

**Modeling neural patterning; exploring the gene regulatory networks in  
developing zebrafish embryos**

by

**Charu Gaur**

An Internship Report presented  
in partial fulfillment of the requirements for the degree of  
PROFESSIONAL SCIENCE MASTER

Major: Computational Biosciences  
Internship Guide: Dr. Ajay Chitnis

Technical Report No: 04-22

Arizona State University

Tempe, Arizona

December 2004

Graduate College  
Arizona State University

This is to certify that this report submitted by Charu Gaur has met the internship requirements  
of the Professional Science Masters in Computational Biosciences Program at  
Arizona State University

APPROVED:

Dr. Rosemary Renaut, Chair

---

Dr. Sudhir Kumar

---

Dr. Jeffrey Touchman

---

Supervisory Committee

ACCEPTED:

\_\_\_\_\_  
Department Chair

\_\_\_\_\_  
Date

# TABLE OF CONTENTS

<b>LIST OF FIGURES .....</b>	<b>V</b>
<b>LIST OF TABLES .....</b>	<b>VI</b>
<b>LIST OF APPENDICES .....</b>	<b>VII</b>
<b>ACKNOWLEDGEMENT.....</b>	<b>VIII</b>
<b>PREFACE.....</b>	<b>IX</b>
<b>ABSTRACT .....</b>	<b>XI</b>
<b>SECTION 1.....</b>	<b>1</b>
<b>INTRODUCTION.....</b>	<b>1</b>
1.1    ZEBRAFISH.....	1
1.1.1 <i>Zebrafish as a model organism.....</i>	<i>1</i>
1.1.2 <i>Zebrafish study in the lab.....</i>	<i>2</i>
1.2    INTERNSHIP PROJECT TASKS .....	2
1.2.2 <i>Studying Gene Regulatory Network (GRN) .....</i>	<i>4</i>
1.2.3 <i>Developing website for the lab.....</i>	<i>4</i>
<b>SECTION 2.....</b>	<b>6</b>
<b>LITERATURE REVIEW.....</b>	<b>6</b>
2.1    DEVELOPMENT .....	6
2.2    NEUROGENESIS.....	6
2.2.1 <i>The mid-hindbrain domain of the embryonic brain.....</i>	<i>8</i>
2.3    MORPHOGEN GRADIENTS AND GENE EXPRESSION .....	8
2.4    PATTERN FORMATION MECHANISMS .....	9
2.4.1 <i>Autocatalysis and lateral inhibition.....</i>	<i>9</i>
2.4.2 <i>Short range autocatalysis coupled with long-range inhibition.....</i>	<i>11</i>
2.5    MODELING AND VISUALIZATION TOOLS.....	14
2.5.1 <i>NetLogo.....</i>	<i>14</i>
2.5.2 <i>Compucell .....</i>	<i>16</i>
2.6    GENE REGULATORY NETWORKS .....	17
2.6.1 <i>NetBuilder .....</i>	<i>17</i>

<b>SECTION 3</b> .....	<b>19</b>
<b>PROJECT IMPLEMENTATION</b> .....	<b>19</b>
3.1 PHASE I: THE LEARNING PHASE.....	19
3.1.1 <i>Modeling Spatial expression of gene under Morphogen gradient: Four Gene Model</i> .....	19
3.1.2 <i>Converting and modifying pre-existing models in StarLogoT</i> .....	24
3.2 PHASE II: MODELING NEW MODELS.....	24
3.2.1 <i>Formation of Isthmic organizer: Xiro1_Model</i> .....	24
3.2.2 <i>Formation of Organizing Centre</i> .....	30
3.2.3 <i>Modeling gene network for organizer development in NetLogo</i> .....	31
3.2.4 <i>Modeling neural patterning with COMPUCCELL</i> .....	34
3.3 PHASE III: PRESENTING THE LEARNING.....	35
3.3.1 <i>Web Development</i> .....	35
3.3.2 <i>Showcasing the NetLogo models: Converting to applet</i> .....	36
<b>CONCLUSIONS</b> .....	<b>38</b>
4.1 CONCLUSION.....	38
4.2 FUTURE WORK.....	40
<b>REFERENCES</b> .....	<b>42</b>
<b>APPENDICES</b> .....	<b>45</b>

## LIST OF FIGURES

Figure 2.1: Gene Activity under morphogen gradient .....	10
Figure 2.2: Gene Activity under zero morphogen level .....	10
Figure 2.4: The Activator-inhibitor system .....	12
Figure 2.5: A ' <i>General</i> ' gene represented in NetBuilder. ....	18
Figure 3.1: Self-catalysis and lateral inhibition: Spatial Expression of genes under morphogen gradient.....	21
Figure 3.2: Gene Expression under shallow morphogen gradient.....	23
Figure 3.3: Effect of increase in morphogen level after development of discrete spatial expression zones.....	23
Figure 3.4: A general model showing gene interactions in the formation of the isthmus organizer at mid-nerula stage. ....	25
Figure 3.5: Modeling gene patterning in formation of the Midbrain-hindbrain boundary (Xiro1 Model) .....	28
Figure 3.6: Gene Expression pattern of MHB genes in absence of Xiro1 .....	29
Figure 3.7: Gene Expression pattern of MHB genes under over expression of Xiro1 .....	30
Figure 3.8: Visualization of single organizer formation.....	33
Figure 3.9: Description of the boundary and diffusion limitation in NetLogo .....	34

## LIST OF TABLES

Table 2.1: Specification of the System used for NetLogo .....	16
Table 3.1: Values used in the simulation of the four-gene model under morphogen gradient .....	22
Table 3.2: Parameter Values used in the simulation of the Xiro1 model .....	28

## LIST OF APPENDICES

Appendix 1	Chitnis & Itoh model of gene network for interpreting morphogen gradient.....	45
Appendix 2	Model describing interaction among MHB genes (Xiro1 Model).....	46
Appendix 3	Models of ectodermal fate choice in response to BMP signaling.....	47
Appendix 4	Four Gene Model setup in NetLogo .....	48
Appendix 5	Xiro1 model in NetLogo.....	49
Appendix 6	Simulating a single organizer setup in NetLogo.....	50
Appendix 7	Simulating Two Organizer setup in NetLogo.....	51
Appendix 8	Neurogenesis: Delta-Notch model in NetLogo .....	52
Appendix 9	AP patterning model setup in NetLogo .....	53
Appendix 10	Screenshot of the 'home page' of the website .....	54
Appendix 11	Screenshot of the 'project page' showing various models .....	55

## **ACKNOWLEDGEMENT**

I thank my guide, Dr Ajay Chitnis, for giving me an opportunity to become a part of the team in the Unit of Vertebral and Neural Development, NICHD at NIH. It was indeed a privilege to get exposure among a wide range of scientists, getting chance to attend lectures, seminars and workshops at NIH during the course of internship. I also thank the other team members at the lab all of who helped in completion of the project.

My gratitude also extends to my committee members including my program chair, Dr. Rosie Renaut, for their guidance and knowledge during graduate courses and lectures that helped me in the internship work.

In my pursuit to complete this degree and specifically this internship project I thank my brothers, who extended their support always encouraged me. Thanks Shailesh, for helping me with programming issues. Thanks to all my friends who have directly or indirectly made this report possible.

Finally, I would like to thank all the people who helped me in arranging the internship. I would particularly thank the International Student Organization (ISO) at ASU that worked promptly to get my CPT document and my friend, Navin, who helped in arranging my accommodation and other logistics at Maryland.

# PREFACE

This is an overview of my internship in the Chitnis lab at Vertebrate Neural Development Unit, Laboratory of Molecular Genetics, NICHD, at NIH campus in Bethesda, Maryland. It gives an insight about the internship program, the work environment and the work process.

## Project Goal

The goal of the project was to design mathematical models to simulate and visualize the experimental results or research work to understand the dynamics of the neural patterning in the zebrafish-developing embryo.

## Internship Requirements

The basic requirements for the internship at NICHD were having an understanding and knowledge in some or all of the following areas:

*Subject Knowledge:* Mathematics, Molecular biology, and developmental biology.

*Programming:* C, C++, Java

*Web Development:* HTML, DHTML, Photoshop, Dream weaver, Style Sheet

## Project Deliverables

- Develop NetLogo based models for anterior- posterior patterning in the zebrafish neuroectoderm. This will involve modifying the preexisting models and creating new models of early patterning events.
- Develop website to publish the models online and help the users to interact with it.
- Source code with detail annotations of the various models and the Gene regulatory network made.
- Documentation if any

## **My Background**

I have good knowledge in mathematics, biology and programming. Additionally industry work as IT professional in the pharmaceutical sector and research work as research assistant in bioinformatics area has honed my logical and technical skills in computational biology. I joined the Professional Science Masters program in Computational Biosciences at Arizona State University, Tempe, AZ, to learn and integrate mathematics, biology and computers, and understand the new dimension that the amalgamation of these three areas. For my internship I was looking to have such a cross-functional exposure. My internship at NIH fulfilled this desire. The three months long summer internship stretched to the fall semester. I feel happy to have gained valuable insights into building biological models and visualization of it through computational representation.

## **Work Environment**

The work environment was friendly, encouraging and it allowed me learn to several technologies. There was NIH library; ongoing seminars and training sessions that we could attend to acquire skills and knowledge on various subject areas. Apart from this a collaborative outlook of the lab helped me to get valuable assistance of the other researchers in the lab. A flexible work time gave me an opportunity to study all details at my own pace. My internship project guide, Dr Ajay Chitnis was very cooperative and understanding in letting me do tasks to the extent of my knowledge and promptly helping me whenever needed.

## **Skills Acquired**

- Understanding of mathematical models for modeling biological processes
- Learning about genetic networks and biochemical processes in neurogenesis
- Introduction to application programming environment for dynamic simulation and visualization of models

## ABSTRACT

Neurogenesis is a complex process involving a lot of rearrangements and pattern formation theories. Neural patterning involves division of the vertebrate neural tube into discrete compartments along the anterior-posterior (AP) axis of the developing zebrafish embryo. This process starts during early gastrulation and goes on till late neural stage. During this process posteriorising factors acting in form of a morphogen gradient cause differential spatial and temporal activation of the genes. In context of AP patterning in the zebrafish neuroectoderm we investigated the formation of the isthmus organizer at the midbrain-hind brain boundary and the gene networks involved in it.

We modeled the process by using Meinhardt's equations that uses a system of partial differential equation to model the effect of a system of genes in response to an external morphogen gradient. To simulate the model NetLogo programming environment was used. NetLogo helped us to visualize the models and see the effects of various factors that in turn explained the various cell-cell interactions and in some cases the gene networks that act together in neural development.

We also studied the gene networks involved in the formation of organizing centers. We modeled the process based on Meinhardt theory of pattern formation by short-range autocatalytic activator and long-range inhibitor. The study, however, could not be fully completed due to the limitations of the tools used: NetBuilder and CompuCell. NetLogo also posed certain limitations in modeling diffusion of substances under certain boundary conditions, though we got some success in modeling single organizing center. However, in general NetLogo modeling and simulations helped to enhance the understanding of the various experimental studies in neural development. We built a website to showcase research activities in the lab and get involvement of researchers worldwide. To be distributed on web the NetLogo models were converted to applets that can run on any browser supporting JVM 1.4 or higher. The preexisting models made in StarLogoT, an earlier version of NetLogo only operable on MacOS, were modified and converted into NetLogo, to be stored as applets.

## SECTION 1

# INTRODUCTION

## 1.1 ZEBRAFISH

### 1.1.1 Zebrafish as a model organism

The zebrafish comes close to being the ideal model organism for studies at molecular level; it appears to combine the best features that make experimentation easier. Some of the key features are as listed below:

- Zebrafish embryos develop externally and can be viewed and manipulated at all stages.
- The development is rapid, the organization of the embryo is simple and the embryo is transparent. The latter makes it easier to view the processes taking place in the embryo with naked eye.
- It is amenable to genetic analysis and has a short generation interval (2-3 months).
- It can produce more offspring in a shorter time. A female zebrafish lays up to 200 eggs per week.
- It is easy to induce new mutations in zebrafish.
- Much is known about the genome of zebrafish, dense genetic maps are available which have been useful for the comparative mapping of human genes.

Comparative genomics shows that there is extensive similarity between the zebrafish and human genomes. The zebrafish genome is 1700 million base pairs in length, about half the size of the human genome. This similarity has the effect that many human developmental and disease genes have counterparts in the zebrafish. Hence studying zebrafish and mutants can give insights into human diseases and maybe their cure too.

### **1.1.2 Zebrafish study in the lab**

The lab is focused on studying the neural development in zebrafish developing embryos. These studies are done with the aim to get findings that may help in medicine and pharmacology to develop better cures for developmental neural disorders in the newborns.

The lab uses an integrated interdisciplinary methodology to study the neural development, the approach being to start with the phenomenon, or the process seen during neural development, and then understand it by using the following disciplines:

- Genetic
- Molecular
- Cellular
- Computational

The Genetic level understanding involves the use of zebrafish mutants [5]. Mutants are an easy way to find the genes of interest and then understand biochemically their function and pathways of action. Molecular and cell biology approach involves designing experiments, creating mutants, to study the particular process, and deriving conclusions based on the observations and results. However, with numerous experimental results it becomes efficient and helpful to model the system in order to understand the effect of various factors that are part of the system and their behavior under given situations. The computational tools help to visualize these models that assist with explaining the observed phenomenon as closely as possible. The lab has in past tried to model the biological processes of neurogenesis computationally by using different schemas of Meinhardt [2, 3, 20, 25]. The lab used the NetLogo programming environment for simulation and visualization. NetLogo can model dynamic interactions that result in emergent patterns. Its ease of operation and efficiency made it a preferred choice for use in the lab.

## **1.2 INTERNSHIP PROJECT TASKS**

### ***Purpose***

The morphogenesis (development process of from zygote stage to a full grown organism) is a result of many sub processes all of which operate simultaneously or exclusively or

synergistically in a spatial and temporal scheme all of which are under genetic control. This makes development of higher organisms a complex network of biochemical reactions. The aim of the internship was to understand these processes and to formulate models in a mathematical precise way that describes essential steps - in spite of the appearing complexity of this process.

There were various aspects to my work, which I categorize under following tasks. These will be presented in detail in subsequent sections of this report.

### **1.2.1 Modeling Neural Patterning in developing embryos**

Stemple and Vincent [11] in their editorial review highlight the idea of modeling and its use in understanding development. They state that, though modeling is not yet part of mainstream development biology, it will soon be with the changing trend; now it is becoming an acceptable additional tool that developmental biologists can use to predict embryonic behavior and explore possible underlying mechanisms.

In my internship I got a chance to learn about modeling neurogenesis and understanding the analysis based on the models. This involved studying the molecular biology theory involved in the neural development of zebrafish and understanding the mechanism of mathematical modeling of these processes. In addition it required me to learn the NetLogo modeling environment for simulating and visualizing the models.

