Modelling Self-structuring Processes in Morphogenesis : Lessons from Programmed Cell Death

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Abstract

We use Differential Adhesion driven self-organisation of cells in a medium as our grounding dynamic mechanism for studying what role does Apoptosis play in deciding the structural details of multi-cellular organism. Behavior of any given cell in a simulation is defined by the retraction and contraction the cell undergoes, which in term decides the spatial pattern a group of cells forms. The model includes an interplay between genetically coded information to and physical cellular rearrangements which emerge. Shape sequences are used to implement a control structure to the medium governed by surface-energy minimization conditions. This sequence is a boolean network (node n) whose state determines the state of the cell, thus implementing differentiation, and hence determining surface energy coefficient Jij. Cell death is implemented by assigning some states to express Programmed Cell Death (ie. killing themselves).

Introduction

"Those interested in the "Big Dig", the city of Boston's heroic attempt to bury Interstate 93 beneath its pavements while maintaining a passable stab, will be acquainted with the idea that any major construction entails a substantial amount of demolition. So too in animal development"
Apoptosis in Development : Meier, Finch, Evan
- Nature , Oct 2000

Essential to natures capability to construct, maintain and repair complex structures, is the capability to induce suicide in its own cells with high specificity. This is observed in all kind of multi-cellular organisms [1]. A general modus operandi of metazoan development in morphogenesis is the over-production of excess cells followed by selective culling at the later stages. This also results in formation of several structures in organisms, which are later removed by cell death. The process is a part of the overall dynamics of morphogenesis, but seems to play a crucial rule in what is described as "self-structuring": which is like sculpting from within.

A high level of plasticity can be described on the basis on apoptosis. This notion of plasticity in later stages of an organism results in highly robust biological systems. It has also been shown that the basic machinery for apoptosis is similar in all multi-cellular organisms, which makes it one of the many essential processes relating a transition from uni-cellular organisms to multi-cellular organisms.

Apoptosis and Limb development

Pattern formation in growing limbs of multi-cellular organisms have been reasoned on the basis of apoptosis. An example is a formation of finger pattern in chicken limb bud, which expresses Hox type gene, known to express program cell death in the expressed cells. This is illustrated below. Thus specificity of cell death is used to self-structure a shape out of an ensemble of cells. And this is achieved in a robust manner without any global control. I was intrigued by this idea and wanted to explore if such a technique could be exploited to be used in artificial mediums.

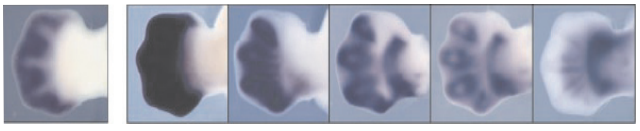

Fig1. Expression of Hox-D gene (known for encoding apoptosis) variants in chicken limb bud responsible for digit formation [2], Nature Nov. 2002

Why would Apoptosis be interesting to an "Amorphous Computer" Scientist?

Various factors which make Apoptosis interesting phenomena for something like an amorphous media are

a. Provides high programmability in the medium, thus greater control to emerging patterns.
b. Changes dynamics of the medium naturally (without any outside intervention).
c. Leads to phenomena of self-sculpting as seen in biological counter parts.
d. Robustness of the system comes for free, since plasticity is inherent in a system implementing programmed cell death.

The specific problem I addressed was to quantify the role of Apoptosis in pattern formation in a developmental cycle (suspended in a dynamic medium). The described model implements cell self-rearrangement (causing organization), cell division, cell growth, differentiation and cell death.

Differential Adhesion driven rearrangements

Cell adhesion is regarded as a mechanism that helps translate basic genetic information into complex three dimensional patterns in cells and tissues [3]. Cell adhesion is intimately related to cell differentiation, cell mobility, cell growth and cell death. Of our particular interest is Cell death and Cell growth, induced by change in cell volume caused by these rearrangements. We have used Differential Adhesion driven self-organisation of cells in a medium as our grounding dynamic mechanism for studying structural changes Apoptosis can induce in a medium. The core of model used for simulating self-organisation amongst cell bodies is adopted from the famous extended-Q potts model first proposed by Garner and Glazier [4]. The following phenomena was proposed as a reasoning for cell sorting as observed in many cells.

