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COMPUTATIONAL AUTOMATA SIMULATION OF BLASTOCOEL ROOF THINNING IN THE *XENOPU LAEVIS* EMBRYO

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ABSTRACT

Computer simulation of the interactions between numerous cells is a novel approach to analyzing and comprehending the spatial and temporal patterns that are formed in a large network of cells within a tissue. Coupled with experimental observation, computational modeling at the cellular level is a powerful method that is capable of providing valuable information about the functions of quite complex biological systems. For this study, the biological system under examination is the *Xenopus laevis* embryo. In the blastocoel roof of the *Xenopus laevis* embryo, thinning of multiple cell layers into two cell layers is accomplished via radial intercalation of the deep layer cells. A cell-based simulation has been developed to analzye the process of blastocoel roof (BCR) thinning event. The cellular automata model can predict important characteristics of the BCR thinning process including total thinning time and spatial fibronectin fibril densities.

1 INTRODUCTION

In the blastocoel roof (BCR) of the *Xenopus laevis* embryo, thinning of multiple cell layers into two cell layers is accomplished via radial intercalation of the deep layer cells. Experimental work involving the observation of individual cells during morphogenetic events in the Xenopus laevis have been previously described (Keller, 1978). That work involved the use of time-lapse cinemicrography to investigate the behavior of individual cells in the superficial layer of the BCR of *Xenopus laevis* embryos. Subsequently, Keller progressed to describing in detail the cellular behavior of deep cells of the BCR (Keller, 1980). Scanning electron microscopy revealed the activities of individual cells in the deep layers of the BCR. Although the behavior of individual cells in the BCR was described, the cellular mechanisms causing such behavior were not revealed by this work. More recently, experiments investigating fibronectin matrix formation on the BCR have been conducted. For example, the mechanisms of fibronectin fibril growth on the BCR have been studied (Winklbauer and Stoltz, 1995), and the conditions necessary for fibronectin fibril formation have been explored (Winklbaeur, 1998). Furthermore, multiple scientific publications have suggested that the fibronectin matrix that forms on the BCR plays an important role in morphogenetic movements which occur subsequent to radial intercalation of cells in the BCR. For example, the importance of fibronectin in governing tissue separation during embryonic development has been described (Wacker, 2000).

At the University of Virginia, Dr. Douglas DeSimone's cell biology laboratory has been striving to acquire a greater comprehension of the cellular mechanisms responsible for BCR thinning. In particular, Dr. DeSimone and the members of his laboratory are interested in exploring the possibility that there may be an interaction between the expression of integrins and the expression of cadherins which affects radial intercalation. Their lab is also interested in examining the relationship between the formation of the fibronectin matrix on the BCR and radial intercalation in the BCR (DeSimone *et al.*, 2002). In 2001, Marsden and DeSimone described their examination of the importance of integrin and fibronectin during epiboly in *Xenopus laevis* (Marsden and DeSimone, 2001).

Systems which are characterized by complex behaviors that arise from the interactions of many components have been termed Complex Adaptive Systems (CAS) (Chan, 2001). The interacting agents in the *Xenopus laevis* embryo are cells. Cell-based simulations allow the nonlinear characteristics of a Complex Adaptive System (CAS) to be replicated. A programming tool called StarLogo was developed for purpose of building simulations of behaviors that emerge from the interactions of a large number of agents (Hayes, 1999). StarLogo has been used for simulat-

ing the behavior of biological systems such as cellular slime molds. NetLogo is a more recently developed programmable modeling tool that was built off of StarLogo and includes additional features (Wilensky, 1999). At the University of Virginia, Dr. Thomas Skalak's laboratory has developed simulations of living systems. For example, StarLogo was used to develop a simulation of microvascular remodeling (Peirce, 2002).

Applying the cell-based modeling technique to the BCR thinning event may help to reveal the underlying mechanisms responsible for individual cell behavior. Additionally, the cellular automata model may generate predictions about the BCR thinning process that will inspire further experimental work.