#### ***Creating New Models***

The project involved creating NetLogo [15, 16, 17] models for anterior-posterior (AP) patterning in the zebrafish neurogenesis process. One focus was on development of a model for modeling the mid-brain hindbrain boundary formation gene interaction that helps in proper positioning of the isthmic organizer (this organizer is involved in development of structures of the mid-brain and hind-brain region). Another major task was development of models for understanding formation of two basic organizing centers: one for the AP axis and the second for the DV (dorso-ventral) axis. The organizing centers are the main managers that are involved in proper positioning and spatial arrangement of various differentiated cells or tissues or organs.

### ***Modification of Previous models***

The internship involved extending the functionality of some of the past models to incorporate certain specific patterning environments in formation of the different boundaries in the brain.

The earlier models were developed in StartLogoT [18, 19] that only works on Macintosh (MacOS) operating system. To make these models available on the web I was given the task to convert them to NetLogo format so that they could be executed in windows environment and distributed as applets over the web.

### **1.2.2 Studying Gene Regulatory Network (GRN)**

Apart from visualizing the model in NetLogo and observing effects of different parameters, it is of importance to understand the gene regulatory network (GRN) involved in the modeled process. This is because the activation, or deactivation of the gene, works in principle at the transcription level. For example deactivation of a gene means a repressor effect on the transcription factor of the gene.

There are currently few freeware tools available for building a GRN. My internship guide, Dr Chitnis had researched some of these tools and to start with the modeling we decided to use NetBuilder [8, 9]. However, since it was being used for the first time we considered exploring other tools in case NetBuilder was found insufficient to create models of our interest. Later in this report I present the use of another tool CompuCell, because NetBuilder was found in appropriate for our task. But, we could not even use CompuCell successfully because of technical issues with the software in the windows environment.

### **1.2.3 Developing website for the lab**

Web site development was included as an easy and efficient platform to showcase the lab research work to a wide range of audiences and for interaction of researchers working in similar areas across the globe. Previously, the lab has produced many exciting results and has been at the forefront in using the computational modeling environment to visualize biological experimentation in Zebrafish. The modeling in some cases has helped in manipulation of the

molecular biology experiments in addition to providing certain new insights to the results observed at the bench. The website would contain information about the various NetLogo models for the users to browse and appreciate the understanding of the experimental process in neurogenesis as it happens. To achieve this all the models will be converted in Java applet form so that they can run from the client browser. With the main idea being to spread a general understanding of the theory of the neural development across a wide variety of readers, we planned to develop the website in a non-technical manner with lots of movie clips and diagrams for easy understanding.

## SECTION 2

# LITERATURE REVIEW

This section presents literature readings on the various topics that I covered as an intern and the software applications and tools that I used. The internship project was in the context of development biology. Having a very basic knowledge in this field, the literature study effectively encouraged by my guide helped me to perform the tasks efficiently.

### 2.1 DEVELOPMENT

Development is a complex process and difficult to understand in entirety as a single step. Hence researchers focus on a certain part of developmental processes and many such researchers in combination have helped to give a panoramic view of the development process. One of the main knowledge discoveries in human developmental biology has been by understanding the morphogenesis in different vertebrate organisms. This is because the basic steps in morphogenesis are conserved among vertebrates. Morphogenesis in general may be defined as the process (growth and differentiation) by which living things develop organized structures to become a fully functional organism. My internship lab, Chitnis lab, at NICHD used zebrafish as the model organism to study neural development. The developmental process, in a crude sense, is a sequential pattern formation schema based on cell-cell interactions, starting with more or less homogenous conditions that have developed certain biases to give rise to spatial polarity.

### 2.2 NEUROGENESIS

Neurogenesis is the process of development of the nervous tissue and the nervous system. The process is conserved in all vertebrates and takes place through a series of steps that include neural induction and neurulation (cell differentiation and neural patterning).

As Chitnis and Itoh [10] report, distinctions that characterize the different compartments of the nervous system begin to be established by early gastrulation. At this stage a diverse range

of cell-cell interactions and signaling mechanisms help divide the prospective neuro-ectoderm into domains with distinct fate. The neural induction is based on signals that act in guiding spatial and positional development. For example, BMP<sup>1</sup> is a caudalizing signal that is present at gastrula stage; to develop neural character we have BMP antagonists that act to maintain low-level of BMP activity and promote neural development [28] (For a model of neural induction see appendix 3). This simultaneously starts the process of neural patterning that completes at later stages. Of course, these are not sufficient to promote neural character and other signals like FGF are required. It is seen that acquisition of anterior character is closely linked with induction of neural character.

Neural patterning is also a dynamic process and eventually leads to neural differentiation when appearance of additional signaling centers, like the IsO (Isthmic Organizer), further organize and differentiate the patterns established by at the shield stage [6, 27]. Neural patterning involves division of the vertebrate neural tube into discrete compartments along the anterior-posterior (AP) axis of the developing embryo. This process starts during early gastrulating and goes on till late neural stage. Initial signals (antagonists to caudalizing factor BMP) induce neural tissue with more rostral (forebrain-like) fate. Later the posteriorising factors act in the form of a morphogen gradient and cause differential spatial and temporal response of the genes. And, as Chitins and Itoh [10] report, differential exposure to caudalizing factors like Wnt<sup>2</sup>, FGF<sup>3</sup>, retinoid signals and nodal signals subsequently transforms the neural plate to progressively more caudal fates.

---

<sup>1</sup> BMP (Bone Morphogenetic Proteins) are signaling proteins that are involved in a variety of developmental processes. They initiate promote and regulate bone development, growth, remodeling and repair. BMPs are also involved in prenatal development and postnatal growth of eye, heart, kidney, skin and other tissues [appendix 3].

<sup>2</sup> Wnt genes constitute a large family of highly conserved cysteine-rich, secreted glycoproteins that are involved in critical aspects of early embryonic development. Wnt genes and Wnt signaling are also implicated in cancer. *Source:* <http://www.stanford.edu/~rnusse/wntwindow.html>

<sup>3</sup> FGF - Fibroblast Growth Factor: family of proteins that possess broad mitogenic and cell survival activities, and are involved in a variety of biological processes, including embryonic development, cell growth, morphogenesis, angiogenesis, tissue repair, tumor growth and invasion.

### **2.2.1 The mid-hindbrain domain of the embryonic brain**

Galvic et al. [1] identify some of the unique genes and the regulatory framework in the formation of the midbrain-hindbrain boundary (MHB). The midbrain-hindbrain domain (MH) of the embryonic neural plate gives rise to crucial structures like the optic tectum (visual center), cerebellum (balance), tegmentum and pons (involved for a large part in the control of social behavior)[12]. Wurst and Bally-Cuif [12] report that at embryonic stages, the MH follows an interesting mode of development: unlike other brain areas, it does not rely on segmentation but rather responds to long range signaling activities originating from the midbrain-hindbrain boundary (MHB). MHB cells, forming the so-called ‘Isthmic Organizer’ (IsO), secrete diffusible factors of the Wnt and FGF families and control growth and patterning across the entire MH. The IsO is considered the paradigm organizer of the vertebrate brain anlage. Thus, investigating MH development is an important step towards understanding brain construction, as well as the function of organizing centers.

In addition, the MH domain is molecularly subdivided at the level of the MHB into rostral (Otx-positive) and caudal (Gbx-positive) domains, which will later acquire different fates. We studied the research of Galvic et al. [1] and modeled the MHB formation scheme as represented in their paper [see appendix 2].

Most of the process, in development, like the formation of midbrain-hindbrain boundary (MHB), as discussed above, starts formation under the influence of certain substances that influences or favors specific differentiation of cells in particular areas. These substances that play the importance role in spatial and temporal patterning are termed morphogens.

### **2.3 MORPHOGEN GRADIENTS AND GENE EXPRESSION**

Pages and Kerridge [4] in their research define morphogens as ‘form-generating substances’ those are capable of organizing distinct territories into different tissue types [4]. In their research on the morphogen gradient they nicely summarize two general mechanisms of action of morphogens on the cells: concentration - dependant and sequential cell mechanisms.

The concentration dependant model describes how the morphogen, acting directly on the cells, triggers a cascade of events and induces distinct cellular responses as genes respond differently to morphogen gradient. In a cell genes that have their required morphogen level present are activated, these genes along with the morphogen then trigger the activation of later genes.

The sequential cell model describes morphogen acting indirectly through secondary signaling events or relay mechanisms. The transcriptional factor (TF) is induced in a sequential manner and the TF along with the signaling molecules allows the expression of a later set of genes. As Pages and Kerridge [4] suggest, this model is like an off/on instruction, where specific response of the cell depends on its prior transcriptional state.

Though scientists still debate which model truly describes the manner in which morphogen acts, in reality it seems that either model or a combination of models may be true, mostly depending upon the system under the study. In our studies we use the concentration dependent scheme for modeling the action of morphogen.

## **2.4 PATTERN FORMATION MECHANISMS**

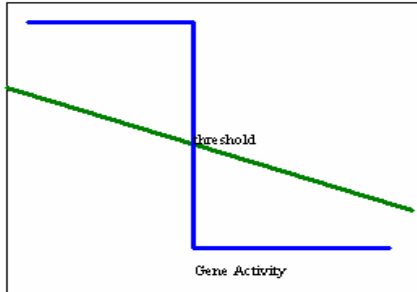
In genes, it is exciting to understand how in development there is a generation of differentiated structures from a more or less homogeneous zygote. Researchers, such as Hans Meinhardt [3, 4] and Turing [14], have tried to capture these phenomenons in mathematical equations, showing that there are certain common principles in the generation of these structures. We further study these mathematical models and their underlying theories.

### **2.4.1 Autocatalysis and lateral inhibition**

#### ***Studying the Activity of a single gene***

Meinhardt [3,4] describes various schemes for pattern formation in biological system and uses mathematical models to describe these schemes. I used Meinhardt's program, 'sp' (works in MS-DOS environment [25]), to understand his models and simulations. A basis to his idea can be understood by the concept of a single gene that has an auto regulatory feedback on its own. Such a gene under the influence of a graded distributed morphogen will be active in all the cells

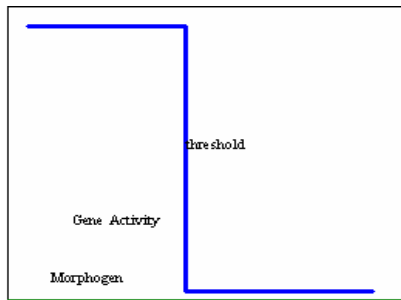
exposed above a threshold of morphogen. This will result in two regions as shown in the Figure 2.1: one with high activity (region above the threshold) and the other with low gene activity (region below the threshold).



**Figure 2.1: Gene Activity under morphogen gradient**

The green line represents the morphogen gradient. Blue line represents the gene activity.

Once the gene is activated the signal is no longer required for maintenance of the gene activity. If the signal is switched off only the tendency to switch to the 'higher' state will disappear, while, the cells that are activated with the gene will not become inactive. The following diagram shows this effect:



**Figure 2.2: Gene Activity under zero morphogen level**

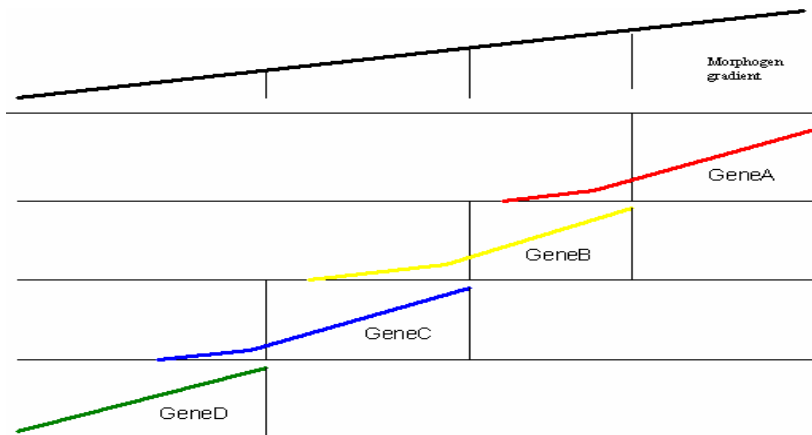
Green line, the morphogen gradient, is zero. The gene activity of the part below the threshold is reduced but the part that is above threshold remains unchanged.

The theory of gene activation based on a graded morphogen level in the cellular environment, as illustrated above, can be extended to demonstrate the behavior of several alternative genes. We assume that the gene has a positive self-autoregulatory feedback and that gene products compete with each other. By feedback of a gene product on the activity of its own gene, stable gene activation is achieved that is independent of the evoking signal. By competition only one of the several alternative possible genes can remain active in one cell.

### ***Studying pattern formation in a system involving many genes***

Consider 4 genes under the influence of a morphogen gradient for space dependant activation of genes in a mutually exclusive manner. Genes that are highly responsive to

morphogen need a lower gradient to get activated and the converse. This coupled with lateral inhibition that creates competition among neighboring gene products, causes discrete gene expression boundaries.



**Figure 2.3: Activation of four genes under the effect of morphogen gradient in a mutually exclusive manner.**

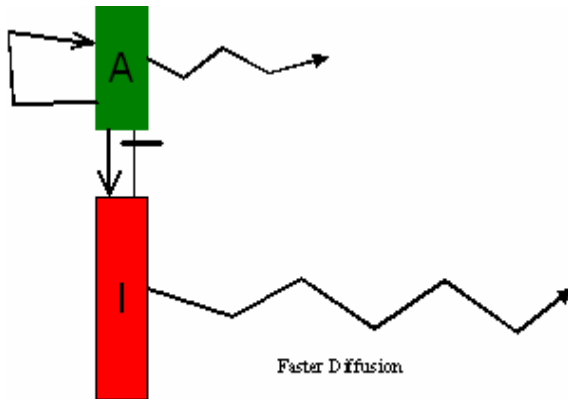
Chitnis and Itoh show the above scheme in their model of rostral-caudal patterning in the zebrafish neuroectoderm [10]. They describe how the genes interpret the morphogen gradient that leads to compartmentalization and patterning. In their schema, the rostral genes' expression in specific domains is only transiently dependant on the morphogen gradient. In line with the Meinhardt explanation they show that the gradient has an initial role in determining the relative size of the individual compartments of the rostral gene expression, which eventually becomes more dependant on auto activation and cross-repression by genes in the network [view appendix 1 for diagrammatic representation of the rostral-caudal patterning model].

## 2.4.2 Short range autocatalysis coupled with long-range inhibition

### *Activator - Inhibitor System*

This model assumes presence of a substance known as an activator, having a positive autocatalytic effect and an inhibitor, inhibiting formation of the activator and having no effect on its own production. The activator also positively influences the inhibitor's production; at equilibrium state there is a uniform concentration of both activator and inhibitor in the system. A small positive deviation in the concentration of activator (due to some bias or local perturbation)

makes the deviation increase rapidly due to positive feedback, causing local maxima of the activator concentration. However, to make a local maximum this should be supplemented by a long-range inhibition (or negative feedback) that will stop the formation of a maximum at other places by inhibiting activator production. Also for this effect to take place the inhibitor should have higher diffusion rate than activator so that it can quickly diffuse into the neighboring cells to stop secondary maxima formation.