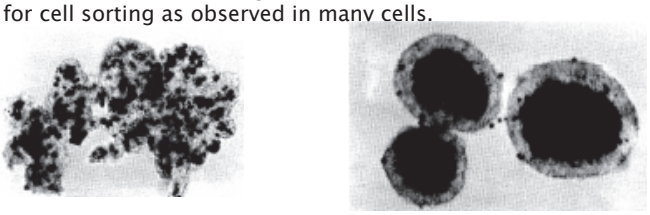

Fig 2. Cell sorting observed in pigmented(dark) and neural (light) retinal cells

The Model

The Model consists of two layers. To the core of the model, in the bottom layer, lies a Cellular Automata model, which models individual cells, there rearrangement, cell division, and cell-death. The top layer consists of a regulation network, modeled as a random boolean network. This acts like a genotype sequence to the generated shape. The final goal of the project is to study various shapes which are generated from the above, and specially how they are affected by cell death occurring at various stages of growth.

Modelling Rearrangement using Differential adhesion

The core of model used for simulating self-organisation amongst cell bodies is adopted from the famous extended-Q potts model first proposed by Garner and Glazier(1993)[4].

Thus in Garner model, several sites in a 2D CA together form a single cell. This initially is a connected set of sites, but as we will observe, the sets can get disconnected due to various reasons like cell growth, random motility of cell membrane modelled with temperature parameter. Glazier and Garner described such cellular rearrangements as an explanation of soap froth formation[] in their work. They later extended the model for multi-cellular systems. The following model is a good model for our experiments since it is a simple but highly extendible model which can incorporate various phenomena's such as reaction diffusion[], thermotaxis etc.

The CA update rule is defined such that a CA cell takes the state of a neighboring cell if this reduces "surface energy" of the cell; subject to some volume constraints. These volume constraints also model one type of cell-death discussed in later sections. Thus behavior of a cell is described in the dynamics of the way it extends and retracts its membranes, which is governed by the above model.

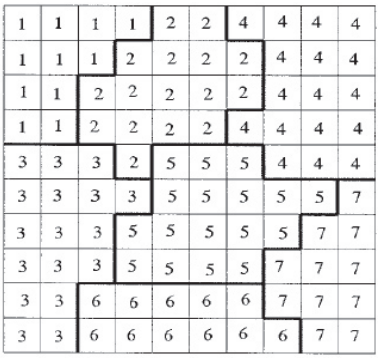

Fig 3. A zoom-in into the CA depicting 7 spin states representing 7 different cells. The cell boundary marked by dark lines.

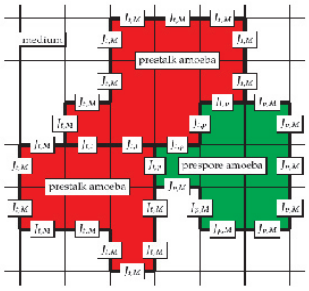

Fig 4. Three different cells depicted in the CA with two cell types represented in the CA. Also medium is represented as another cell. Interaction energies are marked at the cell boundary. The system evolves for minimum surface energy

