The Perfect *C. elegans* Project: An Initial Report

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Abstract The soil nematode *Caenorhabditis Elegans (C. elegans)* is the most investigated of all multicellular organisms. Since the proposal to use it as a model organism, a series of research projects have been undertaken, investigating various aspects of this organism. As a result, the complete cell lineage, neural circuitry, and various genes and their functions have been identified. The complete *C. elegans* DNA sequencing and gene expression mapping for each cell at different times during embryogenesis will be identified in a few years. Given the abundance of collected data, we believe that the time is ripe to introduce synthetic models of *C. elegans* to further enhance our understanding of the underlying principles of its development and behavior. For this reason, we have started the Perfect *C. elegans* Project, which aims to produce ultimately a complete synthetic model of *C. elegans*' cellular structure and function. This article describes the goal, the approach, and the initial results of the project.

Keywords
computational biology, *C. elegans*, development, simulation of biological systems

1 Introduction

When Sydney Brenner proposed the investigation of *C. elegans* to the Medical Research Council, he chose it because it was the simplest of all differentiated organisms [7]. The decision proved wise, generating a number of fruitful results including the complete identification of the *C. elegans* cell lineage [11, 17, 18] and its full neural circuit topology including all synapses and gap junctions [23]. Using a whole mount in situ hybridization, the complete *C. elegans* DNA sequencing and expression pattern mapping are now within our reach [19, 20]. These investigations clarify what *C. elegans* is composed of and how each of its individual components work. However, an understanding of the components and their isolated functions does not lead to an understanding of the dynamics behind the global development and behavior of this organism. Despite the fact that it is the simplest known differentiated organism, *C. elegans*' dynamics are still too complex for us to grasp fully at this time.

This situation typifies the problems that lie ahead in molecular biology. The human genome projects and other genome sequence projects will soon identify complete DNA sequences and clone all genes, giving the ultimate reductionist view of ourselves and other organisms; however, this does not directly lead to an understanding of the full interactions that make up living organisms. Because many phenomena occurring in living organisms are complex and nonlinear, it is almost impossible to understand their characteristics fully without the aid of simulation and modeling. Thus, we claim that an in-depth understanding may only be achievable through reconstructing the phenomena with computer simulation. By creating a detailed model of a specific biological system, (in this article, *C. elegans*), we intend to establish a more solid theoretical method for biology.

In modern particle physics, the work of theorists is essential. They formulate theories and predict hypothetical particles at precise energy levels. Experimental physicists then try to discover these predicted particles. For example, Glashow [9], Weinberg [22], and Salam [16] proposed the *electro-weak theory*, which explains the interaction of electromagnetic forces and weak forces. This theory predicted the existence of a hypothetical particle called an "intermediate vector boson." Carlo Rubia, an Italian experimental physicist, led the UA-1 team at CERN to discover this particle [2, 3], whose discovery was also confirmed by the other team, UA-2 [4]. They were successful and the theory was confirmed. This is a typical success story in particle physics.

We wish to bring such successes to biology, by providing a theoretical model that not only can display known data but can make specific predictions that can then be verified or rejected through experimental effort. Due to the complexity of biological systems, it is natural that a computational approach be taken instead of the analytical approach of theoretical particle physics.

By implementing a detailed model, we can verify how much we really do understand about biological systems such as *C. elegans*. Due to the complexities of genetic and cell-to-cell interactions, and the large number of participating genes and their products, it is extremely difficult for us to understand fully and verify whether a certain hypothesis can be supported by biological findings. A possible use of the simulation model is to bring the genetic interactions of genes into focus to see if hypothetical genetic interactions can consistently recreate phenotypical changes that match known biological observations. If the results of simulation differ from actual observations, there are two possibilities: Either the simulation or the hypothesis is incorrect.

The accuracy of simulations needs to be carefully examined and verified before the model can serve as a testbed for hypotheses. It should be noted, however, that even with a limited-accuracy model, hypotheses can be tested if they focus on specific aspects of genetic interactions and involve logical interrelationships of interactions rather than details concerning the subtle balance of participating components.

By the same token, a well-designed model can be used for predicting the workings of possible molecular mechanisms that are involved in specific phenomena. The ideal approach is for the model to provide a set of possible molecular mechanisms for specific phenomena whose true mechanisms are unknown. Experimental biologists can then design experiments to identify which of these mechanisms actually exist.

To accomplish such a task, a number of basic system infrastructures must be developed. For multicellular organisms, it is vital to develop simulation and visualization systems of cell positions, shapes, and time course changes. Without using such a three-dimensional simulation system, complex cell-to-cell and gene interactions may be near to impossible to grasp, much less implement.

In a first attempt at this approach, we have developed detailed simulation models of *C. elegans* and *Drosophila melanogaster* (another widely studied organism), as a part of the Virtual Biology Laboratories [12]. Given the abundance of data and the expected progress of analytical methods for these organisms, they are the natural target for this approach.