**Figure 2.4: The Activator-inhibitor system**

Modified version of Turing's Reaction-diffusion mechanism [14]

- A- Activator: has autocatalytic effect, positive effect on inhibitor production and slow diffusion
- I- inhibitor: has negative effect on activator production and fast diffusion

Meinhardt and Gierer [20] derived general criterion to model the above schema of activator-inhibitor interactions that forms stable patterns. This is represented in the following partial differential equations:

$$\frac{\partial a}{\partial t} = s \frac{a^2}{h} - r_a a + D_a \frac{\partial^2 a}{\partial x^2} \text{ ----- (1)}$$

$$\frac{\partial h}{\partial t} = s a^2 - r_h h + D_h \frac{\partial^2 h}{\partial x^2} \text{ ----- (2)}$$

where  $a$  represents the activator and  $h$  the inhibitor.  $r_a, r_h$  defines the decay rate and  $D_a, D_h$  the diffusion rate of the activator and inhibitor respectively.  $s$  represents the ability of the activator to undergo autocatalysis. By a suitable choice of the diffusion rates we can achieve local instability with overall stability of the system. This model is a modified form of the reaction-diffusion model [14] and resembles the latter in the sense that it first involves a reaction, i.e. production and decay of the activator and inhibitor and then diffusion of these substances in the system.

**Modification of Activator - inhibitor system: incorporating competence factor in the model**

The above, activator-inhibitor, model is not ideal for biological systems wherein there is more dynamicity and influence of many factors. In case of the above system the reaction-diffusion will not form a stable pattern that is required especially for organizing centers in the development. Hence we modify the above equations to account for the dynamicity of the system such that the result should come out as an emergent property of the system, reproducing experimental observations or some known behaviors. Meinhardt equations for modeling an organizer system are:

$$\frac{\partial a}{\partial t} = S\left(\frac{a^2 + b_a}{h(1 + s_a a^2)}\right) - (r_a \times a) + D_a \nabla^2 a \text{ ----- (3)}$$

$$\frac{\partial h}{\partial t} = S a^2 + b_h - (r_h \times h) + D_h \nabla^2 h \text{ ----- (4)}$$

$$\frac{\partial c}{\partial t} = \frac{a^2}{1 + a^2} + b_c - r_c \times c \text{ ----- (5)}$$

These equations represent ‘Activator – Inhibitor- Competence’ system. *a*- activator, *h*-inhibitor and *c*-competence.

The activator, *a*, has a nonlinear feedback on its own production rate (*a*<sup>2</sup>). *S*, the source density, describes the ability of the cells to perform the autocatalysis. The inhibitor only slows down activator (1/*h*), but its own production. *s<sub>a</sub>* represents the saturation of the autocatalysis because the size of the term (1/ (1 + *s<sub>a</sub>a*)) regulates the activated area if the activator production saturates at a high concentration . In a way *s<sub>a</sub>* can determine the distribution pattern of the activator, for example. *s<sub>a</sub>* > 0 can lead to stripe-like distributions.

The activator and the inhibitor decay by a first order process, i.e., the number of molecules disappearing per time unit is proportional to the number of molecules present (represented by the term  $-(r_a \times a)$  for activator and  $-(r_h \times h)$  for inhibitor). To obtain numerical values for the concentrations around unity, a production rate constant equal to the decay rate constant is assumed. Both substances can spread by diffusion; *D<sub>a</sub>* and *D<sub>h</sub>* are the diffusion constants, and the diffusion is modeled as *D<sub>a</sub>*∇<sup>2</sup>*a* for activator and *D<sub>h</sub>*∇<sup>2</sup>*h* for inhibitor. The spread is faster for inhibitor as compared to the activator.

$b_a$ ,  $b_h$ ,  $b_c$  are the basic activator, inhibitor and competence production respectively. The small basic activator production (activator-independent) helps in initiating autocatalysis in areas with low activator concentration. In contrast, the basic inhibitor production can suppress the appearance of local secondary maxima.

The competence plays a role of suppressing formation of secondary maxima when the embryo grows in size. This needs a feedback mechanism of the system on the competence production. If source has steeper slope than inhibitor then no secondary maxima, also in the system the competence should have time constant higher than the AI system. For pattern formation a minimum size of field is required (visualized with Meinhardt's 'sp' program [25]). His program simulates how during growth a maximum emerges at the end of the field if some critical size is exceeded. This then remains stable on further growth. Such a system has properties of an organizing center. Results of many other schemas and simulations were also presented on Meinhardt's website [26].

To explore the various parameters in the above models and to see the results we need to use computational tools. NetLogo is one such tool that achieves this in an effective and efficient way.

## **2.5 MODELING AND VISUALIZATION TOOLS**

To understand the dynamics and properties of a modeled system it is necessary to simulate it with hypothesized factors and conditions. For achieving this many freeware tools and applications are available. We discuss two of them: NetLogo and CompuCell, which were used in this report.

### **2.5.1 NetLogo**

It is a multi-agent cross platform programming language and modeling environment that uses a set of agents to visualize complex systems, their interactions and emergent properties as a result of these interactions [21]. NetLogo was developed on Java platform in 2002, by a team at Northwestern University, Illinois, led by Uri Wilensky.

The precursor of NetLogo was StarLogo that was originally developed by Daniel Bobrow and Wallace Feurzeig at Bolt, Beranek and Newman, Inc. and Seymour Papert at MIT in 1960s. Starlogo was modified into StartLogoT [18, 19] by the team at Northwestern University. The StarlogoT programmable environment was good for studying emergent properties of complex systems developing over time. NetLogo encompasses this basic characteristic along with some additional features that make it a preferred tool for modeling biological systems.

### ***Advantages of NetLogo***

- i. NetLogo is an easy to learn and use tool. It is fully programmable with a simple language structure. There is no pre requisite of computer programming knowledge. This makes it a preferred choice for biologists with no computer background.
- ii. It is a freeware and standalone application that can easily be installed. The software and documents can be downloaded from <http://ccl.northwestern.edu/netlogo> [21].
- iii. NetLogo has a well-written documentation with built-in examples and tutorials that make the learning faster. There is also a big user community networked by user-groups and forums that provide additional advantage in learning.
- iv. It offers an advantage over the earlier StartLogoT version in being cross platform. While the latter could only operate on MacOS, NetLogo can operate on MacOS, Windows, and linux among many other operating systems.
- v. NetLogo models can easily be converted to applets, which offer an advantage for distribution over the Internet. Also programs built in StartLogoT can be converted to NetLogo using NetLogo version 1.3.

NetLogo is continuously being modified and upgraded with each newer version offering something more based on the user feedback. This makes it a more robust and efficient tool.

### ***Modeling in NetLogo***

In general models help to describe a system. However, with a tool like NetLogo that can help visualize a given model, the task of learning from the models becomes much more efficient and effective. NetLogo has 3 kinds of agent set: turtles, patches and observer. Each of these has their own set of properties, functions and uses. A model can be built using these agents, which

can be programmed into the application. The modeler can specify how these agents interact with each other using defined rules. Modelers can give instructions to hundreds of agents all operating simultaneously. This makes it possible to explore the interactions among the agents (at micro-level) and the patterns that emerge from such interactions (at macro level) [15, 16, 17].

### ***System requirement***

NetLogo is designed to run on almost any type of computer, but some older or less powerful systems are not supported.

**Table 2.1: Specification of the System used for NetLogo**

	<b><i>Window system</i></b>	<b><i>Mac System</i></b>
Operation System (OS)	XP	OS X version 10.3
RAM	512 MB	256 MB
HDD	60GB	40 GB
JVM	Version 1.4.2	Supplied as part of the OS

NetLogo can run on any platform on which a Java Virtual Machine, version 1.4.1 or later, is available and installed. Version 1.4.2 or later is preferred [21]. The application is memory intensive and it requires at least 25MB of free hard drive space. Both the Microsoft Windows and Apple Mac system we used at NICHD lab met the minimum requirements as given in the NetLogo user manual.

## **2.5.2 COMPUCCELL**

### ***Modeling in CompuCell***

Developed by Izaguirre et al. [13], CompuCell is a multi-model software framework especially targeted for modeling and simulation of the morphogenesis process. It follows the basic mathematical definition, and researchers have based the modeling environment of gene regulation on the theory of:

- a) Concepts of cellular automatons with stochastic local rules

- b) System of differential equations, including subcellular ordinary differential equations and extracellular reaction-diffusion partial differential equations; the latter describe diffusible morphogens.

The researchers state that the tool has a flexible computational environment for simulations and simultaneous incorporation of growth and spatial patterning. If we can use this tool it will be helpful to model the gene network involved in the formation of the organizing center. The model for the development of the organizer needs to take into consideration both the growth of the developing embryo and the spatial patterning.

### ***System Requirement***

CompuCell is a free software tool and has binaries both for Microsoft Windows and Linux. The tool can be downloaded from the site: <http://sourceforge.net/projects/compuCell/>

## **2.6 GENE REGULATORY NETWORKS**

A Gene Regulatory networks (GRN) can be considered as a signaling network made of on/off switches that are operating at gene level in the cell. A GRN dynamically controls the expression of the genes in the cell by controlling whether and how fast the transcription of gene to mRNA occurs.

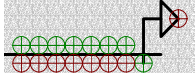
### **2.6.1 NetBuilder**

NetBuilder is a tool developed by the bioinformatics group at University of Hertfordshire, Hatfield, UK, that can model a given GRN.

#### ***Modeling in NetBuilder***

In NetBuilder a GRN is modeled as a system consisting of components made of genes, cell surface receptors and other signaling molecules. The components send, receive and respond to signals [9]. In NetBuilder a gene is modeled to have input and output signals (see figure 2.5). A gene responds to the input signals to modulate the output signals, for example binding of a transcription factor (TF) to a gene is an input signal and the transcription rate change (increase,

decrease or switch on/off) upon TF binding is an output signal .In NetBuilder the signals are transformed at network nodes and transferred between nodes through links.



**Figure 2.5: A 'General' gene represented in NetBuilder.**

The green and the red circles present input and output ports respectively

### *System Requirement*

NetBuilder is built to work in a Microsoft Windows environment. It is freeware and can be downloaded from the site:

[http://strc.herts.ac.uk/bio/maria/NetBuilder/Installation/NB\\_Download.htm](http://strc.herts.ac.uk/bio/maria/NetBuilder/Installation/NB_Download.htm)

## SECTION 3

# PROJECT IMPLEMENTATION

This section is divided into three phases each of which involved implementation of a specific task.

### 3.1 PHASE I: THE LEARNING PHASE

My first task was to understand the modeling environment and programming methods for both NetLogo and StarLogoT; and convert the programs made in the past in the lab in StarLogoT to NetLogo. I used NetLogo version 2.0.2. It was easy and quick to learn. With resources available in form of tutorials, built-in examples, models from user community, and discussions forums it took me no time to understand the programming. The concepts used in most of the models made in StarLogoT were based on Meinhardt's schemas and equations describing processes in biological systems. I started out with understanding a basic spatial patterning model, Four-gene model, and then converted the Four-gene program along with other models made in StarLogoT to NetLogo.

#### 3.1.1 Modeling Spatial expression of gene under Morphogen gradient: Four Gene Model

The Four Gene model represents a concept that we can correlate to processes occurring during AP patterning, somatogenesis and other neurogenesis processes in Zebrafish; shown by Chitnis and Itoh [10] in their model of rostral-caudal gene patterning.

The model also provides a basic understanding of the concepts of morphogen gradient, occurrence of differential gene expression under the effect of gradient, and the modeling of the process as a system of differential equations using the Meinhardt [2] theory of self-activation and lateral inhibition. These concepts are important to know as different manipulating of them can achieve patterns. The model is based on the Meinhardt gene activation model [2,3,20] and is represented by the following non-linear system of partial differential equations:

$$\frac{\partial gene_A}{\partial t} = \frac{sA \times gene_A^2 + mA \times morphogen}{sA \times gene_A^2 + sB \times gene_B^2 + sC \times gene_C^2 + sD \times gene_D^2} - gene_A \times decay_A = f_A$$

$$\frac{\partial gene_B}{\partial t} = \frac{sB \times gene_B^2 + mA \times morphogen \times gene_A}{sA \times gene_A^2 + sB \times gene_B^2 + sC \times gene_C^2 + sD \times gene_D^2} - gene_B \times decay_B = f_B$$

$$\frac{\partial gene_C}{\partial t} = \frac{sC \times gene_C^2 + mC \times morphogen \times gene_B}{sA \times gene_A^2 + sB \times gene_B^2 + sC \times gene_C^2 + sD \times gene_D^2} - gene_C \times decay_C = f_C$$

$$\frac{\partial gene_D}{\partial t} = \frac{sD \times gene_D^2 + mD \times morphogen \times gene_C}{sA \times gene_A^2 + sB \times gene_B^2 + sC \times gene_C^2 + sD \times gene_D^2} - gene_D \times decay_D = f_D$$

In the above equations the term ‘*m*’ represents the responsiveness to the morphogen, ‘*s*’ represents the production rate and *decay* the decay rate of the given gene. We set up the morphogen gradient as a linear graded, non-moving gradient that can be modeled as a straight line.

$$morphogen = m \times x + C, \text{ where } m \text{ is the slope of the line and } C \text{ is the intercept}$$

Converting the above equation into NetLogo environment we will write as:

$$morphogen = \left( \frac{source - baseline}{screen\_edge\_x} \right) \times (p\_xcor + screen\_edge\_x) + baseline$$

where *source* represents the maximum morphogen concentration and *baseline* the base level or basal activity of the morphogen.

*screen\_edge\_x* represents the size of the screen from centre to the edge (screen in net logo is the display monitor that represents the area of the system) and *p\_xcor* is the distance of the particular patch from the center of the screen (this is what determines the concentration of gradient on that patch as other terms are the same for all the patches).

### **Algorithm in NetLogo: Four-gene model**

1. Set up  $t=0$ ,  $\Delta t=1$ . *t* represents the time or the number of iterations. Use the monitor object for *t* to view it on the screen. Set slider object for all the parameters that will be

manipulated to carry out simulations. These are the production rates( $s$ ), decay rates ( $decay$ ) and morphogen sensitivity ( $m$ ) for each of the genes (view appendix 4 for a screenshot of the model setup). For the morphogen set up sliders for the source (max morphogen) and the baseline.

2. Set the initial level of the gene parameters. Set up the morphogen gradient.
3. Set the new level of gene at time  $t=t+1$ . The general equation for modeling this is:  $gene(t + 1) = gene(t) + \Delta t \times f_{gene}$  where  $f_{gene}$  is substituted by the equations shown above for each gene, equivalently Euler's method of integration with step size 1.
4. Increment  $t = t+1$  for the next iteration
5. Repeat step 4 and 5. Stop once a stable pattern is reached (i.e. no more change is seen in the pattern).