Consider a 2 spin Potts model with CA sites having only 2 states. The following has been extensively used for explaining ferromagnetic properties in materials. An extension to it is the Q Potts model, where every site can now have any one of the total Q states possible in the system. State or also referred as spin of a site determines the contact surface energy of a cell to its neighbor. This is represented as a J_{ij} which models the surface energy of contact between cells of type i to cells of type j. Thus the total surface energy of cell i is given by :

$$Hi = \Sigma J_{ij} + 2 \Sigma J_{i,\,medium} \qquad \text{eq.1}$$

where Ji,medium models the contact surface energy of a cell due to contact with the medium. In the implementation of the 2D CA, the medium is also modeled as type of cell with spin σ which determines the type of the cell. Medium cells on the other hand no area constraint, ie. they can extend as much with no elasticity.Since this should not be true for cells other than the medium, a limitation factor proportional to the current updated volume of the cell is added to the surface energy. Thus

$$Hi = \Sigma J_{ij} + 2 \Sigma J_{i,\,medium} + \lambda(Vi - vi) \qquad \text{eq.2}$$

where Vi is the predicted cell volume for spin i and vi is the current volume of the cell.

The iteration and the update rule of the CA is designed such as to minimize the above described surface energy. Thus A random site is chosen at the border of a cell i, and another random neighbor is chosen (neighborhood factor 8) which does not belong to cell i. Now the state of this site is really copied to the previous site only if the flip amounts to a decrease in the total surface energy of cell i. Also some random mobility of the membrane is taken into account by implementing a thermal noise factor. Thus is P is the probability of flipping site belonging to cell 1 to that of cell j then

$$P(i \text{ to } j) = \exp(-\Delta H/T) \text{ if } \Delta H>0 \qquad \text{eq.3}$$
$$= 1 \text{ if } \Delta H<0$$

Where ΔH is the gain in the surface energy for cell i due to flipping. A set of such flips are defined as a single step.

Shape sequences as Boolean Network

The notion derives its inspiration from work by Kauffman [5] and others, showing that gene regulatory networks in organisms can be realistically modelled as Boolean Networks of fixed size. Since the mapping of a genotype to phenotype is complex, there are no mechanisms to predictably determine what state space in a genotype corresponds to what structure in the phenotype. This is also studied in RNA structures[] which shows the mapping is highly nonlinear. Later studies by Kauffman[] shows in spite of the above it is sufficient to model complex genetic networks as updated boolean network.

Shape sequences as described can be thought of as implementing a control structure to what is happening in the medium governed by surface-energy minimization. The same are modeled as a n node Boolean network with connectivity K <=2, and choosing only those networks which have attractor cycles of state more than 2.

Thus every simulation initially is assigned a boolean network and a state vector (random initially), which specifies a fixed state of the given cell, and its surface energy properties. For our simulations, I started with 20 node networks. For evaluating surface energy from the state vector, the following factors are crucial. Firstly energy coefficient for Cell i to j ie. Jij should be equal to Jji. Consider interaction of two cells with state vectors A and B. Thus degree of matching between vector A and B is evaluated which gives a value of surface energy coefficient Jij.

A simple scheme to implement the evaluation of Jij , which is symmetrical for i and j is :

a. Consider two state vectors A and B, representing two cells A and B.
b. Mirror B around to obtain B'
c. AND A and B' to obtain a MATCHING vector
d. OR left and the right half of the MATCHING vector to evaluate surface coefficient energy strength.

We also introduced small amount of cross talk between networks of neighboring cells. This is biologically analogues to Juxtacrine signalling in delta-notch patterns. Thus inter-cellular signalling is only limited to neighboring cells, forming a local neighborhood for each cell. This is done by using particular bits of a state vector (1st and 2nd bit always) from neighboring cells as input to the boolean network. Thus changes in state of a neighbor cell, can cause perturbation of the boolean network of a considered cell, causing it to differentiate into a new cell. Also, since Cell death suddenly causes new kind of cells in contact to each other, we speculate cell death of a region would trigger extensive cell differentiation and rearrangements.

Implementation of the above behavior required to maintain a neighborhood matrix for every single cell. This informs the cell which other cells to poll while updating its own network. Since the whole medium is a dynamic system, the neighborhood matrix of the cell is dynamically changing and needs to be updated. Overheads in this evaluations made the simulations very slow, which I have not been able to currently resolve.