2 *Caenorhabditis elegans*

C. elegans is a small worm found in soil and ubiquitously observed throughout the world. It accounts for the largest biomass on earth. It has a life span of about 3 days and feeds on bacteria. *C. elegans* has no female sex (only hermaphrodites and males), so most every worm in the population is a genetic clone. This, plus the simple nature of the organism, means that lineages, positions, and interactions of *every single cell* can be mapped out. The adult male *C. elegans* has exactly 1,031 somatic nuclei, and the adult hermaphrodite has 959 somatic nuclei. The lineage of these cells has been fully identified by the extensive work of various research groups (one in particular: [18]).

The nervous system of *C. elegans* is relatively simple. Its hermaphrodites have only 302 neurons and 56 glial and associated support cells. This accounts for 37% of all somatic cells. In the adult male, the number of neurons is 381, and there are 92 glial and support cells, which is 46% of all somatic cells. White differentiated these neurons into 118 classes and reported that there are about 5,000 chemical synapses, 2,000 neuromuscular junctions, and 600 gap junctions [23].

A haploid *C. elegans* genome is composed of 8×10^7 nucleotide pairs. The *C. elegans* genome project is being carried out by the Sanger Center and Washington University, which has already sequenced more than 25% of its genomes. Current progress suggests that all genes, estimated to number about 13,000, will be identified within a few years.

The mechanism of fate determination involving maternal genes has been intensively investigated. However, fate determination in later cells is largely unknown because downstream genes have not been identified. To investigate the genetic interactions for fate determination, a project to identify the genes that are expressed in a specific cell lineage has been initiated at the National Institute for Genetics [20]. This project uses in situ hybridization on whole mount embryos to identify the expression of genes at specific cells at specific times during embryogenesis.

Many mutants have been isolated that can be used for genetic analysis. For example, the *ced* family affects programmed cell death, and mutations in the *lin* family of genes cause cell lineage abnormality. There is a large list of genes and their phenotypical disorders, allowing for a wide range of manipulations to help in the investigation of cellular development. Other manipulations are possible during embryogenesis through laser ablation or direct micromanipulations.

3 Project Goals

Given these biological accomplishments and on-going efforts, the Perfect *C. elegans* Project aims to create a detailed simulation model of *C. elegans* to promote our understanding of the organism and of life in general. Implementing the model can be extremely useful for biologists studying *C. elegans*. It can be a comprehensive visual database of the organism and can assist in cell identification. Currently, the most widely used computer-assisted system for *C. elegans* is the Angler system, also called the 4D system, developed by the Sanger Center. Angler is essentially a collection of tagged images taken by Nomarski confocal microscopy. However, the Angler system does not provide the capability to rotate images or to animate the embryogenesis process. A good computer model can complement the Angler system by providing computer graphic images and simulations linked with a set of optical images.

While modeling *C. elegans* on a computer is useful in its own right for augmenting biological knowledge, the visualization of embryogenesis is one of the most persuasive tasks of the project. Visualization helps researchers to conceptualize both global and local issues in embryogenesis, and to identify cells during experiments. This requires tools to assist biological observations using three-dimensional computer graphics of the complete *C. elegans* development process (possibly coupled with image understanding techniques for automatic data acquisition and semi-automatic cell identification), a simulator for a complete neural circuit, and an integrated database on *C. elegans*.

To begin meeting these goals, we have so far developed three modeling and visualization systems for *C. elegans* cellular data. The first, an embryogenesis visualization system, uses a sophisticated dynamics model to display the embryogenesis process cell by cell. The second, a neural simulation system, models the known neural circuitry involved in *C. elegans* thermotaxis. The third, a portable Java-based three-dimensional visualizer, displays simple cell position, neural connections, and other information using new positional data recently made available, without attempting the sophisticated cellular modeling and three-dimensional rendering of the first system.

4 Visualization and Simulation of Embryogenesis

As the first step toward these goals, we have developed a computer graphics system that helps visualize the development process of *C. elegans*. This visualization system is an appropriate starting point because it provides a three-dimensional model of *C. elegans*, so that the cell-to-cell interaction dynamics, at both the physical and chemical levels, can be superimposed on the current model. This could greatly help research in developmental biology. The current system is based on the cell lineage and cell location data published in [18]. The system generates a computer graphics image from the division of the first cell to around 600 minutes after the first cell division.

It is no trivial task to create a reasonably accurate computer graphics image based on the Sulston data because the information necessary to create a full three-dimensional model is missing. The following information was available:

- the complete cell lineage chart
- hand-drawn pictures in 2 1/2 dimensions
  - all 28 cells at 100 minutes
  - 55 of 180 cells at 200 minutes
  - 137 of more than 350 cells at 260 minutes
  - 156 of more than 350 cells at 270 minutes
  - nearly all cells at 430 minutes
- qualitative descriptions of the embryo shape
- qualitative descriptions of the disparity in the size of divided cells
- general information on migration

In addition, the lineage chart indicates only approximate division time and a rough direction of the division, such as anterior-posterior, dorsal-ventral, or left-right. For example, the cell lineage of two ring interneurons (ADAL and ADAR) are given as `ADAL AB.plapaaaapp` and `ADAR AB.prapaaaapp`. This means that the `ADAL` cell was derived from the founder cell `AB` through a series of divisions: The first division was anterior-posterior, and ADAL is derived through the posterior child. The second division was left-right, and ADAL is derived through the left child. The remaining divisions are anterior (`a`), posterior (`p`), four anterior (`aaaa`) divisions, and two posterior divisions (`pp`). ADAR is the symmetrical counterpart to ADAL, and has a similar lineage, but made a division to a right cell after the posterior division.