### Result

The simulations based on the above algorithm give the following result

**Figure 3.1: Self-catalysis and lateral inhibition: Spatial Expression of genes under morphogen gradient**

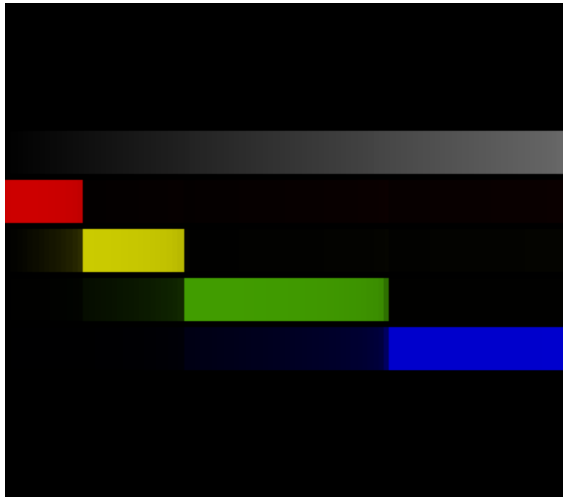


Figure 3.1(a): Spatial expression of the 4 genes red- geneA, yellow-geneB, green- geneC, blue-geneD

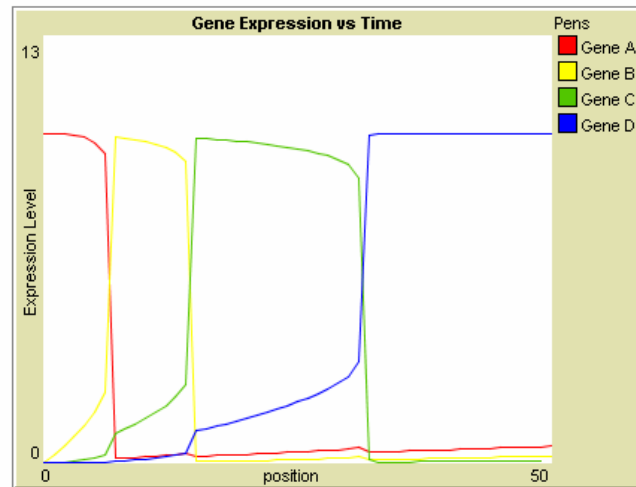


Figure 3.1 (b): Plot showing the positional expression level of 4 genes

The *geneA* has the highest responsiveness to the morphogen i.e. it gets activated at low morphogen level and the lowest production rate that makes it be expressed first. Other genes,

*geneB*, *geneC* and *geneD* have relatively lower responsiveness to morphogen but higher production rate in order i.e.  $m_A > m_B > m_C > m_D$  and  $s_A < s_B < s_C < s_D$ .

**Table 3.1: Values used in the simulation of the four-gene model under morphogen gradient**

Parameter	Gene			
	A	B	C	D
Production Rate (s)	1.5	2.4	3.4	4.3
Decay Rate (decay)	0.1	0.1	0.1	0.1
Importance of Morphogen (m)	2.1	1.75	1.30	.95
Max. Morphogen (source)	4.6			
Morphogen baseline	0			

### ***Observation***

Once a stable gene spatial pattern is formed, it remains unchanged if we then lower or switch off the morphogen. We also simulate the pattern so formed under following conditions.

#### *Case 1: Influence of increase in morphogen 'baseline' (making the gradient shallow)*

An increase in the lowest morphogen level causes the pattern to shift rostrally. This is because an increase in morphogen at the rostral gene makes the threshold nearer to the activation of the gene next in line. For example, we observe that keeping other factors same as in earlier simulation (table 3.1), an increase in baseline to 1.5, makes the gradient shallow and causes the threshold in *geneA* region to be a value that activates *geneB*. Hence, *geneB* expression is seen in *geneA* area. Figure 3.2 shows the results of NetLogo simulation.

**Figure 3.2: Gene Expression under shallow morphogen gradient**

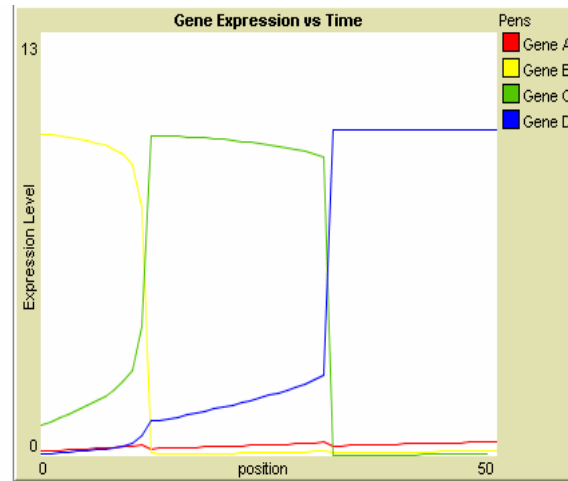
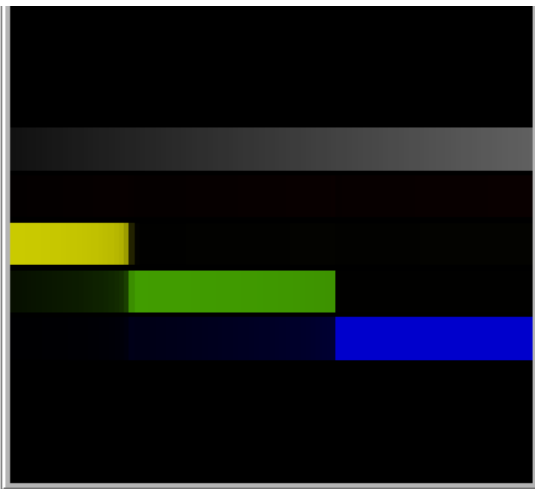


Figure 3.2 (a): Spatial expression of the 4 genes

Figure 3.2 (b): Plot showing the positional expression level

The gene A (red bar) is missing and all genes have moved rostrally

*Case 2: Influence of increase in morphogen level*

A similar effect is observed if we increase the morphogen level (but not the gradient, which remains 0) to a high level of around 8 from 4.6. However in this case the shift is not very drastic as compared to the change in the gradient level.

**Figure 3.3: Effect of increase in morphogen level after development of discrete spatial expression zones**

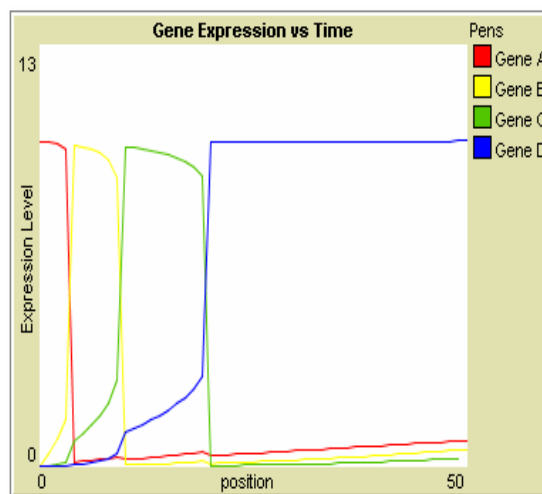
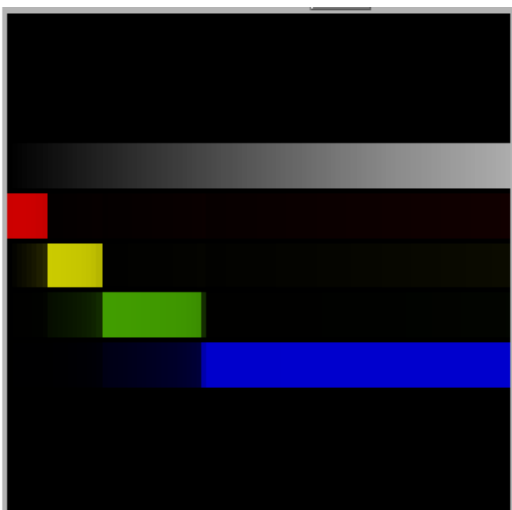


Figure 3.3 (a): Spatial expression of the 4 genes

Figure 3.3 (b): Plot showing the positional expression level

## ***Inference***

Under the influence of the morphogen when the change from one gene to the next occurs in a concentration-dependant manner, particular genes remain active at a particular position initially because of morphogen gradient and later because of self activation and cross repression. Hence, though local morphogen concentration determines which gene becomes activated at particular position, the cells have to remember what they learned to maintain the pattern at later stages when the morphogen signal is no longer present. For a sharp mutually exclusive spatial patterning the condition:  $s_A < s_B < s_C < s_D$  must be satisfied.

The above model was explored in various forms in past in the lab. These involved the study of somatogenesis, Delta-Notch signaling [22].

### **3.1.2 Converting and modifying pre-existing models in StarLogoT**

The earlier models made in StarLogoT were required to be converted to NetLogo to save the models as applets for display on the website.

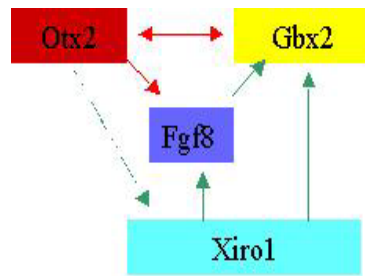
This required installation of NetLogo version 1.3 on the Mac system (Apple machine). NetLogo1.3 has inbuilt functionality for backward integration with StarLogoT. The converted models were then imported to NetLogo2.0.2 application on windows machine and all of the syntax errors were fixed. Though there was not a real need to convert the models into NetLogo2.0.2 version as version 1.3 also has capability to save the model as applet; we did the conversion for future variations in the models that may require the additional functionalities only provided in the newer version (i.e. version 2.0.2).

## **3.2 PHASE II: MODELING NEW MODELS**

### **3.2.1 Formation of Isthmic organizer: Xiro1\_Model**

This model is based on the research conducted by Glavic et al. on Midbrain-hindbrain boundary (MHB) formation [1]. In their research the researchers show how homeoprotein, Xenopus Iro gene, Xiro1, is involved in the formation of the isthmic organizer (IsO) that patterns the midbrain hindbrain domain.

Galvic et al. [1] represent a schema that describes the interactions of Xiro1 with other genes at the MHB boundary (refer appendix 2 for the model). Xiro1 has a different role to play at different stages, viz: gastrula, late gastrula (or early nerula) and mid nerula. Considering the dynamicity of these systems it is imprecise to model the entire system and find a mathematical description of the process. However, if we modularize the process then we can model them assuming their independence. Hence we model the mid neural stage in the MHB boundary formation, involving the positioning of isthmus organizer. The interactions at this stage are shown in the diagram below.



**Figure 3.4: A general model showing gene interactions in the formation of the isthmus organizer at mid-nerula stage.**

The green arrow represents positive effect, the dashed green arrow represents weak positive effect and the red represents a negative effect on expression

The above figure represents the relationship among the genes involved in positioning the isthmus organizer at mid-nerula stage, as discussed in the research by Glavic et al. [1]:

- Otx2 and Gbx2 genes participate as mutual repressors in positioning the isthmus organizer genes Fgf8, En2<sup>4</sup> (not included in the figure 3.4).
- Xiro1 is co expressed with Otx2 and Gbx2 at the MHB.
- Xiro1 is absolutely necessary for Fgf8 induction independent of Gbx2 and Otx2 expression. Presence of Xiro1 acts like an on-off switch for Fgf8 expression.
- Though Xiro1 acts as transcriptional repressor promoting Otx2 expression in early gastrula it has no effect during mid nerula stage. At this stage Otx2 may have some influence on Xiro1. At early and mid nerula Xiro1 promotes Gbx2. But unlike Otx2, Gbx2 is unable to induce Xiro1 expression.

<sup>4</sup> En2: Engrailed gene, encodes for transcription factor that is expressed in the midbrain domain

For simplicity of the model we consider 5 genes: Otx2 (geneA), Gbx2 (geneB), Fgf8 (geneD), Xiro1 (geneX) and a caudal gene (geneC), and represent the system by varying the Meinhardt four-gene model schema. The Xiro1 system can be represented by the following equations:

$$\frac{\delta geneA}{\delta t} = \frac{sA \times geneA^2 + mA \times morphogen}{sA \times geneA^2 + sB \times geneB^2 + sC \times geneC^2 + sD \times geneD^2} - geneA \times decayA = f_A$$

$$\frac{\delta geneB}{\delta t} = \frac{sB \times geneB^2 + mB \times morphogen \times geneX}{sA \times geneA^2 + sB \times geneB^2 + sC \times geneC^2 + sD \times geneD^2} - geneB \times decayB = f_B$$

$$\frac{\delta geneC}{\delta t} = \frac{sC \times geneC^2 + mC \times morphogen \times geneB}{sA \times geneA^2 + sB \times geneB^2 + sC \times geneC^2 + sD \times geneD^2} - geneC \times decayC = f_C$$

$$\frac{\delta geneD}{\delta t} = \frac{sD \times geneD^2 + geneA \times geneB}{sA \times geneA^2 + sB \times geneB^2 + sC \times geneC^2 + sD \times geneD^2} - geneD \times decayD = f_D$$

$$\frac{\delta geneX}{\delta t} = \frac{sX \times geneX^2 + mX \times morphogen \times geneA}{sA \times geneA^2 + sB \times geneB^2 + sC \times geneC^2 + sX \times geneX^2} - geneX \times decayX = f_X$$

The variables *geneA*, *geneB*, *geneC*, *geneD* and *geneX* represent the concentration (or the expression levels) *sA*, *sB*, *sC*, *sD*, *sX* the production rates, *mA*, *mB*, *mC*, *mD* and *mX* the responsiveness to the morphogen constants, and *decayA*, *decayB*, *decayC*, *decayD* and *decayX* the decay rates for the 5 genes involved. All the variables mentioned above are for genes A (Otx2), B (Gbx2), C (caudal gene), D (Fgf8), and X (Xiro1) respectively.

### ***Algorithm in NetLogo***

1. Set up  $t=0$ ,  $\Delta t=1$ .  $t$  represents the time or the number of iterations. Use the monitor object for  $t$  to view it on the screen. Set slider object for all the parameters that will be manipulated for the simulations. These are the production rates(s), decay rates (decay) and morphogen sensitivity (m) for each of the genes. (See appendix 5 for a screenshot of

the model setup). For the morphogen set up sliders for the source (max morphogen) and the baseline.

2. Set up on-off switch to represent presence or absence of Xiro1 in the system.
3. Set the initial level of the gene parameters. Set up the morphogen gradient.
4. Set the concentration of gene under the condition that ***Xiro1 is present***.

$$geneA(t+1) = geneA(t) + \Delta t \times f_{geneA} \dots\dots (Gbx2)$$

$$geneB(t+1) = geneB(t) + \Delta t \times f_{geneB} \dots\dots (Otx2)$$

$$geneC(t+1) = geneC(t) + \Delta t \times f_{geneC} \dots\dots (caudal\ gene)$$

$$geneD(t+1) = geneD(t) + \Delta t \times f_{geneD} \dots\dots (Fgf8)$$

$$geneX(t+1) = geneX(t) + \Delta t \times f_{geneX} \dots\dots (Xiro1)$$

where  $f_{gene}$  is substituted by the equations shown above for each gene

If ***Xiro1 is absent*** (i.e. the switch is off) then fgf8 (*geneD*) is not produced, and Gbx2 (*geneB*) production lowers. However, Otx2 (*geneA*) and caudal gene (*geneC*) expression is not affected directly by Xiro1. Under this condition *geneA* and *geneC* remain unchanged and we set the concentration of *geneB*, *geneD* and *geneX* as:

$$geneB = geneB + \frac{sB \times geneB^2 + mB \times morphogen}{sA \times geneA^2 + sB \times geneB^2 + sC \times geneC^2 + sD \times geneD^2} - geneB \times decayB$$

$$geneD = 0$$

$$geneX = 0$$

5. Increment  $t$  for next iteration
- Repeat step 4 and 5. Stop once a stable pattern is reached (i.e. no more change is seen in the pattern).