2 BLASTOCOEL ROOF THINNING

The BCR thinning process is accomplished via radial intercalation of multiple cell layers. The blastocoel roof is the region of approximately three to four cell layers in the animal cap of the embryo. Prior to thinning, the BCR consists of one layer of superficial or epithelial cells, and approximately two to three layers of deep or inner cells. As development of the embryo progresses, the three to four cell layers radially intercalate into two layers causing the BCR to thin.

As thinning proceeds, a matrix of fibronectin fibrils is assembled on the inner layer of cells (Winklbauer and Stoltz, 1995). The fibronectin matrix is thought to play an instructive role in the BCR thinning process (Marsden and DeSimone, 2001).

3 THE NETLOGO MODELING TOOL

The NetLogo modeling tool was used to develop the computer simulation of the BCR thinning process. NetLogo is a programmable modeling package that was developed for the purpose of simulating complex, multi-component systems (Wilensky, 1999).

3.1 The NetLogo Interface

The NetLogo main window includes an interface tab, an information tab, a procedures tab, and an errors tab (Wilensky, 1999). The interface tab includes a graphics window and is the screen where the running of the simulation is viewed and monitored. The procedures tab contains the code for the model, the information tab contains a description of the model, and the errors tab displays a warning when an error in the code is encountered.

3.2 NetLogo Agents

NetLogo uses agents that carry out commands. NetLogo has its own programming language that can be used to write procedures for the agents. In NetLogo, the agents that can move around are called turtles. Furthermore, turtles of different breeds can be created where each breed has specific behaviors. Models consisting of hundreds or thousands of turtles can be developed, and the complex patterns that arise from the interactions of these agents can be observed.

4 DEVELOPING A CELLULAR AUTOMATA MODEL

Dr. DeSimone's cell biology laboratory at the University of Virginia acquired the experimental measurements and observations necessary for the development of a model of BCR thinning. The computer simulation of BCR thinning was created using NetLogo. In the computational automata simulation of BCR thinning, the agents are cells. Hundreds of individual cells in the BCR interact, and the emergent behavior is the thinning of the BCR into two cell layers.

4.1 Experimental Measurements and Observations

Information about the BCR thinning process was obtained through collaboration with Dr. Douglas DeSimone's cell biology laboratory. Members of the cell biology laboratory obtained several experimental measurements and observations from *Xenopus laevis* embryos. Through experimentation, Dr. DeSimone's lab has determined that the time necessary for the completion of BCR thinning is approximately four hours, there are approximately 200 cells in the region of interest for the initial computer simulation, there is little if any lateral movement of cells during radial intercalation, the superficial cell layer is necessary for intercalation, and cell to cell contact is maintained throughout the thinning process (DeSimone *et al.*, personal communications, 2002). The BCR thinning model was constructed using the independent experimental data provided by Dr. DeSimone's laboratory.

4.2 Cell Types

There are different breeds to represent the different cell types in the model. All superficial cells are of breed "top" and are represented as blue squares. Deep cells that do not begin in the bottom cell layer are of breed "middle" and are represented as orange, red, and green squares depending on their initial location. Deep cells that begin in the bottom layer are of breed "bottom" and are represented as yellow squares. Figure 1 shows the initial configuration of cells in the BCR model where each cell type is represented as previously described.

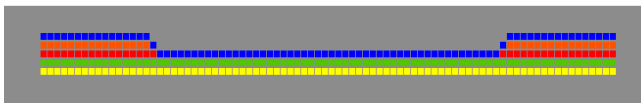

Figure 1: The Initial BCR Configuration

4.3 Cell Variables

Each cell has several variables. All turtles have predefined variables such as breed, color, shape, xcoordinate, and ycoordinate. For the BCR thinning model, additional variables have been created for each cell. Global variables are variables that can be accessed by all agents. The global variables that have been created for this model include variables such as "tempxcoor" which keeps stores the x coordinate of the most recently moved cell and "RndNbr" which is a random number used to determine the direction of cell movement. Furthermore, additional variables have been created for "bottom" cells. For example, a variable has been created in order to keep track of how long the cell has resided in the bottom cell layer.