In reality, the direction of cell divisions are not necessary aligned with these axes. The simulator has to deal with this issue carefully. By the same token, the location of cells in Sulston's article [18] are drawn as a series of two-dimensional figures. The vertical position of cells are shown only by using circles having three levels of thickness. While the approximate two-dimensional location of cells can be estimated from these figures, it only provides a crude idea of the depth (or vertical position) of cells. Again,

Table 1. Data record for cell lineage and cell positions.

R	Cell name	d_1	d_2	x	y	z	vol	time	x_1	y_1	z_1	r_1	t_1	x_2	y_2	z_2	r_2	t_2
f	Egg	AB	P1	0	1	0	.6	0	0	0	0	5.4	0					
p	AB	*a	*p	0	1	−1	.5	18										
p	ABa	*l	*r	−1	0	0	.5	35										
p	ABp	*l	*r	−1	0	0	.5	35										
p	P1	EMS	P2	0	1	−1	.6	19										
p	EMS	MS	E	0	1	0	.6	38										
p	P2	C	P3	0	1	1	.6	44										
									⋮									
p	ABalaaappr	l	r	−1	0	0	.5	287	5.0	15.8	−2	1.8	260	−3.9	15.3	−5	1.7	270

the simulation method needs to develop a good estimate of the true three-dimensional location of these cells.

These efforts are necessary to make a best guess on the three-dimensional position of cells. This is essential to create accurate computer graphics images. Obviously, a straightforward way would be to collect three-dimensional position and shape data in a series of time steps, to create graphics based on these data. This approach is extremely labor-intensive because each cell must be manually identified at each time step. To collect this data, researchers need a crude three-dimensional visualization tool to assist in the identification of cells and their position. In addition, such an approach cannot be applied to visualize the development of organisms that have only a limited amount of position information. Therefore, we need to develop a system that can generate reasonably accurate three-dimensional computer graphics images based on limited data sets.

Our strategy to overcome this problem is to merge simulation with data. First, to assure the accuracy of the computer graphics image, cells must be in the position given by the observed data. Second, various simulation techniques are used to fill in missing information, such as the location of cells not provided in the data. To do this, the system computes forces between cells, such as the force that pushes back colliding cells. However, if only a dynamic simulation is used to decide the position of cells, some cells will not be in the position described in the observed data because of cell movement. To compensate for this discrepancy, the force that a cell generates for its movement is estimated using inverse kinematic techniques and is added to the cell's force vector. Cell movements are computed as objects in a viscous fluid.

4.1 Data Records

A set of data obtained from actual observation must be represented in machine readable form. Table 1 shows part of a data record that represents cell lineage and cell positions taken from the Sulston data. R is the record type (f and p mean a full record, x means a terminal record), and d_1 and d_2 are the names of daughter cells. When an asterisk (*) precedes a symbol, such as *a, it is concatenated to the name of the mother cell to create the name of the daughter cell. For example, AB's daughters are labeled *a and *p in the table. The full names of these daughter cells are ABa and ABp. The direction of division is indicated by x, y, and z. The relative volume of the cell d_1 is shown under "vol." The time of the division is indicated under "time." The notations x_1, y_1, z_1, r_1, and t_1 are the x, y, and z positions and cell radius at time t_1 (similarly, later columns continue describing x, y and z positions and cell radii at additional time points).

This database contains most of the information provided in the Sulston article. Even when we obtain more accurate data, we can continue to use this data record by making minor changes. One point that needs to be changed is the way the cell division direction is specified. In the current data structure, it is represented as a three-dimensional vector where each dimension has only three discrete values (1, 0, and −1). This might be changed to allow continuous values. Cell shape information is also not included in the current version, but we hope it will be added in the future.

4.2 Interpolation and Kinematics

Although the database contains some data on cell lineage, position, and other information, this is not sufficient for visualization. The simulation system uses interpolation and kinematics to estimate positional values when data are not available.

When a cell divides, its two daughter cells divvy up the volume of the mother cell. The volume conservation rule imposed at division time can help determine the approximate position of the center of each daughter cell for daughter cells. Immediately after each cell division, the new cells are moved to positions consistent with known dynamics. The position of the cell must also be consistent with the estimate provided by the observed positions of the daughter cells. Given the center-of-cell positions of the two daughter cells (x_1, y_1, z_1 and x_2, y_2, z_2), the center-of-cell position of the mother cell is assumed to be in the middle of the daughter cells ($x = \frac{x_1+x_2}{2}, y = \frac{y_1+y_2}{2}, z = \frac{z_1+z_2}{2}$).

