent to Turing Machines, so in principle, any model in $\mathfrak{J}$ may be simulated in a P system device. Nevertheless, despite the similarities mentioned, we should note that: a) mapping a $\mathfrak{J}$ model into a P system is not always an easy task; b) in addition, the resulting P system could lose the structural similarity with biological systems, making it too abstract for biologists.

We illustrated the use of $\mathfrak{J}$ in the modeling of the Delta-Notch system and its participation in the patterning of neuronal precursors. In the simulations, we have obtained compatible results with the expected patterns: occurrence of lateral inhibition with, between 20 and 30% of the cells having adopted primary fate (neuroblasts) and between 70 and 80% cells having adopted secondary fate (epidermoblasts). However, refinements in the model are still necessary as well as new improved simulations and analysis in order to identify structural and functional properties in the model proposed for the Delta-Notch system.

Natural deficiencies of $\mathfrak{J}$ are those intrinsic to the discrete modeling. In addition, due to its abundant details, the complexity of computational time and space involved can represent a drawback of $\mathfrak{J}$, depending on the model type that is created. However, it is interesting to observe that juxtacrine signaling is typical in the initial phases of the embryonic development, where the amount of cells is relatively small; what is typically complex is the signaling pathway. Although we have not had problems with the simulations that we have done, it is reasonable to suppose that in situations where the amount of cells is immense, the computational demand can grow swiftly. This suggests that extensions of this work can be related to more efficient computational solutions. For instance, the structure of the model supplies a natural ease in performing its paralleling, which could be explored.

Other possible extensions for this work include: a) exploring dynamical properties of $\mathfrak{J}$ models, such as the development of an algorithm for automatic generation of attraction basins for small boolean models; b) application of $\mathfrak{J}$ for a more detailed analysis of the Delta-Notch system, which could incorporate multi-level variables and environmental signals and c) application of $\mathfrak{J}$ to model and to analyze other biological events, for instance the planar polarity generation in ommatidia [3].

REFERENCES

[1] F. Fagotto and B. M. Gumbiner. Cell contact dependent signalling. *Dev. Biol.*, 180:445–454, 1996.

[2] B. Castro, S. Barolo, A. M. Bailey, and J. W. Posakony. Lateral inhibition in proneural clusters: cis-regulatory logic and default repression by suppressor of hairless. *Development*, 132(15):3333–3344, 2005.

[3] S. Bray. Planar polarity: out of joint? *Curr. Biol.*, 10:155–158, 2000.

[4] J. Lewis. Notch signalling and the control of cell fate choices in vertebrates. *Semin. Cell Dev. Biol.*, 9:583–589, 1998.

[5] J. R. Collier, N. A. M. Monk, P. K. Maini, and H. L. Lewis. Pattern formation by lateral inhibition with feedback: a mathematical model of Delta-Notch intercellular signalling. *J. Theor. Biol.*, 183:429–446, 1996.

[6] M. R. Owen and J. A. Sherratt. Mathematical modelling of juxtacrine cell signalling. *Math. Biosci.*, 152:125–150, 1998.

[7] M. R. Owen, J. A. Sherratt, and H. J. Wearing. Lateral induction by juxtacrine signalling is a new mechanism for pattern formation. *Dev. Biol.*, 217:54–61, 2000.

[8] H. J. Wearing, M. R. Owen, and J. A. Sherratt. Mathematical modelling of juxtacrine patterning. *Bull. Math. Biol.*, 62:293–320, 2000.

[9] H. J. Wearing and J. A. Sherratt. Nonlinear analysis of juxtacrine patterns. *SIAM J. Appl. Math.*, 62:283–309, 2001.

[10] M. R. Owen, J. A. Sherratt, and S. R. Myers. How far can a juxtacrine signal travel? *Proc. R. Soc. Lond.*, B 266:579–585, 1999.

[11] M. R. Owen. Waves and propagation failure in discrete space models with nonlinear coupling and feedback. *Physica D*, 173:59–76, 2002.