Modelling Cell Division

Cell division is crucial in our model for studying evolution of patterns in a medium. In morphogenesis, cells divide and differentiate in developmental time scales. Thus all the genetic information from the mother cell is replicated from in both the daughter cells. Also, all the cell states encoded in the protein concentrations at the time of cell division, are also duplicated.

We model cell division based on a single criteria of cell volume. Thus whenever updated cell area of a given type of cell, exceeds its pre-set value by a factor, the cell is divided into two. Since we are using a fixed size 2D CA, and the number of sites in the CA representing individual cells is fixed, we introduce cell division, by assigning half the values of a given parent cell to two daughter cells. This removes any overhead of actually assigning new sites. Though the overall sites representing a cell thus decreases in developmental time, we argue that since we would be interested in a fixed number of divisions only it does not hurt us much.

Thus whenever a cell divides at a particular site, all the state vector of the given cell is duplicated and transferred as new state vector to the daughter cells. Also, since every cell needs to maintain a neighborhood matrix, they are also copied over to the new cells from the parent. At this point the neighborhood matrix is updated due to likely change in neighborhood.

Modelling Cell Death during morphogenesis

Cell Death has been shown to play a crucial role in spatial arrangements of cells during developmental cycle.

Our model captures Cell Death in two different scenarios. First is when cell death is caused by squeezing action of neighbors[6], thus causing sufficient loss in cell volume, which in turn triggers cell death.This depends on the surface bond energy and non-elasticity term in equations() described previously. Thus for a low value of λ the cells can squeeze other cells causing them to be killed. This is done by killing all the cells which fall below a fixed threshold of cell volume.

Also, cell death can be triggered by a cell **within itself**. Thus a given state of gene expression, which corresponds to a state vector of the given cell, rather than causing differentiation or growth, can cause Programmed Cell Death. This is because in the state space of the Boolean Network used, some of the states have been specifically demarcated to map to Apoptosis. Thus whenever any cells expresses that state, it is destined to die. The elimination may not be sudden though, since we would like this state to affect surrounding states. We need to be careful not to assign large attractor basins as states expressing apoptosis, to avoid large number of cells dying in our simulations. Thus the states chosen for Cell Death are hand picked from the state space based on the above considerations.

Also, when a cell dies, the CA is updated to fill the empty region with medium cells. This takes place in a single step, and is not biologically defensible. But it is known that average time it takes for a Apoptotic cell to disappear is far shorter than developmental time scale. Since in Biology, this kind of a clean-up process is implemented by a complex mechanism of phagocytes, where certain cells digest the ruminates of other cells. But we assume this does not have large affect in pattern formation which we are largely concerned about.

Model initialization and steps involved

The CA starts as base matrix of size n (=200) , with only a single cell. This is surrounded by a uniform medium. As in Garner model of cell rearrangement, this medium is modelled as a single cell with no area constraint. This brings uniformity in the model, and thus the medium can be treated like another cell.

The simulation starts as a single cell, with cells dividing for later growth. The differentiation is initially caused by manually flipping particular bit, used for solving symmetry breaking. This is true for the first and the second division in the starter cells. This is crucial so as to generate "interesting shapes". This is similar to "maternal genes" known to cause symmetry breaking in morphogenesis of various multi-cellular organisms.

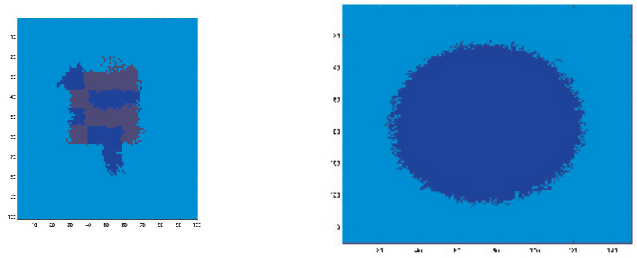

Fig 5. Depicted two initial configurations. a. Used for cell-sorting experiments, b. Initial single cell before the onset of differentiation

The Boolean Network is upgraded independently to determine the state of a given cell at any point of time. This is not currently integrated with the update of the CA. As cell-division occurs, parent cell state is copied to daughter cells, with duplicate state-vector of the boolean network. This is also accompanied by update of neighbor list of every single cell in the medium. Thus every cell-divison causes this huge overhead of updating all the lsits, which is currently unhappy in the current version of the code. Thus no results on the emergence of shapes in the medium could be simulated, as of right now.