However, this assumes that there is no cell movement, a consideration that requires a more complex estimation strategy. Although the division time of each cell is known, the location of the division is not described in the Sulston article. Thus, we had to interpolate the position where the cell division takes place. For data, we can use the position of the mother cell (cell number = 1) at time t_1 as $\vec{x}_1(t_1)$ and the positions of the daughter cells (cell numbers 2 and 3) at time t_3 as $\vec{x}_2(t_3)$ and $\vec{x}_3(t_3)$. The center-of-cell position of the mother can be obtained as the middle position of the center of two daughter cells: $\vec{x}_c = \frac{\vec{x}_2+\vec{x}_3}{2}$. Assuming that the division took place at time t_2, the position of cell division can be obtained by using the linear interpolation as

$$\vec{x}_1(t_2) = A\vec{x}_1(t_1) + B\vec{x}_c(t_3) \tag{1}$$

$$A = \frac{t_3 - t_2}{t_3 - t1} \tag{2}$$

$$B = 1 - A \tag{3}$$

This is used for simulations when less computation power is available. The spline interpolation method is used for the default simulation model:

$$\vec{x}_1(t_2) = A\vec{x}_1(t_1) + B\vec{x}_c(t_3) + C\vec{x}_1''(t_1) + D\vec{x}_c''(t_3) \tag{4}$$

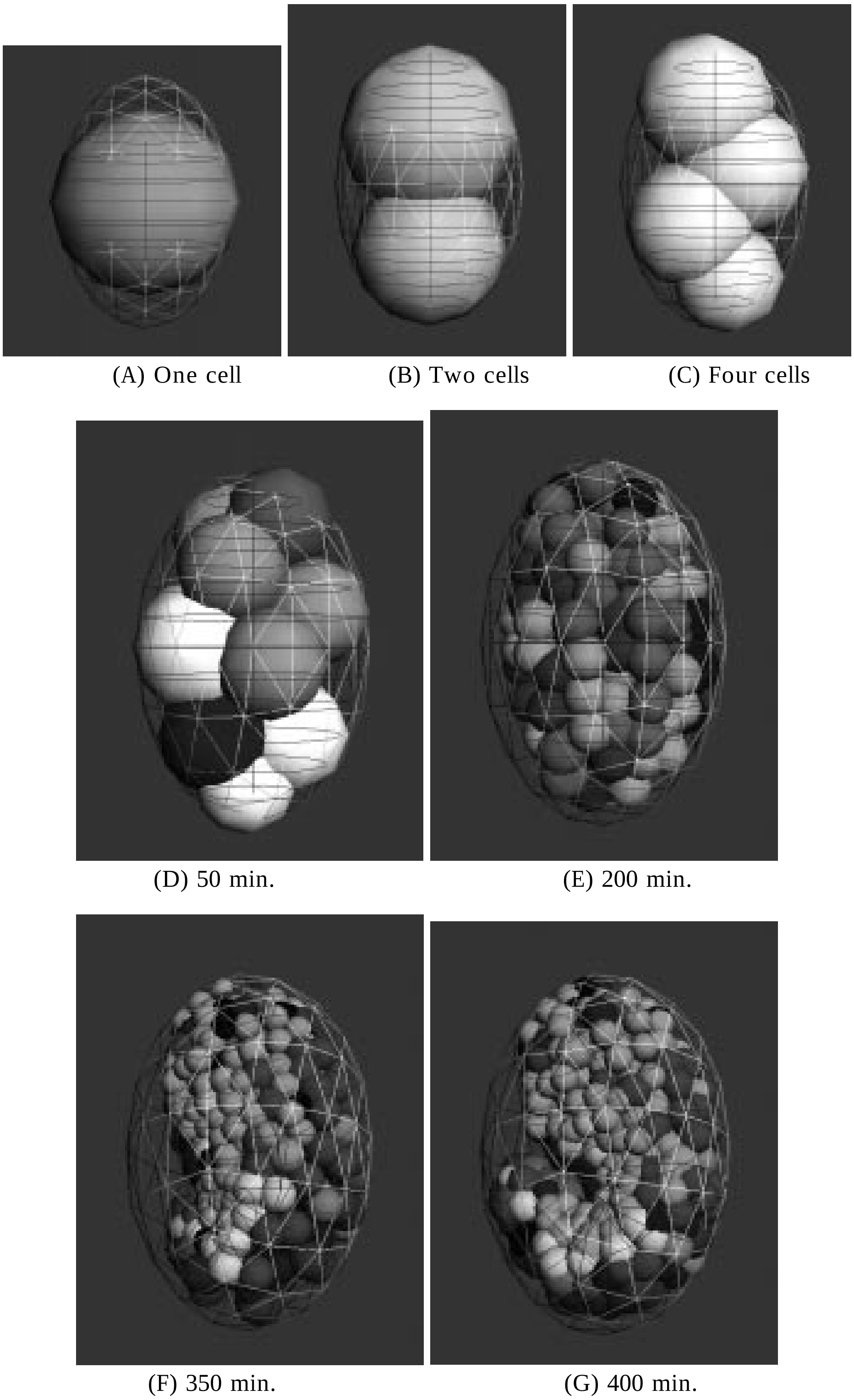
$$A = \frac{t_3 - t_2}{t_3 - t1} \tag{5}$$

$$B = 1 - A \tag{6}$$

$$C = \frac{1}{6}(A^3 - A)(t_3 - t_1)^2 \tag{7}$$

$$D = \frac{1}{6}(B^3 - B)(t_3 - t_1)^2 \tag{8}$$

These are the basic techniques for interpolation based on the available data.