[12] S. D. Webb and M. R. Owen. Oscillations and patterns in spatially discrete models for developmental intercellular signalling. *J. Math. Biol.*, 48:444–476, 2004.

[13] N. A. M. Monk. Restricted-range gradients and travelling fronts in a model of juxtacrine cell relay. *Bull. Math. Biol.*, 60:901–918, 1998.

[14] P. O. Luthi, B. Chopard, P. Preiss, and J. J. Ramsden. A cellular automaton model for neurogenesis in *Drosophila*. *Physica D*, 118:151–160, 1998.

[15] S. D. Webb and M. R. Owen. Intra-membrane ligand diffusion and cell shape modulate juxtacrine patterning. *J. Theor. Biol.*, 230:99–117, 2004.

[16] G. von Dassow, E. Meir, E. M. Munro, and G. M. Odell. The segment polarity network is a robust developmental module. *Nature*, 406:188–192, 2000.

[17] G. von Dassow and G. M. Odell. Design and constraints of the drosophila segment polarity module: Robust spatial patterning emerges from intertwined cell state switches. *Journal of Experimental Zoology*, 294:179–215, 2002.

[18] F. J. Bruggerman, H. V. Westerhoff, J. B. Hoek, and B. N. Kholodenko. Modular response analysis of cellular regulatory networks. *J. Theor. Biol.*, 218:507–520, 2002.

[19] J. A. Papin, T. Hunter, B. O. Palsson, and S. Subramaniam. Reconstruction of cellular signalling networks and analysis of their properties. *Nature*, 6:99–111, 2005.

[20] E. E. Allen, J. S. Fetrow, L. W. Daniel, S. T. Thomas, and D. J. John. Algebraic dependency models of protein signal transduction networks from time-series data. *J. Theor. Biol.*, 238:317–330, 2006.

[21] R. Thomas. Boolean formalization of genetic control circuits. *J. Theor. Biol.*, 42:563–585, 1973.

[22] R. Albert and H. G. Othmer. The topology of the regulatory interactions predicts the expression pattern of the segment polarity genes in *Drosophila melanogaster*. *J. Theor. Biol.*, 223:1–18, 2003.

[23] J. Lewis. Neurogenic genes and vertebrate neurogenesis. *Curr. Op. Neurobiol.*, 6:3–10, 1996.

[24] T. Whitfield, C. Haddon, and J. Lewis. Intercellular signals and cell-fate choices in the developing inner ear: origins of global and of fine-grained pattern. *Sem. Cell Dev. Biol.*, 8:239–247, 1997.

[25] S. S. Huppert, Jacobson, T. L., and M. A. T. Muskavitch. Feedback regulation is central to Delta-Notch signalling required for *Drosophila* wing vein morphogenesis. *Development*, 124:3283–3291, 1997.

[26] E. C. Lai. Notch signaling: control of cell communication and cel fate. *Development*, 131:965—973, 2004.

[27] A. M. Bailey and J. W. Posakony. Suppressor of hairless directly activates transcription of enhancer of split complex genes in response to notch receptor activity. *Genes Dev.*, 9:2609–2622, 1995.

[28] E. Meir, G. von Dassow, E. Munro, and G. M. Odell. Robustness, flexibility, and the role of lateral inhibition in the neurogenic network. *Curr. Biol.*, 12:778—786, 2002.

[29] Z. Paroush, S. M. Wainwright, and D. Ish-Horowicz. Torso signalling regulates terminal patterning in drosophila by antagonising groucho-mediated repression. *Development*, 124:3827–3834, 1997.

[30] Andrew Wuensche. Discrete dynamical networks and their attractor basins. In *Complex Systems '98*, 1998.

[31] D. I. Strutt. The assymmetric subcellular localisation of components of the planar polarity pathway. *Sem. Cell Dev. Biol.*, 13:225–231, 2002.

[32] G. Păun. Computing with membranes. *J. Comput. Syst. Sci.*, 61(1):108–143, 2000.

[33] F. Bernardini and F. Manca. Dynamical aspects of P systems. *BioSystems*, 70:85–93, 2003.