The model so developed simulated the gene interaction and helped in the study of the emergent properties and analysis of the effect of variation in the parameters (or the genes) involved.

## Result

Figure 3.5: Modeling gene patterning in formation of the Midbrain-hindbrain boundary (Xiro1 Model)

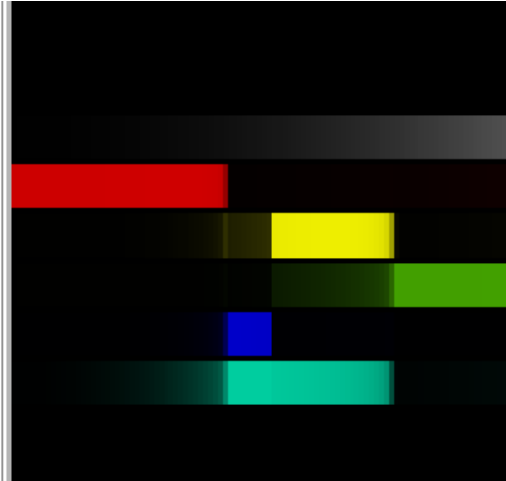


Fig 3.5 (a): Spatial expression of the MHB genes

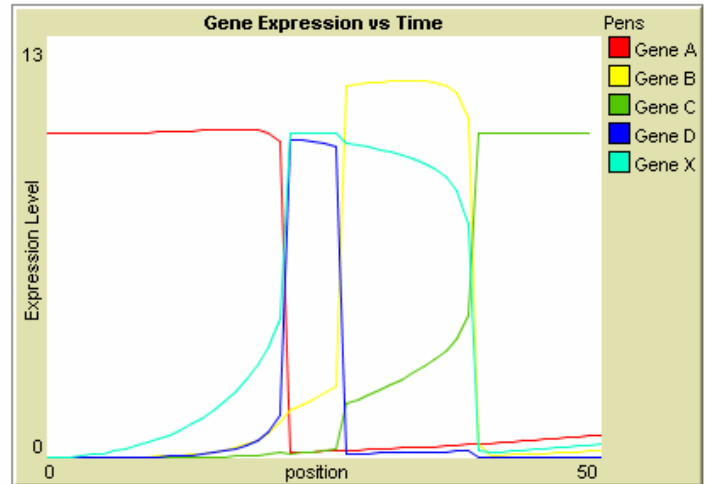


Fig 3.5 (b): Plot of expression level of MHB genes corresponding to a given position in (a). We made the field size = 50

Table 3.2: Parameter Values used in the simulation of the Xiro1 model

Parameter	Gene				
	A (Otx2)	B (Gbx2)	C (caudal)	D (fgf8)	X (Xiro1)
Production Rate (s)	1.1	1.6	2.4	2.1	2
Decay Rate (decay)	0.1	0.1	0.1	0.1	0.1
Importance of Morphogen (m)	2.6	1.95	1.25	1.55	2
Max. Morphogen (source)	7				
Morphogen baseline	0				

The result of the NetLogo mathematical model is in close proximity to the experimental based model [see appendix 2] as represented by Glavic et al. [1]. This affirmed the correct hypothesis for modeling the system. However, the system modeled only the mid-nerula stage wherein we brutally assumed the presence of a certain level of Otx2 and Xiro1 already present in

the system. The model developed was not fully robust to model the development of the Isthmic organizer from the start to the end stage of its formation i.e. the gastrula stage to the mid-nerula stage.

### Observations

The Xiro1 model was simulated to test if the observations that were reported by the researchers in their Xiro1 molecular experiments could also be seen in the modeled system.

#### Case 1: Xiro1 is switched off

Figure 3.6: Gene Expression pattern of MHB genes in absence of Xiro1

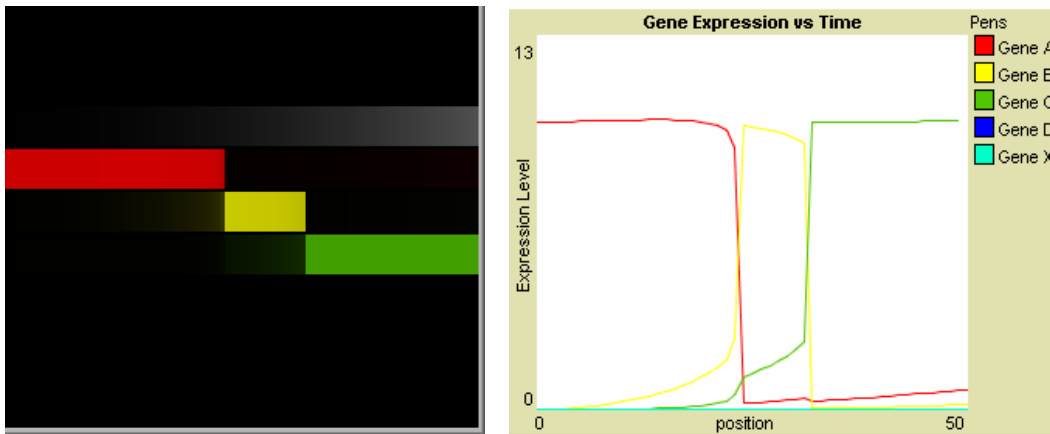


Fig 3.6 (a): Spatial expression of the MHB genes

Fig 3.6 (b): Plot of expression level of MHB genes corresponding to a given position in (a)

In our model we remove Xiro1 from the system by changing the on/off switch from on to off position before we start simulation of the model. As hypothesized there is no expression of Fgf8 (*geneD*), since Xiro1 acts as an on/off switch for stimulating Fgf8 production and also there is very low expression of Gbx8 (*geneB*). This is consistent with the observations reported by Glavic et al. [1].

#### Case2: Over expression of Xiro1

If there is over expression of Xiro1 at later stage in development then Xiro1 is no longer able to activate Otx2, but it can activate Gbx2; the latter displaces the midbrain-hindbrain (MHB) boundary anteriorly through Otx2 down regulation. This phenomenon can be seen in the

following figure. In comparison to the figure 3.5 (a), figure 3.7 (a) has bigger posterior domain, as the MHB boundary moves anteriorly.

**Figure 3.7: Gene Expression pattern of MHB genes under over expression of Xiro1**

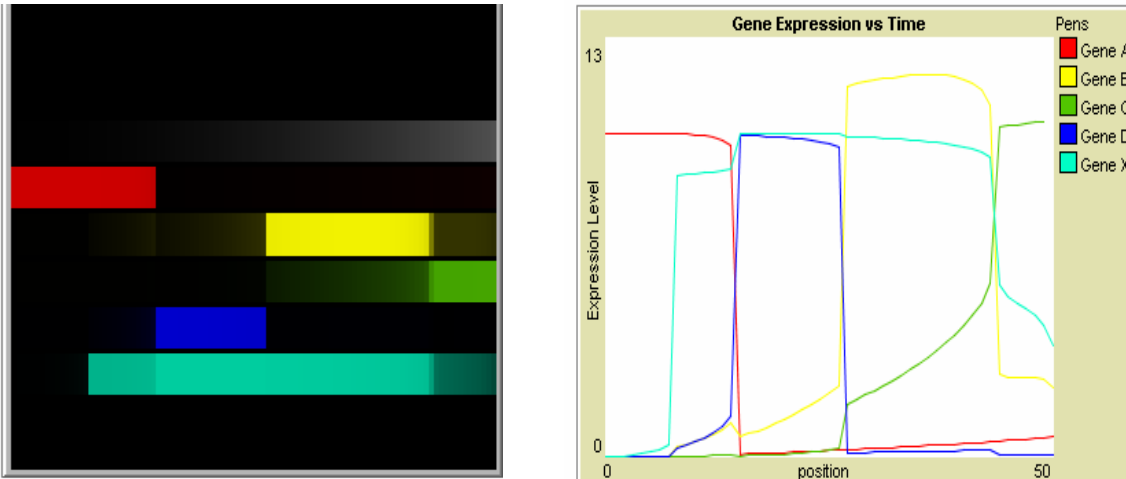


Figure 3.7(a): Spatial expression of the MHB genes

Figure 3.7(b): Plot of expression level of MHB genes corresponding to a given position in (a)

### 3.2.2 Formation of Organizing Centre

Development involves proper spatial and temporal patterning to give rise to structures at proper position. There are two major ways of organization: a) along the dorso-ventral axis and b) along the anterior-posterior axis.

The formation of organizing centers in zebrafish involves a complex genetic regulatory network. We planned to model this early differentiation process in order to get insights about molecular level interactions among cell that may help to redefine/reiterate/fine-tune experiments or previous models.

#### *Modeling in NetBuilder - Gene Regulatory Network*

We used the NetBuilder [8] application tool for making a GRN. The choice was based on the developmental models presented by the researchers that showed the genetic network for endo-mesoderm specifications.

In NetBuilder GRN is modeled as a signaling network, in which the components (gene/promoter/transcription factor) respond to signals. For the component gene the input signal is the transcription factor that binds to the gene and modulates the transcription rate (output signal). Network nodes do this transformation.

### ***NetBuilder a failure***

After making a few simple test models we could not generate the required model. It is useful to understand the gene regulation at the genomic level but initially we were more interested in the visualization of the cellular automata and their interaction, which assists in forming organizer regions in the zebrafish embryo. NetBuilder did not have this capability. It could only make a model of Gene network that can be simulated to see the affect of switching on or off certain signals in the pathway.

Additionally, the program was not very user friendly and it was tedious to set up a genetic network.

### **3.2.3 Modeling gene network for organizer development in NetLogo**

In Zebrafish there are two organizing centers that form tissue polarity during morphogenesis. We modeled the formation of these organizing centers based on the Meinhardt's theory of activator-inhibitor systems involving reaction-diffusion mechanism and competence factor. The reaction involves an activator inhibitor system with autocatalysis where an activator has positive affect autocatalytic effect on its own production and also that of an inhibitor while an inhibitor does not have any effect on its production though it inhibits activator production. Diffusion involves activator and inhibitor diffusion in the system with a short range diffusing activator and a long range diffusing inhibitor. Together with feedback on the source density only one maximum is formed.

#### **Modeling a single organizing center: Dorso-ventral organizer**

The neural tube of zebrafish embryos is, like that of other vertebrates, highly polarized along its dorso-ventral axis. In the spinal cord, sensory neurons form at dorsal aspects, whereas

motor neurons and the floor plate develop at ventro-lateral and ventral positions, respectively, and interneurons occupy intermediate regions [23].

In Zebrafish neurogenesis patterning it is observed that the regeneration of the organizing centre can occur. To capture this, we model the component, competence, in the system. It acts same as source density, however, *per se*, it is formed by the activator system.

*Activator – Inhibitor - Competence system:* Meinhardt’s theory was used as a basis to model this system for zebrafish [7]. We represented the system by the following set of equations:

$$\frac{\partial a}{\partial t} = competence \left( \frac{a^2 + b_a}{i(1 + saturation \times a^2)} \right) - a \times decay_a + D_a \nabla^2 a$$

$$\frac{\partial i}{\partial t} = (competence \times a^2) + b_i - i \times decay_i + D_i \nabla^2 i$$

$$competence = c * \frac{a^2}{1 + a^2} + b_c - competence \times decay_{competence}$$

*a*-activator, *i*- inhibitor

*b<sub>a</sub>* , *b<sub>i</sub>* , *b<sub>c</sub>* basic activator , inhibitor and competence production

*D<sub>a</sub>* and *D<sub>i</sub>* diffusion rates of activator and inhibitor

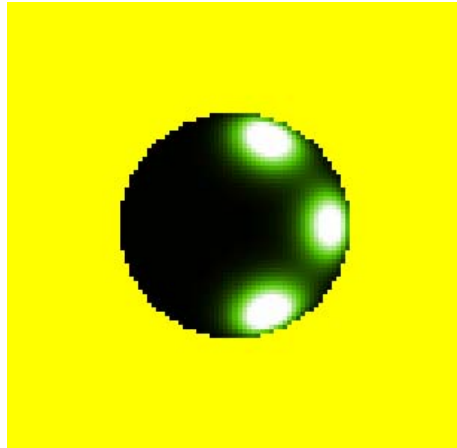
In terms of the above equations the crucial condition for pattern formation is that the diffusion of the inhibitor is much higher than that of the activator i.e. the condition  $D_i \gg D_a$  must be fulfilled [7]. As shown by Granero et al. [24] the inhibitor must diffuse at least 7 times faster than the activator.

### ***Algorithm in Netlogo***

1. Set up sliders for the user-defined parameters: decay rates (*decay*), diffusion rates for the activator and inhibitor. (See appendix 6 for a screenshot of the model setup).
2. Setup a boundary condition for the system. The system components within the boundary interact with each other.
3. Set the initial conditions. The decay rate and basic production rate are kept constant.

4. Set up the concentration change in the A-I-C system (reaction change) and then diffuse the activator and the inhibitor based on the strength of diffusion rate values.
5. Repeat step 4 till some stable pattern is observed.

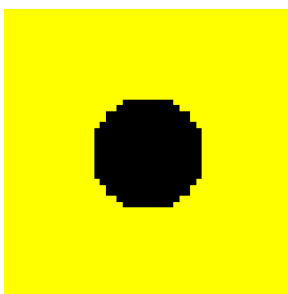
### ***Result***



**Figure 3.8: Visualization of single organizer formation**

The model did not give precise results; instead of one single organizer center we got three organizers as shown in the above figure. This was because of the in-built NetLogo diffusion function that was utilized; the diffusion of the cellular automaton as represented by the agents in the NetLogo model was not in line with the needs of the model. We then tried to write our own diffusion function. We tried different versions of model, defining boundary conditions or enforcing user-defined diffusion mechanism. However, it was a complicated process to handle a number of agents changing values simultaneously and it gave no better result than the in-built function. We also tried to model the two-organizer system formation (see appendix 7 for setup in NetLogo) and the visualization was not in accordance with the schema modeled. NetLogo posed two limitations:

- a. Definition of the boundary condition
- b. Diffusion in a limited boundary condition



**Figure 3.9: Description of the boundary and diffusion limitation in NetLogo**

The above figure shows this limitation. In its present version the diffusion function performs diffusion on the entire set of patches exposed in the graphics window (i.e. the entire yellow area in the figure) though we want diffusion to take place only in the desired area (colored black in the figure). Hence we used another tool, CompuCell to model the formation of organizing centers.

### **3.2.4 Modeling neural patterning with COMPUCELL**

As a first read the CompuCell applications and capability of the software looked viable to model a system involving the formation of organizer centers in Zebrafish neurogenesis. However, even though the theory seemed to fit our description of modeling environment, the technical difficulties with the software made it unfit for use.