Results and Discussion

Several phenomena in the domain of self-reorganisation were observed in the sorting simulations. This include a complete cell sorting, with more than 100 cells of two types in a medium. Cell engulfment and No-cell adhesion were also seen in a setting of 100 different cells. This also validated the functioning of the very first layer of the model.

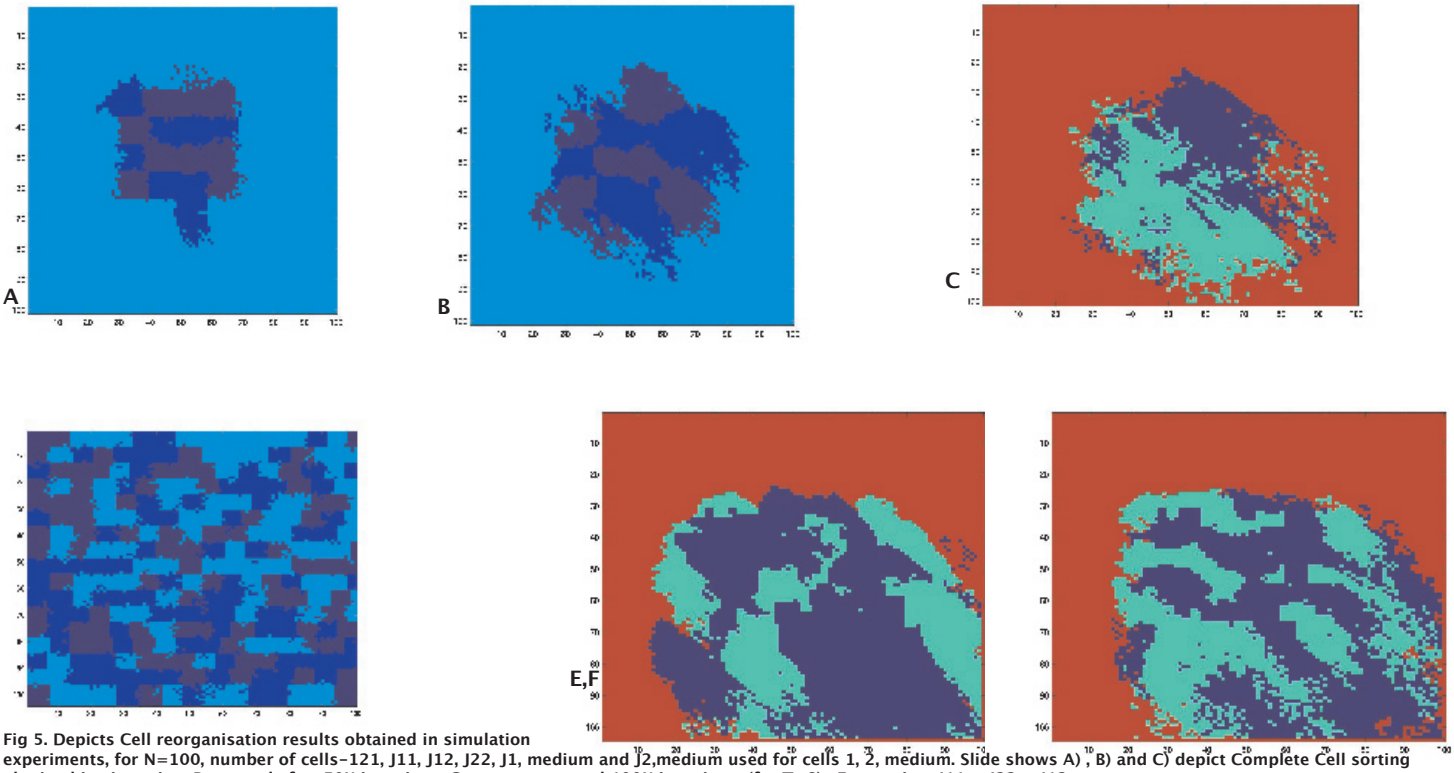

Fig 5. Depicts Cell reorganisation results obtained in simulation experiments, for N=100, number of cells-121, J11, J12, J22, J1, medium and J2,medium used for cells 1, 2, medium. Slide shows A) , B) and C) depict Complete Cell sorting obtained in simuation. B occured after 50K iterations, C occurs at around 100K iterations. (for T=6) . For sorting, J11 = J22 < J12
Slide D) shows no-cell cell adhesion with J11 > 2J1,medium ; J22> 2J2,medium. Thus as you can see clearly, cells move apart from each other with no noticable adhesion features. Slide E) and F) depict cell engulfment (with green cells engulfing the blue in red medium). with J1,2 < J1,medium; J2,medium < J 1, medium

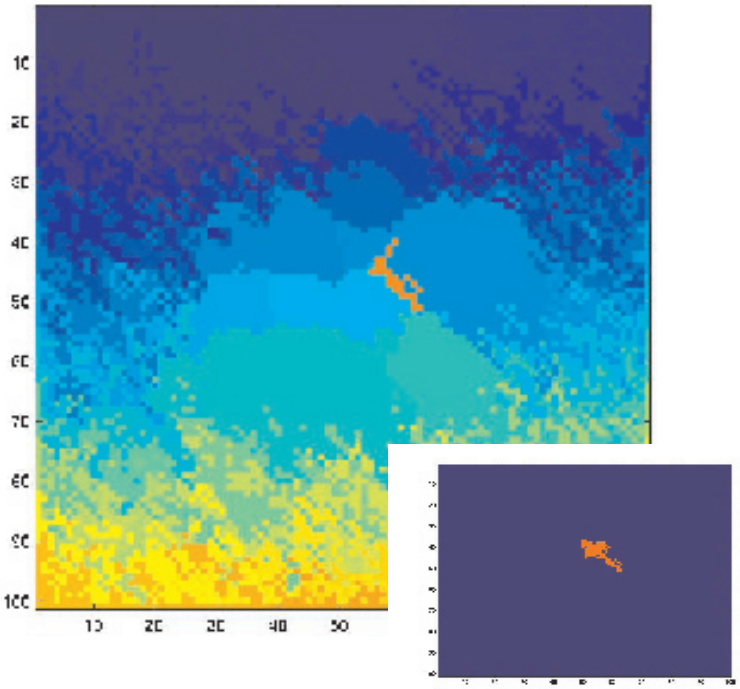

Fig 6. Cell division algorithm implemented in this slide. The window shows a sigle cell, marked orange; which after division is only half the volume of the older cell. Division took place since Area A of the given cell is above a threshold. The more jittery sites depict the medium cells (they break up since they have a negative area). Other color sites depict organic looking cells with rough boundaries.

Due to bugs in the neighborhood update rules I missed completing the loop and testing the whole system for shape generation. Thus though the Boolean network update and hence differentiation was implemented separately, it could not be tested with the Garner extended Q potts CA model.

Summary

I was able to mathematically formulate ways to model Cell death in existing Q-potts model used for morphogenesis. Also, the use of a layered model depicts the nature of developmental process itself, where information in set of micro “rules” (shape sequence) is converted into a phenotype shape with underlying physical laws which govern the dynamics of the whole system.

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