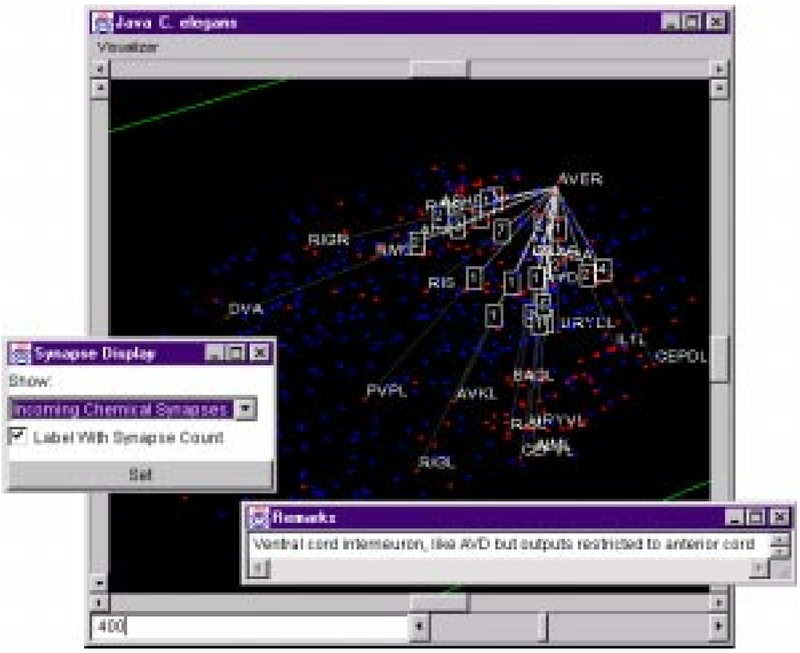


(A) One cell (B) Two cells (C) Four cells

(D) 50 min. (E) 200 min.

(F) 350 min. (G) 400 min.

Plate 1. Snapshots of computer graphics images of *C. elegans* embryogenesis.



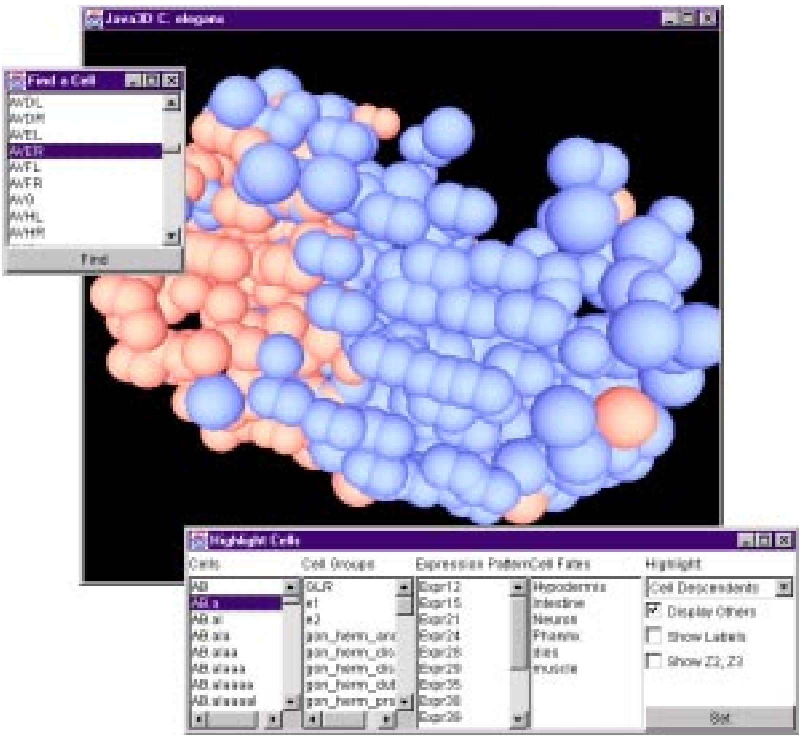
Show: Incoming Chemical Synapses

☑ Label With Synapse Count

Set

Ventral cord interneuron, like AVD but outputs restricted to anterior cord

(A) Chemical synapse connections from neurons to the AVER neuron.



Highlight: Cell Descendents

☑ Display Others

□ Show Labels

□ Show Z2, Z3

Set

(B) The *C. elegans* embryo 375 minutes after the first cell division, showing the first stages of gastriculation. Lighter cells are descendants of cell AB.a; darker cells are all others.

Plate 2. The Java 3D visualization system for *C. elegans*.

Table 2. Color Maps.