#### ***Issues with CompuCell***

- The tool was not organized properly for having a single place that gave link to all the resources needed to run the tool.
- With no proper installation documentation it took a lot of wasteful hours to learn how to run the tool.
- The Windows version had lots of bugs. It had a linux version too but that was not tried. However, the windows version was not functioning properly. When the authors of CompuCell were contacted detailing this problem they replied that they are working on a newer version of the program that will not have the bugs that were crucial for proper and full functioning of the tool.
- It had an extensive software requirement that was very tedious and time-consuming process. On windows with the CompuCell version I used, required installation of 5

different software packages. Some of the tools were difficult to obtain s no proper links for the appropriate version(s) to be downloaded were available. One of the requirements was installation of the .NET framework. Luckily, I had a student copy of the software application with me else it would have been difficult to run CompuCell. And these requirements were not exhaustively documented. Even after installation of all the requirements as per information available from user groups the program was not able to run properly.

Due to the above reasons our work on CompuCell tool was stalled. In September a new bug-free version of CompuCell as a standalone program was launched. However, this newer version has not been tried due to time constraints.

### **3.3 PHASE III: PRESENTING THE LEARNING**

My final task was to make a dynamic website for the Chitnis lab at Unit of Vertebrate and Neural Development, NICHD. Though I started coding during the last part of my internship the process of collecting information started quite early. During free time and breaks my guide would explain to me the contents and the basic design of the website. Infact, it was quite an experience to take down notes while he used to give me detail explanations about the various aspects he wanted to present on the web. The notes where then organized into prototype web pages that were subsequently modified based on suggestions of my guide and other lab researchers.

The basic idea behind making the website was to showcase the research activities of the lab to other researchers and students, for generating their interest, which might help in collaborative work in future.

#### **3.3.1 Web Development**

As a first step to website development I made a prototype of the various feature content pages that were needed. Once the prototype was approved I compiled the information into a fully functional website (view appendix 10,11 for screen shots of the web pages).

**Software used:** Microsoft FrontPage, Dreamweaver, Photoshop for making pictures for the website, NetLogo 2.0.2 for converting models to applet

The programming mainly involved HTML, JavaScript and style sheets (for giving a uniform look to all the pages).

### **3.3.2 Showcasing the NetLogo models: Converting to applet**

The applet requires Java 1.4.1 or higher to run. It will not run on Windows 95 or Mac OS 8 or 9. Mac users must have OS X 10.2.6 or higher and use a browser that supports Java 1.4 applets. On other operating systems, one may obtain the latest Java plug-in from [Sun's Java site](http://java.sun.com/getjava/download.html) (<http://java.sun.com/getjava/download.html>).

To convert to applet the model file and the file *NetLogoLite.jar* must all be in the same directory. One can create a separate model folder and can make applets by copying *NetLogoLite.jar* from the directory in which NetLogo is installed into the directory where models are placed. A number of models depicting various processes involved in neural development were converted into applets. Among these two of the models, made by earlier intern Ei-Ei Gaw [22] need mention as they relate to the models and Meinhardt schemas as presented in this report and were used in our study.

#### **i) Delta Notch signaling model**

This model is based on the idea of autocatalysis and lateral inhibition (as seen earlier in the four-gene, model). In developing embryo cells express an “activator” that promotes neural fate and drives its own expression. When the level of activator exceeds some defined threshold cells become neurons; however, all cells that initially express activator do not become neurons because of lateral inhibition provided by delta-notch signaling. The signaling process stimulates *her*<sup>5</sup> gene expression and the latter acts as an inhibitor, inhibiting activator in neighboring cells.

---

<sup>5</sup> *Her* – Her genes are family of receptors that are involved in signaling pathways. In development they are expressed during somatogenesis in the nodal network.

There were many different versions made of this model that helped in understanding various aspects and models. For display on the website, the model was converted into applet and interactive help buttons were added [see appendix 8].

## **ii.) AP Patterning model**

This model is to explore how gene expression patterns may form under the influence of a morphogen gradient. Here there is slight modification in determination of the morphogen gradient; it is not calculated as a simple straight-line distribution but as a function of the morphogen and *tcf3*<sup>6</sup> gene expression levels. The effective morphogen gradient is proportional to morphogen and inversely proportional to *tcf3* [see appendix 9 for the model setup in NetLogo].

The above models were studied as they were supposed to be modified, incorporating growth and formation of organizer center that would influence the gene expression as an emergent property. However, since the modeling of growth and organizer center formation was not achieved in NetLogo, we could not do the integration and modification in the above models.

---

<sup>6</sup> Tcf3 (Transcription factor 3) – homeobox gene involved as transcriptional repressor for axis induction in early embryo. Involved in a form of pre-B-cell acute lymphoblastic leukemia.

## SECTION 4

# CONCLUSIONS

### 4.1 CONCLUSION

The process of neural development, from a mathematical perspective, is seen as a combination of various sub processes; and for modeling, each of these processes is hypothesized to be independent. Identifying the various factors, and their parameters, influencing the model or the biological process follows this. For example modeling the process of neurogenesis might involve identifying how a uniform environment in the embryo breaks symmetry and develops specific differentiated neural cells and tissues. From a biological perspective the same study may involve investigating what genes are promoting and suppressing each other, and how the proteins they encode interact. My internship was an effort to understand both the aspects and use them in a synergistic manner to improve understanding of the neural development process at molecular level.

We have studied various models that can be matched to different processes involved in the development. The reaction-diffusion mechanism forms patterns that are transient since they depend on the size and the geometry of the fields. Since both size and geometry change during development this model can be used in describing formation of a morphogen gradient wherein the graded concentration profiles are created by reaction-diffusion mechanisms and provide positional information for the cells [2, 26].

The mechanism of autocatalysis and lateral inhibition plays a role both at early and late stages in the development. During initial stages of development this schema is involved in initiating the pattern formation. During later stages it again plays an essential role in allowing the spacing of repetitive structures such as in the somatogenesis (formation of somites) process in zebrafish. This mechanism was explored in the four-gene model and allows the selection of a small region out of a larger possible area. This type of pattern formation is based on competition and external influences. For example the presence of graded morphogen gradient can determine which region will dominate over others.

We next studied the formation of an organizing center that is a primary step in correct development process. The development of such a center involves generation of polarity from a more or less homogeneous medium. A reaction in which a short-range autocatalysis is coupled with a long-range inhibition in presence of competence factor (helps in suppressing secondary maxima) is able to generate such a pattern in a very reliable and stable way. The mechanism also accounts for the re-establishment of an “organizing centre” after its unspecific induction.

There are two basic primary organizing centers involved in development in zebrafish. The first one is along the anterior-posterior (AP) axis that is used for orientation, and the second one is along the dorso-ventral axis, which is required to organize the dimension perpendicular to the AP axis. For the developing organism it is essential that both patterns not be parallel to each other. In my internship, models representing development of the organizer centers could not be achieved due to the shortcomings of the programs NetBuilder and CompuCell, which were utilized for modeling and visualization of the system. NetBuilder models a system of cells and tries to stimulate the gene network. However, our studies were not exactly at the genomic level hence it was not suited to model the emergence of two different organizing systems. The system also could not be modeled with CompuCell: a tool made specifically to model and simulate morphogenesis process, due to bugs in the windows version of the tool. In the NetLogo environment we achieved partial success in developing a basic model for a single organizer center. Enhancement of the basic model to incorporate dynamics of growth of the region was hampered by the limitation of the tool as it did not have a relaxed definition of the in-built diffusion function and boundary condition as needed by the model describing the formation of the organizer center.

The mechanisms of pattern formation during development discussed above do not represent an exhaustive and self-sufficient list to model a complete set of processes involved in neural development. The intention of this internship was to gain knowledge about neural developmental process, understand the basics of pattern formation during neural development using models to study the emergent property, and compare the latter to the observations as seen in the experimental work. The internship introduced me to the NetLogo programming environment for observing new results or appreciating the known ones during simulation of the model.

The models developed in NetLogo were found, by computer simulation, to be able to account in a quantitative way for some initially chosen basic experimental observations. Though some of the models were not close to the biological system being modeled (e.g.: organizer center development model), once a model consistent with the experimental observations was developed, it was also found often to be able to account for additional phenomenon for which it was not originally designed (e.g.: Four-gene model- observations found for the effect of removing morphogen at various stages). This of course, does not prove the validity of the model. However, it does suggest that the models are close to the development process in the biological system and can help in unveiling of some emergent properties that may not be observed on experimental desk. The possibilities that can be explored using mathematical and computational tools in study of developmental biology are many and future work in this area is required to understand and explore various biological systems and experimental studies all of which aim in benefiting the human life.

## **4.2 FUTURE WORK**

Some of the work presented in this report, tried for the first time, is at a basic level. These models can be enhanced to study further details about the neural development processes and involved gene networks. Basically we have used the Meinhardt equations with little adjustments to suit our precise model. I feel in future that it may, additionally, be required to understand the modeling based on theories by other researchers.

Our model of Xiro1 gene, describes the formation of the isthmic organizer (the mid-brain hindbrain boundary), and shows how the spatial patterning takes place at the mid- neural stage. This model is made for the interactions at the final stage (the process starts at the gastrula stage) and takes into account only the major genes; those play a role in the positioning of the isthmic organizer. The model can be modified and made more robust by incorporating temporal patterning, i.e. modeling the gene interaction from gastrula stage and letting the system evolve and grow over time so that the final result that we observe at the nerula stage is an emergent property of the system. Also the model can be modified to incorporate additional genes like En2 that also play a role in the midbrain hindbrain boundary formation.

In the organizer model we were unable to exactly achieve the formation of an organizer center in NetLogo environment. Future work may involve overcoming the limitations of the NetLogo environment by exploring various diffusion functions. Though we tried to write our own diffusion functions the search and effort was not exhaustive. While modeling organizer region it is important to account for the growth in the size of the regions. This is important in order to get insight into how cell growth plays a role in pattern formation. We did try to achieve this but the efforts were not successful. With the new version of CompuCell, for windows system, being launched one may retry forming the organizer model in this application to explore how the organizing center leads to specific polarity and patterning during development. One may also try the Linux version that is bug-free and on which we may get in-built model examples.

The developed website was mostly a static collection of web pages that gave information about the lab. Apart from exploring the models online, there was not much interaction with the users. Future work may involve making the website can be made more dynamic and interactive by incorporating feedback forms, online discussion groups. However, due to time constraints this feature was not added. Also, in achieving this security issues had to be checked with the NIH IT division, which would have required a good amount of time. In future the functionality of the website can be extended by not only making it a showcase of lab activities, but also a information board wherein various news/upcoming events/seminars can be posted for lab users and other researchers. To achieve this authorized lab users may be given the administrator access to update content on the site or, if it is possible (by crosschecking with the IT department about the server access), one can create the updating of news or posting of events dynamically.

Future work in mathematical modeling of biological processes can play a vital role in enhancing the understandings of the animal system and the learning can be used to improve experimental design for molecular level studies. Additionally development of computational tools for visualization of the model such that the modeling environment acts like a biological system can help in making the virtual models close to the real system.

## REFERENCES

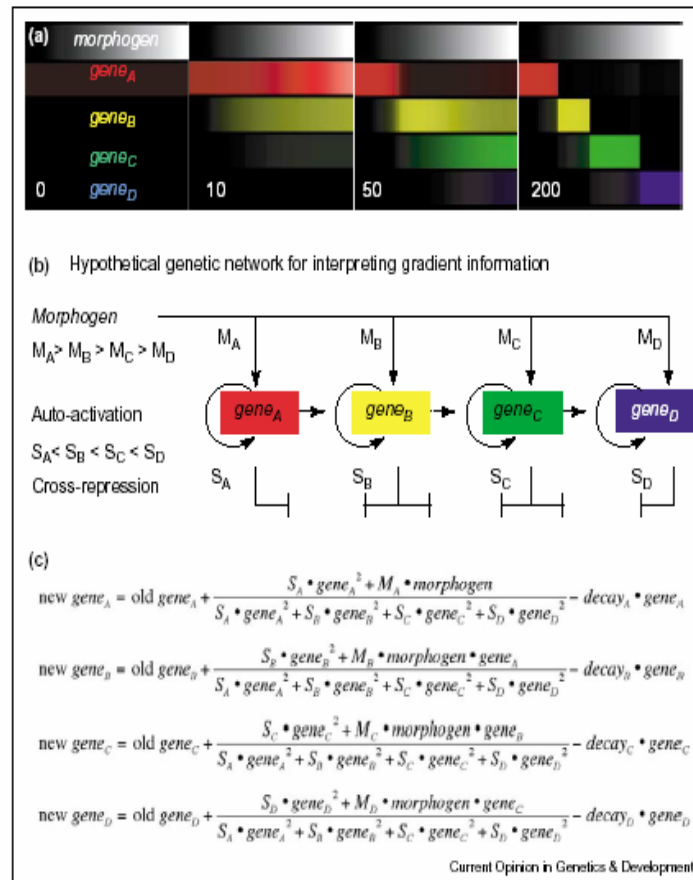
1. Glavic A, Gomez-Skarmeta JL, Mayor R. (2002). **The homeoprotein Xiro1 is required for midbrain-hindbrain boundary formation.** *Development*. 2002 Apr; 129(7): 1609-21
2. Meinhardt, Hans. **Models of Biological Pattern formation.** (1982) Academic Press, London
3. Meinhardt, Hans. **The Algorithm beauty of seashells.** (1995)
4. Pages F and Kerridge S. (2000) **Morphogen gradients. A question of time or concentration?** *Trends in Genetics*. 2000 Jan; 16(1): 40-4.
5. Kim, C-H, Oda T, Itoh M, Jiang D, Artinger KB, Chandrasekharappa SC, Driever W, Chitnis AB. **Repressor activity of Headless/Tcf3 is essential for vertebrate head formation,** *Nature*. 2000 Oct 19; 407:913-6.
6. Wilson SW, Houart C. **Early steps in the development of the forebrain.** *Dev Cell*. 2004 Feb; 6(2): 167-81
7. Meinhardt H. **Organizer and axes formation as a self-organizing process.** *Int J Dev Biol*. 2001; 45(1): 177-88.
8. Davidson EH, Rast JP, Oliveri P, Ransick A, Calestani C, Yuh CH, Minokawa T, Amore G, Hinman V, Arenas-Mena C, Otim O, Brown CT, Livi CB, Lee PY, Revilla R, Rust AG, Pan Z, Schilstra MJ, Clarke PJ, Arnone MI, Rowen L, Cameron RA, McClay DR, Hood L, Bolouri H. **A genomic regulatory network for development.** *Science*. 2002 Mar 1; 295(5560): 1669-78.
9. Schilstra, MJ and Bolouri, H. **Modelling the regulation of Gene Expression in Genetic Regulatory Networks (NetBuilder Theory)** *Biocomputation group, Science & Technology Research Centre, University of Hertfordshire, UK*
10. Chitnis AB, Itoh M. (2004). **Exploring** alternative models of rostral–caudal patterning in the zebrafish neurectoderm with computer simulations. *Curr Opin Genet Dev*. 2004 Aug; 14 (4): 415-21.
11. Stemple DL, Vincent JP. **Pattern formation and developmental mechanisms: super-models of development.** *Curr Opin Genet Dev*. 2004 Aug; 14(4): 325-7.