4.4 Cell Behavior

There are multiple rules to govern the behavior of individual cells. For example, the movement of individual cells is dictated by a distinct set of rules. For instance, once a deep "middle" cell has entered the bottom cell layer, this cell is "captured" by the fibronectin matrix. The breed of this cell will be changed from "middle" to "bottom," and the cell will not leave the bottom layer.

Another example of a rule for the cells' behavior is that the superficial cell layer must always cover the entire region of the BCR being modeled. Therefore, as the deep cells intercalate and the BCR extends, the blue superficial layer of cells will extend to cover the entire length of the BCR.

A final example of a rule incorporated in the BCR thinning simulation is the rule for generating fibronectin fibrils on the bottom cell layer. This rule uses conditions such as the amount of time the "bottom" cell has spent in the bottom cell layer to determine whether or not that cell will produce fibronectin fibrils. Each bottom cell has a fibril variable whose value is recalculated at each step based on this rule.

4.5 Developing a Timing Mechanism

A timing mechanism was generated by using experimental results obtained from collaborators in the Cell Biology department. The measurement that was made by the experimental biologists was the "dilution rate" of the bottom cell layer. The dilution rate is the percent of cells entering the bottom cell layer per time. Experiments done in Dr. DeSimone's lab revealed that the dilution rate is approximately twenty percent in the first hour. A simulation clock was created by relating the dilution rate to the number of cells that have moved when a certain dilution had been reached. Assuming that the bottom cell layer will be "diluted" by twenty percent in the first hour allows the number of cells that have moved in one hour to be counted. The amount of time by which the model clock should be incremented each time a cell moves can be calculated by dividing one hour by the number of cells that have moved when the bottom layer has been diluted by twenty percent.

4.6 Model Assumptions

Several assumptions were made in order to simplify the initial model. For example, the model does not incorporate cell division. In addition, the model assumes that lateral cell movement is negligible; cells can move into an upper layer or into a lower layer, but they cannot move from side to side. Furthermore, the model assumes that the size and shape of the cell remains constant throughout the BCR thinning process. Although some assumptions are necessary to simplify such a complex biological process, the accuracy of the model may improve by decreasing the number of assumptions incorporated in the model's design.

5 EMERGENT THINNING PATTERN

In the BCR of the *Xenopus laevis* embryos, many individual cells interact and the system behavior that emerges from these individual cells is the thinning of multiple cell layers into two cell layers. The same pattern emerges in the computer simulation of the system, because the set of BCR cells thin eventually thins to two cell layers. Figure 2 shows a time sequence of a simulation of the BCR thinning process.

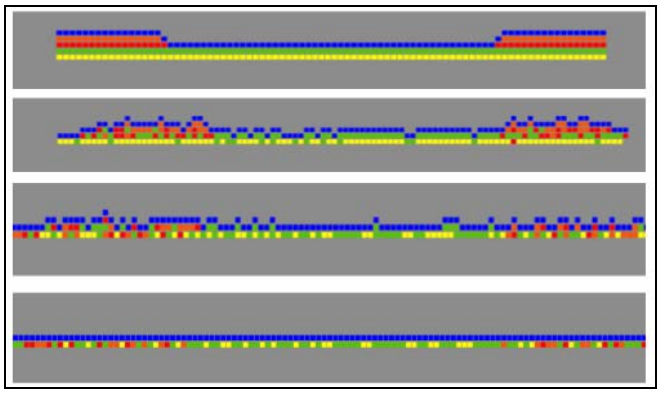

Figure 2: Snapshots of the Simulation During Thinning

6 PREDICTING THINNING TIME

The simulation was run 100 times to the point at which twenty percent dilution of the bottom layer was achieved,

and the average number of cells that had moved at this point was calculated. Based on this measurement, the clock was programmed to increment by 1.415 minutes each time a cell moves. The simulation was then run to completion and the average total time necessary for thinning to two layers was calculated to be 4.78 hours. This time approximates the experimentally observed time necessary for thinning.