Color	Cell Fate
Red	Dermal cells
Green	Neural cells
Blue	Digestive system cells
Cyan	Body muscle cells
Magenta	Germ cells
Yellow	Excretory system cells
White	Miscellaneous blast cells (also mixed lineage cells)
Dark Green	Tail spike cells
Dark Grey	Cells which will die
Black	Dead cells (not yet absorbed by other cells)

(a)

Color	Precursor Cell
Red	AB
Green	MS
Blue	E
Cyan	P, Z
Magenta	D
Yellow	C
White	Early generation (ex. Egg)

(b)

However, because the time intervals of existing data are so large that simple interpolation does not capture the complex movement of cells, we need a simulation of dynamics as well. Such an approach is also essential to simulate phenotypical changes due to the microscopic operations of cell positioning, laser ablation, and mutant analysis where some cell movements are affected. The current version models these kinetic interactions of cells as a coupled continuous elastic system, where many masses are coupled by elastic springs. Although cells are displayed as spheres and no elasticity is visualized, it appears from the graphics image that cells are overlapping each other. In such cases, a repulsive force is imposed on overlapping cells. The simple way to approximate this force is a linear increase of force proportional to the length of the overlap. A more detailed simulation demands a more concrete force calculation. The details of the algorithms and equations used in these kinematics methods are beyond the focus of this article.

The shape of the embryo changes after 200 minutes to create the so-called “comma” or “2-fold” shape. Because the driving force that bends the embryo’s shape is not identified biologically, the computer simulation imposes a top-down kinematic. At 200 minutes, a cylindrical coordinate system is imposed to describe the cell locations, and the cylindrical coordinate system itself is bent slowly to agree with the observed shape so that cells change their absolute positions according to the bending of the cylindrical coordinates (Plate 1, F and G). As soon as a mechanism for bending is identified, we will incorporate it into our system and remove this top-down part of the simulation.

4.3 Implementation

The current implementation of the system uses the C++ programming language, the OpenGL graphics library, and the SGI sphere library on a Silicon Graphics workstation. The simulation gives a real-time animation of the embryogenesis process. Some of the images are shown in Plate 1. Colors assigned to cells are based on the cell fate color map. A, B, and C are snapshots of the computer graphics image at the initial stage (egg), two-cell stage, and four-cell stage, respectively. D, E, F, and G are snapshots at 50 minutes, 200 minutes, 350 minutes, and 400 minutes after the first cell division, respectively. F and G show the gradual bending of the embryo into the "comma" state.

The coloring of cells can be selected from two basic classifications: either by cell fate or by their mother cells (Table 2). Coloring of cells can be selected to suit the purpose of the visualization. Information is held in the cell color assignment map in the system. The default assignment map is based on cell fate, as shown in Table 2a. An alternative color map assigns colors to each cell based on the cell precursor (shown in Table 2b). All images shown in Plate 1 use the default color map. If "transparent" is assigned to a cell, the cell will not be visible on the image, although it exists for computing dynamics. Using this feature, it is possible to display only a certain cell, making the other cells transparent. The system allows for zooming in and out, and rotation around the x, y, or z axes. Simulation can be paused at any time step during the execution, allowing users to examine the visualized image as a still picture. Currently, all cells are shown as spheres. The use of more accurate cell shapes is possible, but at a much greater computing cost. Deformations in a cell's shape and the forces involved are approximated by overlapping spheres, and the repulsive force is proportional to the degree of overlap. A future system will implement cell shape deformation and an elastic model.

5 Neural Circuits

The neural system of *C. elegans* consists of 305 neurons with over 5,000 connections. The circuit topology is fully identified, although it may contain some errors, and various biological investigations are being made. In addition, a machine readable form of circuit topology data is available [1]. A few reports exist on the simulation of parts of the neural system of *C. elegans*, such as a mechanosensory subsystem [24, 25].

In conjunction with the embryogenesis model, we are developing a simulator for neural systems to identify the functions of each component in the circuitry. Initially, we have built a simulator that focuses on a neural subsystem involved in thermotaxis. Thermotaxis is an example of *C. elegans'* learning and memory behavior. When *C. elegans* is kept in a thermal gradient dish and given a food at a certain place, it memorizes the temperature of the area where it got the food. Later, it tends to move toward the temperature zone where it got food in the past. A neural subsystem for thermotaxis is mainly composed of 20 neurons (12 sensory neurons, 7 interneurons, and a single motor neuron) [14].

Our simulator can generate arbitrary input stimuli and simulate the possible voltage levels of each neuron. Information about even local neural dynamics in *C. elegans* is far from complete. We hope to improve the simulator so that it can incorporate new results from laser ablation experiments, mutant analysis, and so forth.

Figure 1 shows a screen of a simulation system for the neural subsystem responsible for thermotaxis. Arbitrary stimuli can be given in the form of an electrical voltage change on the sensor neurons. In this neural circuit, a continuous voltage change is propagated instead of spikes, which reflects the real physiological characteristics of the *C. elegans* neural system (*C. elegans* neurons are believed to have no action