12. Wurst W, Bally-Cuif L. **Neural plate patterning: upstream and downstream of the isthmic organizer.** *Nat Rev Neurosci.* 2001 Feb; 2(2): 99-108.
  
13. Izaguirre JA, Chaturvedi R, Huang C, Cickovski T, Coffland J, Thomas G, Forgacs G, Alber M, Hentschel G, Newman SA, Glazier JA. **CompuCell, a multi-model framework for simulation of morphogenesis.** *Bioinformatics.* 2004 May 1; 20(7): 1129-37. Epub 2004 Feb 05.
  
14. Turing AM. **The Chemical Basis of Morphogenesis.** *Philosophical Transactions of the Royal Society B (London)*, 237, 37--72, 1952.
  
15. Tisue, S., & Wilensky, U. **NetLogo: Design and Implementation of a Multi-Agent Modeling Environment.** SwarmFest, Ann Arbor, May 9 - 11, 2004.
  
16. Tisue, S., & Wilensky, U. **NetLogo: A Simple Environment for Modeling Complexity.** International Conference on Complex Systems, Boston, May 16 - 21, 2004.
  
17. Wilensky, U. **Modeling Nature' s Emergent Patterns with Multi-agent Languages.** Proceedings of EuroLogo 2002. Linz, Austria.
  
18. Wilensky, U. **Modeling Emergent Phenomena with StarLogoT .** CONCORD.org, Winter 2000.
  
19. Wilensky, U. & Reisman, K. **Learning Biology through Constructing and Testing Computational Theories - an Embodied Modeling Approach.** Proceedings of the Second International Conference on Complex Systems. Nashua, 1998, NH: New England Complex Systems Institute.
  
20. Meinhardt, H. and Gierer, A. **Application of a theory of biological pattern formation based on lateral inhibition.** *J.Cell Sci.* 1974 15,321-346.
  
21. Wilensky, U. (2002). **NetLogo. Center for Connected Learning and Computer-Based Modeling.** Northwestern University, Evanston, IL. <http://ccl.northwestern.edu/netlogo/>.
  
22. Gaw E, Chitnis AB. **Simulating gene expression patterns of neurogenesis, somatogenesis and morphogenesis during early zebrafish embryo development.** Internship report, Jan 2004, CBS program, Arizona State University.

23. Blader P, Strahle U. **Zebrafish developmental genetics and central nervous system development.** *Hum Mol Genet.* 2000 Apr 12;9(6):945-51.
24. Granero M.I., Porati A, Zanacca D. (1977). **A bifurcation analysis of pattern formation in a diffusion governed morphogenetic field.** *J. Math. Biology.* 1977; 4, 21-27
25. Meinhardt H. “**SP program for modeling simulation**”. Free software tool available with the book “The Algorithmic beauty of sea shells”
26. Max-Planck-Institut für Entwicklungsbiologie, “**Meinhardt webpage**”  
<http://www.eb.tuebingen.mpg.de/dept4/meinhardt/biuprog.html> (Sept 2004)
27. Meinhardt H. **Models of biological pattern formation: common mechanism in plant and animal development.** *Int J Dev Biol.* 1996 Feb; 40(1): 123-34.
28. Wilson PA, Hemmati-Brivanlou A. **Vertebrate neural induction: inducers, inhibitors and a new synthesis.** *Neuron.* 1997 May; **18**, 699–710.

# APPENDICES

## Appendix 1 Chitnis & Itoh model of gene network for interpreting morphogen gradient

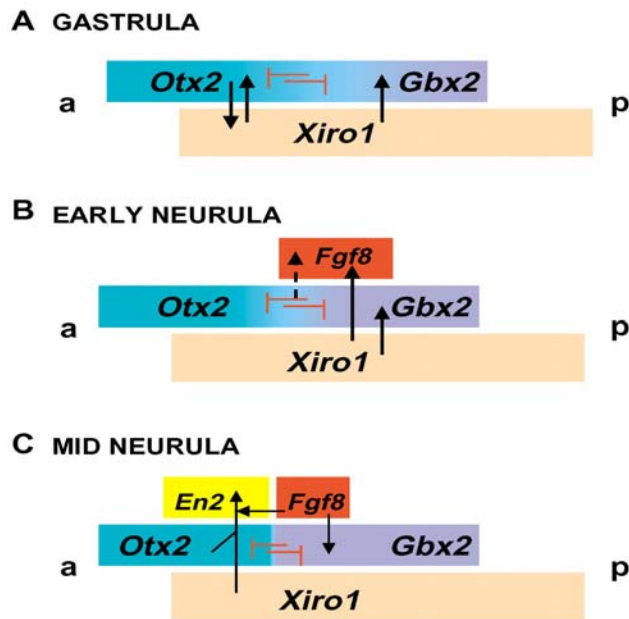


Dynamics of a hypothetical genetic network for interpreting gradient information. Panel (a) shows a graphical output of the network after 0, 10, 50 and 200 iterations of the program. Relative brightness of colors reflects level of gene expression along the rostral-caudal axis (100 cells).

(b) A schematic representation of genetic interactions implemented for the output shown in (a). Note the systematic differences in the influence of the morphogen ( $M_A, M_B, M_C, M_D$ ) and in auto-regulation and cross-repression ( $S_A, S_B, S_C, S_D$ ). Dashed arrows between rostral and caudal genes show that rostral genes help initiate expression of caudal genes in this version of the network. Panel (c) shows the equations that were used to implement this genetic network in the computer program. For the simulations shown in (a),  $M_A = 5, M_B = 4, M_C = 3, M_D = 1; S_A = 0.1, S_B = 1, S_C = 3, S_D = 7$ ; The program was initiated with  $gene_A = 0.1$  and  $gene_B, gene_C$  and  $gene_D = 0$ . Maximum morphogen = 5; basal activity = 0. Final level reached for each of the genes was 10.

**Source:** Chitnis AB, Itoh M. (2004). Exploring alternative models of rostral–caudal patterning in the zebrafish neuroectoderm with computer simulations. *Curr Opin Genet Dev.* 2004 Aug; 14 (4): 415-21.

## Appendix 2 Model describing interaction among MHB genes (Xiro1 Model)



**Xiro1 Model:** A model for the induction and positioning of the isthmus organizer. (A) Gastrula. *Xiro1* encompasses *Gbx2* expressing domain and the presumptive midbrain territory of *Otx2* and participate in the activation of both genes (arrows). In addition, *Otx2* also activates *Xiro1* expression in the midbrain. At this stage *Otx2* and *Gbx2* expression domains overlap in the prospective isthmus and the mutual repressive activities between the corresponding proteins begin (red lines) (B) early neurula. The expression domains of *Otx2* and *Gbx2* start to separate although a faint overlapping is still detected. At this stage, *Xiro1* is no longer able to activate *Otx2*. In addition, *Fgf8* expression, and therefore the establishment of the isthmus, begins as a result of the overlapping domain created by *Otx2* and *Gbx2* (broken arrows) and the activity of *Xiro1* in this region (arrow). (C) Mid neurula. A sharp boundary between *Otx2* and *Gbx2* arises, which is probably due to an equilibrium reached by their cross-inhibitory activities (red lines). The interaction between *Otx2* and *Gbx2* maintains *Fgf8*, which reinforces the expression of *Gbx2* in the caudal face of the isthmus (arrow). In addition, *Fgf8* induces *En2* expression in the competent territory defined by the co-expression of *Otx2* and *Xiro1*. a, anterior; p, posterior.

**Source:** Glavic A, Gomez-Skarmeta JL, Mayor R. The homeoprotein *Xiro1* is required for midbrain-hindbrain boundary formation. *Development* 2002 Apr; 129(7): 1609-21

### Appendix 3 Models of ectodermal fate choice in response to BMP signaling.

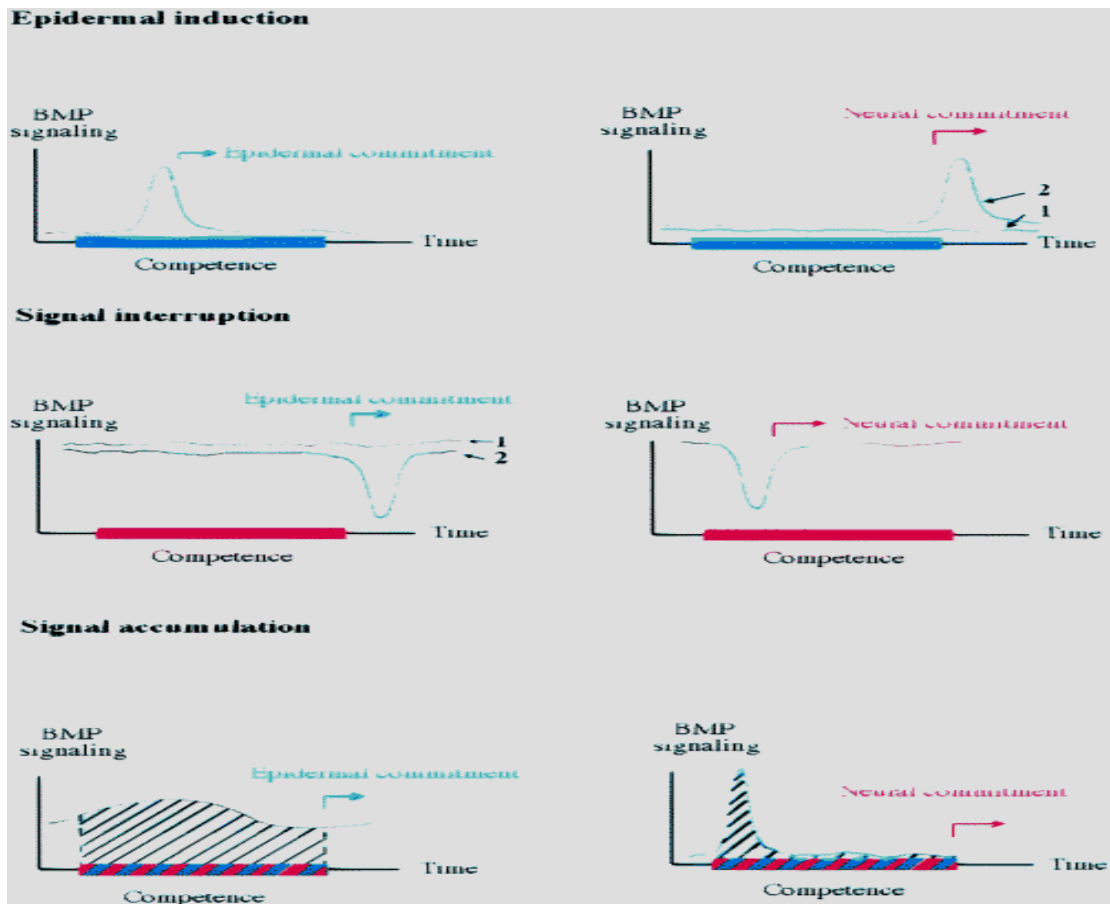


Figure 3. Three general ways in which ectodermal cells might choose between neural and ectodermal fates in response to BMP signaling, with different consequences for the nature of competence and commitment. The three models are described in more detail in the text. In each case, neural is the default fate of cells in the absence of all BMP activity, and neural inducers act by reducing or interrupting the BMP signaling experienced by ectodermal cells. In the scenario diagrammed at the top, cells that receive a BMP signal of sufficient intensity at any time during a period of competence (blue bar) become committed to form epidermis (left). Cells that do not receive a BMP signal (1), or receive a signal after competence has expired (2), form neural tissue (right); commitment occurs at the end of competence. In the signal interruption model, epidermal specification requires continuous BMP activity (left). If this signaling is interrupted at any time during a certain period (red bar), cells become committed to neural development (right). Interruption that happens too late has no effect (left: 2). Finally, cells could evaluate the total amount of BMP activity throughout a critical period, represented by the area under the curves in the bottom drawings. In this case, a brief exposure to a strong signal (right) might not be as effective in specifying epidermis as weaker signaling of longer duration (left).

**Source:** Wilson PA, Hemmati-Brivanlou A. Vertebrate neural induction: inducers, inhibitors and a new synthesis. *Neuron*. 1997 May; **18**, 699–710.

## Appendix 4 Four Gene Model setup in NetLogo

**NetLogo: 4\_Gene\_Morph\_positive**

File Edit Tools Zoom Tabs Help

Interface Information Procedures Errors

Edit Delete abc Button abc 5 Slider On Off Switch abc 5 Choice abc Monitor abc Plot abc def ghi jkl Text