7 PREDICTING CELL DISTRIBUTION

The distribution of cells from the initial cell layers in the final bottom cell layer was examined. Figure 3 shows the average number of cells from each initial cell layer that ended up in the left third, middle third, and right third of the bottom layer of the BCR at the end of thinning.

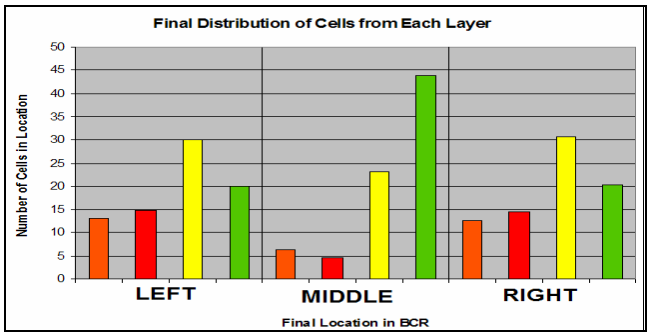

Figure 3: Distribution of Cells from Each Initial Cell Layer in the Bottom Cell Layer of the BCR Upon the Completion of Thinning

This graph suggests that, following thinning, more of the "green" cells (i.e. cells that began in the second layer) end up in the middle third of the bottom cell layer than on either the left or right third. The graph also suggests that more of the "orange", "red", and "yellow" cells (i.e. cells that began in fourth, third, and bottom cell layers respectively) end up on the left and right thirds of the bottom cell layer than in the middle third. Experimental work has not yet been done to verify this model prediction.

8 PREDICTING FIBRONECTIN MATRIX ASSEMBLY

The distribution of assembled fibronectin over the length of the bottom cell layer was inspected. Figure 4 shows a time sequence of the fibronectin fibril distribution over the length of the bottom cell layer as the BCR thins.

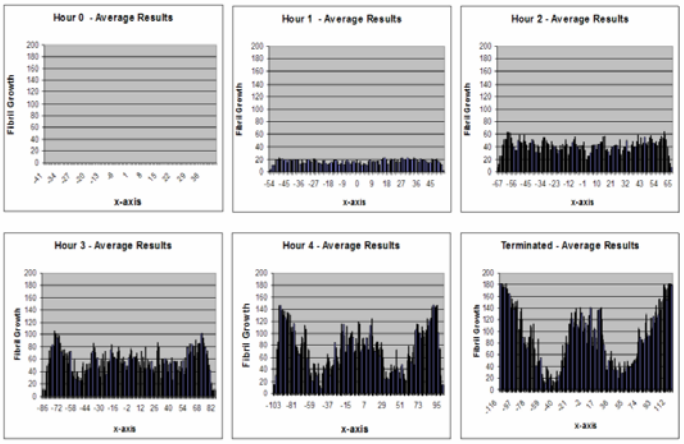

Figure 4: Fibronectin Fibril Distributions in the BCR Model

The model predicts that, at the end of thinning, the density of fibronectin fibrils will be relatively higher at the very center of the BCR and on either side of the BCR. This predicted distribution seems to resemble experimentally observed fibronectin densities. However, Dr. DeSimone's lab is currently performing experimental work involving the examination of spatial and temporal fibronectin intensities in an effort to quantitatively verify the model's prediction.

9 CONCLUDING REMARKS

The initial BCR model is capable of making important predictions about the thinning process. However, further modifications to the model may be necessary to fully realize the potential of the simulation method.

This paper descirbes a first attempt to develop a cellular automata model of one aspect of frog embryogenesis, BCR thinning. The objective of developing this model was to analyze the spatial and temporal patterns formed in the assembly of BCR cells during the morphogenetic thinning event. The results of the simulation indicate that such a model can be useful in investigating the underlying mechanisms responsible for BCR thinning. The cell-based model can predict important characteristics of the BCR thinning process including total thinning time and fibronectin fibril distribution.

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