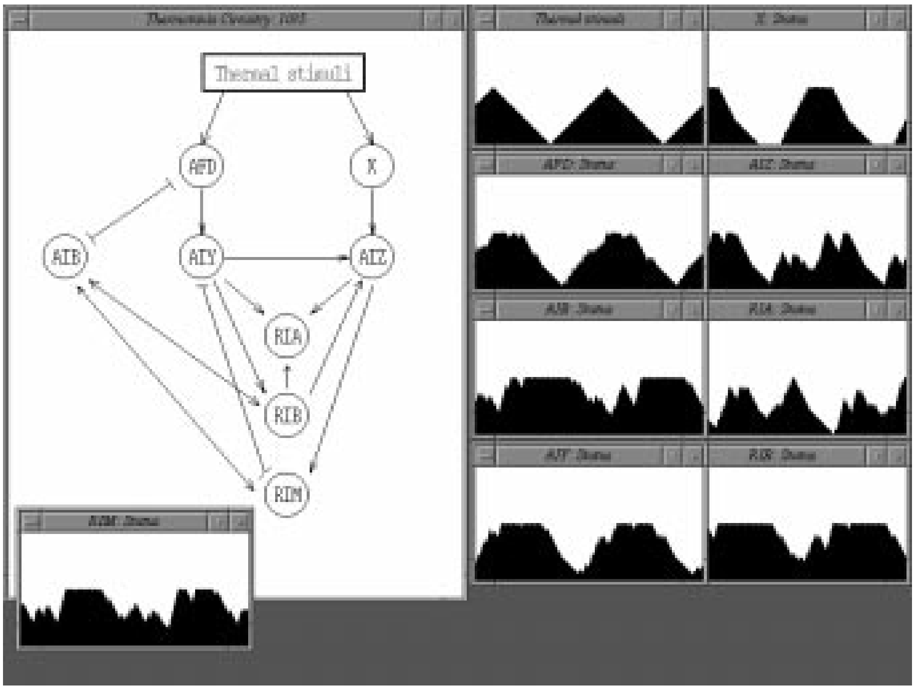


Figure 1. A simulator for a thermotaxis subsystem.

potential). The polarity, delay, and conductance of the connection can be changed with the simulator.

6 Three-Dimensional Visualization in Java

After the development of our three-dimensional *C. elegans* model, microscopy data is now becoming available that, to a limited extent, gives accurate three-dimensional position information for many preembryonic cells [10]. This data, along with gene sequencing, cell lineage data, and known neural connections, are now freely available at the *C. elegans* database on-line at `http://probe.nalusda.gov:8300/cgi-bin/browse/acedb`. This data is still sparse, but we will soon incorporate it into our three-dimensional C++ cell model to assist its model-based cell position estimation.

We have also created a three-dimensional visualization system written in 100% pure Java that uses this new data to show the position and early migration of preembryonic *C. elegans* cells. Plate 2 shows a screenshot of this system. This system is different from our C++ model in that its intent is not to *simulate* cell dynamics, but only to display visually known biological data available on the Web. The system not only displays three-dimensional position data, but also neural synaptic connections, cell fates and expression patterns, lineages, and so forth. For completeness, the visualization system can also show postembryonic cells; because postembryonic cell location and migration information is currently not available (and there is a *lot* of postembryonic cell migration, beginning with the "comma" migration), the system just assigns postembryonic cell positions near their parents' positions based on a simple cell-splitting lineage heuristic.

The visualization system comes with a number of options. The system allows the user to find cells by name, display chemical synapses and gap junctions, and highlight neurons, cells of a given cell fate, cells with a given expression pattern, or the descendents of any particular cell.

Because we have implemented the system in Java, it is highly portable. However, Java is new and necessitates some compromises. As efficient, portable three-dimensional rendering technology for Java is currently nonexistent for most platforms, on platforms without support for Java 3D, the Java visualization system draws cells simply as points indicating the position of the center of the cell. Java 3D-enabled platforms render cells as spheres whose volume is approximated with a simple cell-splitting heuristic. Synapses are similarly displayed simply as lines connecting cells. Because known three-dimensional cell data is sparse (perhaps two data points per cell on average), the system must interpolate positions between known data time stamps. In contrast to our more sophisticated C++ visualization system, for efficiency's sake the Java system currently uses only simple linear interpolation.

Nonetheless, we have found the Java simulator to be a useful tool for visualizing known three-dimensional embryogenesis data, cell types, and especially neural interconnections in *C. elegans*, primarily because of the wide range of data it can display. When better data becomes available, we hope to augment this system with postembryonic cell migrations and positioning, giving the user a full tour of *C. elegans* cell lineage from the single zygote to the final worm, complete with neural connections and other useful cell data.

7 What's Next: Cell Fate Determination

It was once believed that the cell fate of *C. elegans* is autonomously determined, without the interaction of other cells. However, recent findings suggest that extensive cell-to-cell interactions are necessary to determine cell fate. In the first phase of the Perfect *C. elegans* Project, we intend to develop a simulation system that can replicate various biological experiments related to cell fate determination in the early stage of embryogenesis. We will particularly focus on cell fate determination until the 46th cell stage, which is about 2 hours after the first cell division. This is because there have been extensive investigations at this stage concerning cell-to-cell interactions, the genes involved, and their mutations. This stage also represents important aspects of cell fate determination mechanisms, which can be generalized to clarify the cell fate determination of other model animals.

A brief explanation to illustrate this process would aid in understanding this issue. When an egg divides, P granules are localized on one side of the cell; as a result, when the egg divides into the daughter cells P1 and AB, the P granules wind up primarily with the P1 cell. At the division of the P1 cell, P granules are again localized to one side to appear subsequently primarily in one of P1's daughters, P2. Hence, the localization of P granules determines cell fate autonomously. However, the determination of cell fate for the cells EMS and ABp, for example, are influenced by cell-to-cell communication. At the surface of the P2 cell, the genetic agent APX appears; through contact with the P2, the ABp and EMS cell membranes receive APX, which causes them to express the agents GLP-1 and mom-2, respectively. Should those cells be isolated from the P2 cell, these genetic agents are not expressed and cell fates of ABp and EMS will change. An overview of this process can be found in various sources [15]. Currently, we are developing a simulation model that enables the replication of such mechanisms.