**Parameters for Morphogen Gradient**

source 4.8

baseline 0.00

Clear All

Setup Region Go Region Info/ Tips

**Production Rates**

sA 1.5

sB 2.4

sC 3.4

sD 4.3

**Decay Rates**

decayA 0.10

decayB 0.10

decayC 0.10

decayD 0.10

**Effect of Morphogen**

mA 2.10

mB 1.75

mC 1.30

mD 0.95

Time 0

Morphogen 0.0

**Gene Expression vs Time**

Expression Level

position

Pens

- Gene A
- Gene B
- Gene C
- Gene D

**Morphogen Gradient**

morphogen

position

Pens

- Morph

Graphic showing the localization of the 4 genes

Command Center Clear

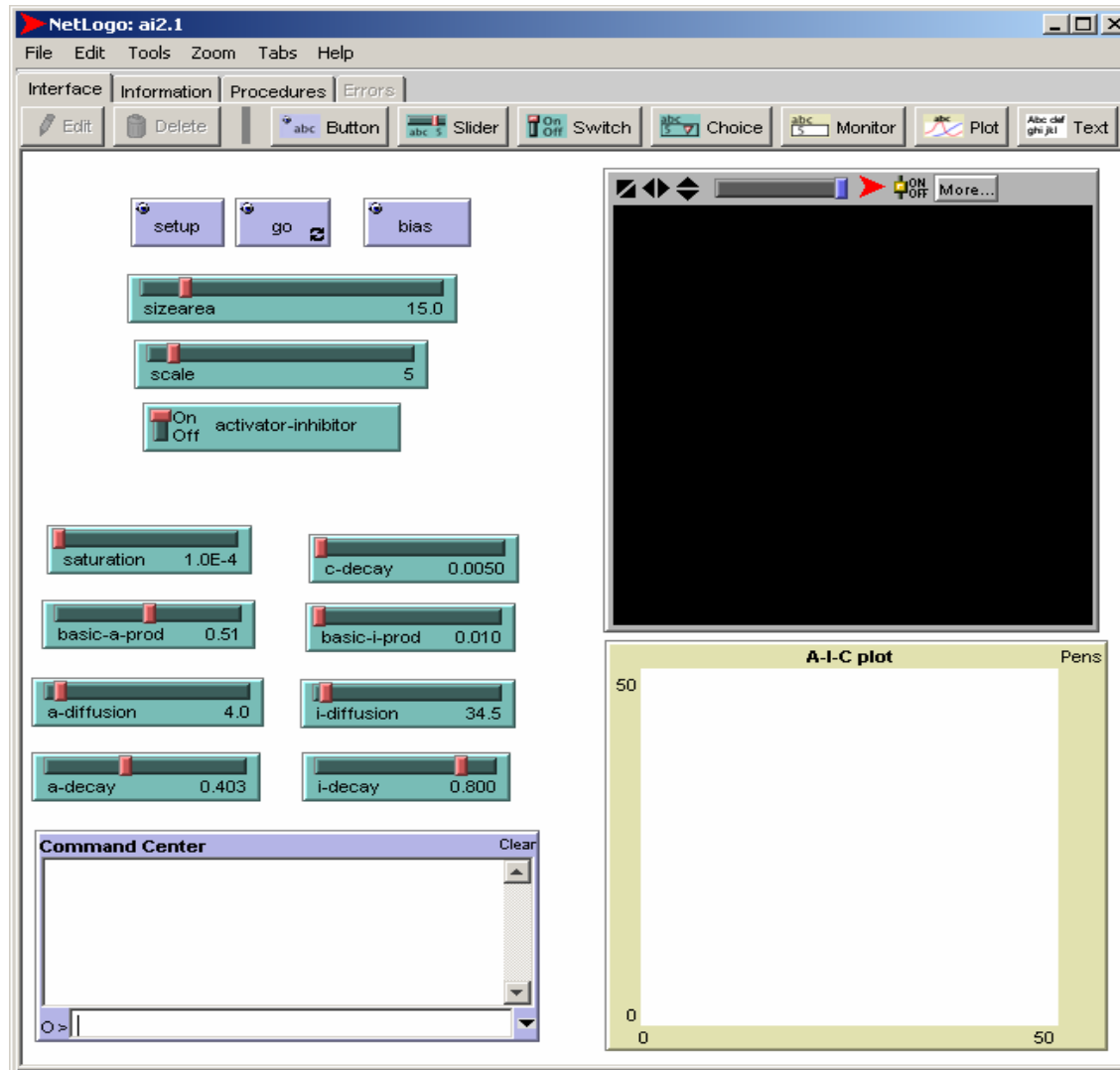
0 >

## Appendix 5 Xiro1 model in NetLogo

The screenshot shows the NetLogo interface for the Xiro1 model. The window title is "NetLogo: Xiro1\_mhb1.2". The menu bar includes File, Edit, Tools, Zoom, Tabs, and Help. The interface is divided into several sections:

- Parameters for Morphogen Gradient:** Includes sliders for "source" (7.0) and "baseline" (0.00), and buttons for "Clear All", "restore default values", "Setup Region", and "Go Region".
- Production Rates:** Sliders for sA (1.1), sB (1.6), sC (2.4), sD (2.1), and sX (2.00).
- Decay Rates:** Sliders for decayA (0.10), decayB (0.10), decayC (0.10), decayD (0.10), and decayX (0.10).
- Effect of:** Sliders for mA (2.30), mB (1.95), mC (1.25), mD (1.55), and mX (2.00).
- Monitors:** "Time" (0) and "Morphogen-level" (0).
- Gene Legend:**
  - Gene A: Obx2 (Red)
  - Gene B: Gbx2 (Yellow)
  - Gene C: caudal gene (Green)
  - Gene D: fgf8 (Blue)
  - Gene X: Xiro1 (Cyan)
- Graphs:**
  - Gene Expression vs Time:** A plot with "Expression Level" on the y-axis (0 to 12) and "position" on the x-axis (0 to 50). A legend identifies the colors for Gene A, B, C, D, and X.
  - Morphogen Gradient:** A plot with "morphogen" on the y-axis (0 to 15) and "position" on the x-axis (0 to 50). A legend identifies the color for "Morph" (black).
- Control Elements:** A switch for "xiro\_present" (currently On), a "Command Center" window, and an "Info about model" button.

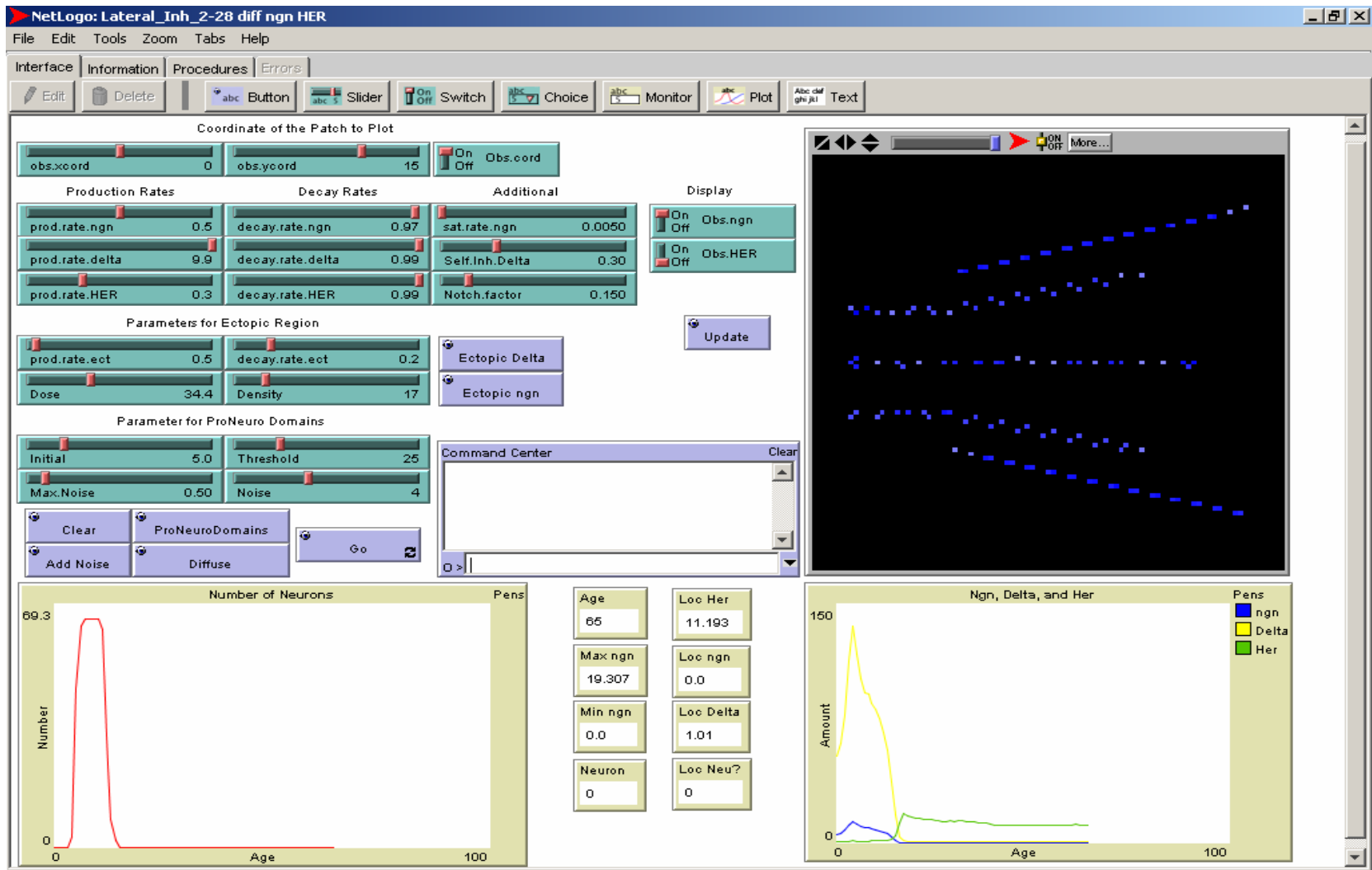
## Appendix 6 Simulating a single organizer formation in NetLogo



## Appendix 7 Simulating Two Organizer formation in NetLogo

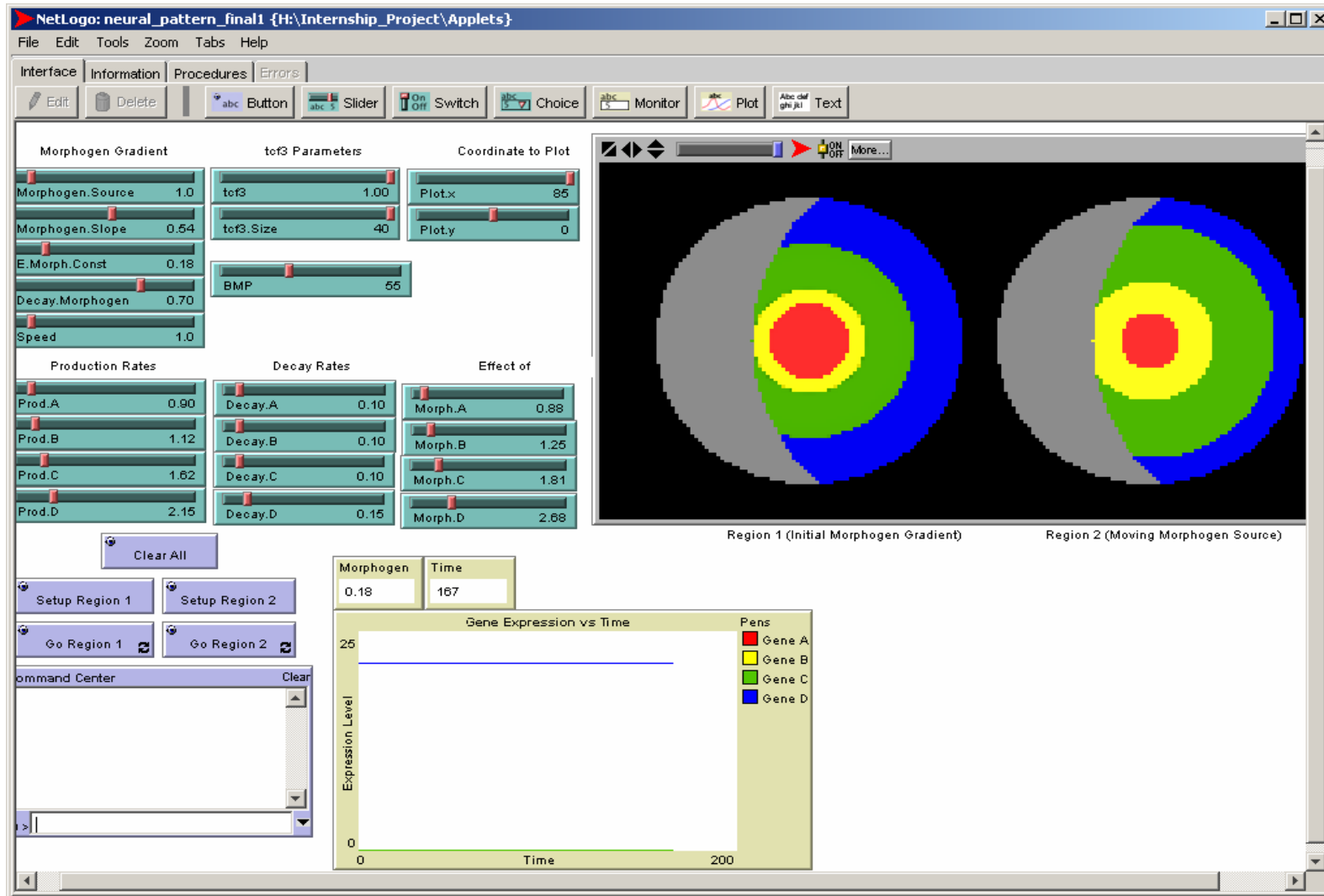
The screenshot displays the NetLogo environment for a simulation titled "ai\_2sys\_Nocomp". The interface includes a menu bar (File, Edit, Tools, Zoom, Tabs, Help) and a toolbar with various control elements. The main workspace features a central yellow square with a dark circular spot in the center, representing the simulation's state. To the left of the workspace are several control elements: "setup" and "go" buttons, a "bias" button, an "activator-inhibitor" switch, and five sliders for "sizearea" (20.0), "scale" (12.0), "scale2" (3), "saturation" (1.0E-4), and "saturation2" (0.217). To the right of the workspace are four sliders for "i-diffusion" (45.7), "a-diffusion" (3.0), "i2-diffusion" (50), and "a2-diffusion" (16). At the bottom of the workspace is a "Command Center" window with a "Clear" button and a "O >" prompt.

## Appendix 8 Neurogenesis: Delta-Notch model in NetLogo




Source: Ei-Ei Gaw and Ajay B Chitnis (2004) Simulating gene expression patterns if neurogenesis, somatogenesis and morphogenesis during early zebrafish embryo development.


## Appendix 9 AP patterning model setup in NetLogo



Source: Ei-Ei Gaw and Ajay B Chitnis (2004) Simulating gene expression patterns if neurogenesis, somatogenesis and morphogenesis during early zebrafish embryo development.

Appendix 10 Screenshot of the 'home page' of the website

 **The Laboratory of Molecular Genetics**  
National Institute of Child Health and Human Development  
National Institutes of Health



---

**Home**      Chitnis Lab

[What we do](#)

[Members](#)


[Models](#)

[Publications](#)

[Interesting Links](#)

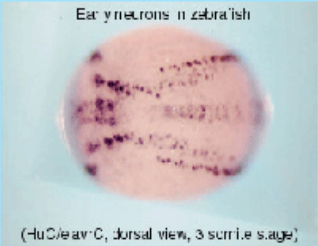
[Contact Us](#)

**Welcome to Chitnis Lab**




**Research Interests:**

Our goal is to understand how neurons are made in the correct number and location in the developing nervous system. To explore this problem we have been studying neurogenesis in the zebrafish embryo where neurons are distributed in a relatively simple pattern. In the caudal neural plate, which eventually forms the spinal cord, early neurons are distributed in three longitudinal domains where they form sensory neurons, interneurons and motor neurons, respectively.




[Early neurons in the zebrafish neural plate](#)

## Appendix 11 Screenshot of the 'project page' showing various models



**The Laboratory of Molecular Genetics**  
National Institute of Child Health and Human Development  
National Institutes of Health



[Home](#)      **Project List**

[What we do](#)

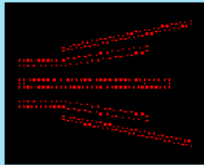
[Members](#)

**Models**

[Publications](#)

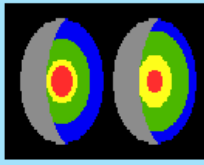
[Interesting Links](#)

[Contact Us](#)




**Lateral Inhibition**  
The basic idea is that cells express an "activator" that promotes neural fate. When the level of activator exceeds some defined threshold cells become neurons. The activator is autocatalytic and drives its own expression, however, all cells that initially express activator do not become neurons because of lateral inhibition (provided by delta-notch signaling). The model also studied the affect of initial noise with her expression in determining the cell fate

- [More Details...](#)



**Neural Patterning**  
This model is to explore how gene expression patterns may form under the influence of a morphogen gradient. The effective morphogen gradient is related to morphogen gradient and inversely related to tcf3.

- [More Details...](#)



**Four-Gene Model**  
This model is to explore how gene expression patterns may form under the influence of a morphogen gradient

- [More Details...](#)