Specifically, we will create a simulation system that can replicate known experiments on the effect of cell positions and interaction on axis determination. These experiments include loss-of-function mutant analysis for

- the anterior-posterior axis determination (*par* family)

- cell fate determination of the founder EMS and P2 cells (with mutation of genes *skn*-1 [5, 6], *pie*-1 [13], and *mex*-1)
- cell fate determination for ABa and ABp, with dorsal-ventral axis and left-right axis determination (using mutants of genes *glp*-1, and *apx*-1, and laser ablation)

Other genes such as *emb*-5 and *lin*-12 will be modeled after establishing the simulation technique. Once we develop a simulation system that can accurately reproduce the above phenomena, it will be applicable to a wide range of biological systems. To make this part of the simulation as accurate as possible, we are planning to design a system to accept the National Institute for Genetics' *in situ* hybridization data [20].

8 Concluding Remarks

The ultimate goal of this work is to establish a powerful scheme for computer-aided biology. Our approach may be best characterized as *systems biology*, because it attempt to introduce simulations, dynamical systems analysis, and other methods generally applicable to the understanding of complex nonlinear systems. Contrary to many artificial life research approaches, which start from a biologically inspired but usually abstract dynamic model, our research is based on biological findings available at present and intends to look carefully into what actually takes place in life as we know it.

Because this project has just been started, the Perfect *C. elegans* Project is nowhere near the stage necessary to predict unknown and significant biological mechanisms. The work reported here is only a first small step toward a synthetic approach to *C. elegans* biology. Simulation techniques have been developed for *C. elegans* that are applicable to the development of various organisms.

First and foremost, we have developed a useful, realistic simulation of *C. elegans* embryogenesis. The accuracy of the model can be improved with more data and by implementing detailed kinematic simulations. Simulation beyond 600 minutes can be made possible by adding this data. Although the current system is primarily for the visualization of the development process, it can be augmented to integrate genetic data such as a DNA sequence database, the pattern of gene expressions obtained by whole mount in situ hybridization, and other genetic information, to do modeling and prediction.

We have also created a Java-based visualization tool that displays known embryogenesis, neural interconnection, and other cell data. As available data grow, we think they will become very useful tools for visualization. Further, we have implemented a simulator of the neural subsystem for thermotaxis and are currently using it for analysis. Lastly, we are now implementing a genetic interaction simulation system, specifically targeting cell fate determination at and before the 46th-cell stage.

In developing such systems, there are a number of issues that need to be properly identified. Some obvious ones:

- Choosing the right level of abstraction is essential. If we tried to build a simulation from the bottom up starting with protein dynamics, we would never reach a sophisticated enough model to simulate *C. elegans* embryogenesis. Not enough is known about molecular dynamics to simulate macroscopic phenomena such as embryogenesis. However, to ignore totally the molecular level is also not desirable, because any meaningful simulation must capture gene expressions and their interactions. We chose to simulate genetic interactions at the level of concentrations of promoters and repressors, and phenotypical effects are modeled functionally. We feel this is the right level of abstraction at this moment.

- Major parts of the simulation system can be applied in a general manner to simulate other animals simply by changing the underlying database. A general morphogenesis simulation system consists of (a) a kinetics simulation, which computes forces between cells, and (b) a genetic interaction simulation, which simulates the expression and repression of genes, the interaction of the products of genes with other genes, and cell-to-cell interactions.
- A study should be carried out to search systematically for all possible interactions of specific phenomena. When focusing on a specific phenomena, such as the cell fate determination of a specific lineage, there are fewer genes that might produce identical effects. A method needs to be developed to generate systematically a set of hypotheses and to define a critical experiment to identify which one of these hypotheses is correct.
- A well-designed visualization, even with a crude simulation, can be a useful tool. Due to the complexity of the phenomena, an intuitive understanding of the overall process of embryogenesis and neural dynamics is extremely difficult. Any system that assists in the understanding of complex phenomena would be welcomed by biologists and is essential to help gather detailed data for more elaborate simulation systems. In this regard, the current version of the simulation system, particularly the visualization module, has been very successful. It generates reasonably accurate simulations, and visualization is very persuasive; many biologists working on *C. elegans* have welcomed our work and express interest in data exchange.

The future course of our research will therefore run along the following lines:

- Establishment of a simulation technique and the development of a general morphogenesis simulator.
- Establishment of a systematic method to generate possible genetic interactions and neural functions and to design critical experiments.
- Completion of the first version of the Perfect *C. elegans* System, which focuses on functions as a research tool for *C. elegans* biologists.

While major efforts are required to achieve the level of detail and accuracy necessary for significant predictions to be made, the project has already identified some key issues in modeling biological systems that can be generalized to other simulations. We believe that we have made a promising first step on what is undoubtably a long journey.

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