A Methodology for Simulating Biological Cell Systems With Cellular Automata: The Case of the Human Heart

Research report

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Abstract

This these presents a study that focuses on finding a methodology for simulating biological cell systems such as the heart by using cellular automata. Cellular automata have given good results when simulating other evolving biological system. The goal is to use them to find the rules that the heart follows during its development. These rules are unknown but more knowledge about those rules that the heart follows during its development could provide for other medical areas such as the detection and prevention of birth defects and heart diseases during fetal development.

We present the key aspects of the methodology that can be used. The model will initially be a 2D multi-scale model. The neighborhood size of the cellular automata cells will be eight cells that surround the cell, but on the lowest level, which is the molecular level, rules will be used to represent the diffusion of molecules. Cell signaling drives the heart development and therefore the rule sets need to contain rules for at least nine interactions between cells and molecules. A new approach for generating the rules is introduced, that uses finite state machines (FSMs) as intermediates. The cell signals are simplified and the cell signaling processes are showed with FSMs. The FSMs are then used to generate simple rules for the rule sets. By using this approach we can be sure that the rules mirror the biology, which is very important.

Keywords: Heart development, cellular automata, cell signaling

Útdráttur

Þessi ritgerð fjallar um rannsókn þar sem markmiðið er að finna aðferðafræði til að herma líffræðileg kerfi líkt og hjartað með því að nota fylkjaaðgerðir. Fylkjaaðgerðir hafa reynst vel þegar verið að herma þróun annarra líffræðilegra kerfa. Markmiðið er að nota þær til að finna reglurnar sem að hjartað fylgir við þróun þess. Þessar reglur eru óþekktar, en meiri þekking á þessum reglum sem hjartað fylgir í þróun sinni gæti gefið mikilvægar upplýsingar fyrir önnur læknisfræðileg svið og gæti orðið til þess að í framtíðinni væri hægt að finna og koma í veg fyrir hjartagalla og hjartasjúkdóma í fóstrum.

Við kynnum lykilatriði aðferðafræðinnar sem hægt er að nota. Í upphafi verður módelið í tvívídd á tveimur stigum, þar sem annað stigið er frumustig og hitt sameindastig. Nágrenni hverrar frumu í módelinu eru átta frumur sem umkringja hana. En á sameindastiginu verður auk þess notaðar reglur fyrir flæði sameinda. Samskipti frumna er það sem að knýr þróun hjartans og þess vegna verða reglusettin að innihalda reglur fyrir að minnsta kost níu samskiptaferla á milli frumna og sameinda. Ný nálgun til að búa til reglurnar er kynnt, en þar eru notaðar stöðuvélar sem millistig. Frumusamskiptin eru einfölduð og stöðuvélar búnar til fyrir ferlana. Stöðuvélarnar eru svo notaðar til að búa til einfaldar reglur. Með því að nota þessa aðferð, þá getum við fullvissað okkur um að reglurnar sem við búum til muni endurspegla það sem gerist í alvörunni.

Lykilorð: Þróun hjartans, fylkjaaðgerðir, samskipti frumna.

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1 Introduction

There are three fundamental aspects of developmental biology, namely morphogenesis, cell growth and differentiation of cells. Morphogenesis is a biological process that is concerned with the shapes of entire organisms. It is also concerned with the positioning of many different specialized cell types. (Bartman and Hove 2005) The process of morphogenesis is self-organizing, that is, it displays emergent properties that are the results of both positive and negative feedback loop along with other interactions. During the process, the complexity of the tissue, organ or the organism is increasing, without external guidance. The complexity increases due to the emergent properties, that is, when multiplicity of components, where each components follows a set of relatively simple rules, form complex systems and patterns.

The human body is an extremely complex system that starts as a single zygote and develops to a full-grown human body, without being guided or managed by an external source. The knowledge of the rules that the human body follows during its development is negligible but this project will focus on the methodology for finding the rules that the heart follows. Although researchers have already become able to grow entire body parts, the rules behind the growth and the development are not known. However, if they were known, we could in the future be able to further advance similar processes. The potential of such technology is immense, stretching even to other medical areas such as detect and prevent birth defects and heart diseases during fetal development.

The heart development is divided into five stages: cardiomyte determination and specification, formation of two endocardial tubes, fusion of the two tubes into a primitive heart tube, heart looping and finally septation and valve formation.

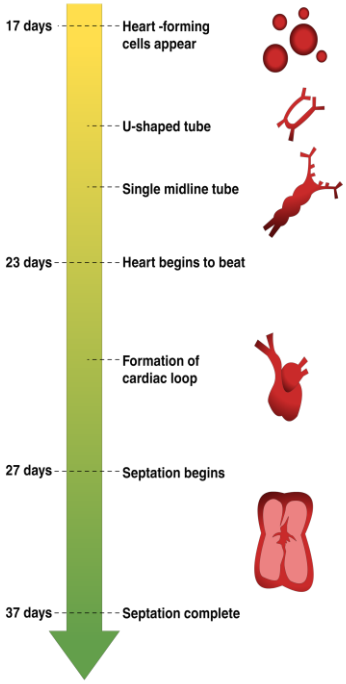

Figure 1: The Heart Development

When modeling a multidimensional system the choice is most often to use partial differential equations (PDEs). The PDE model is a continuous model and they are often transparent in terms how parameter changes affect outcomes and thereby are often more understandable. On the other hand, when using PDEs for modeling a biological system, such as the heart, a detailed knowledge of formulas of physical terms, especially kinetic terms, is required. A model of the heart development has never been done before. The reason why nobody has made that model is the little knowledge we have about all the physical terms of the development. The goal of this research is to infer new insights into how the human heart develops by finding and using new approaches and methodology.

- We will use a different methodology, cellular automata, for modeling the development of the heart. CAs have given good results when simulating biological systems and they don't require a detailed knowledge like PDEs.
- The model will be a 2D model on two levels, i.e. molecular and cellular level. That will give us insights into what rules the heart follows during its development. The starting point of the simulation is the second stage of the heart development.
- Each cell in the body has a set of rules that it follows. We will determine cells' neighborhood size and minimum size of the rule sets. We will also give a new approach to generate the rules and

answer if different rule sets can be used to simulate the stage and the effect that a little change in the rule set will have on the model.

This thesis is arranged as follows: it starts with a background section, where related work and motivation are discussed. That section is followed by a chapter about the heart, where we discuss the heart development and cell signaling. After that we talk about cellular automata and the methodology we use for the research. In the next chapter after that we present our results and finally we have conclusion, future work and acknowledgement.

2 Background

In this chapter we will introduce the background of the project. We will start by talking about the motivation behind this research and then we will talk about related work, that is, simulations using cellular automata.

2.1 Motivation

For a very long time may interests have been biology and mathematics. When I started my study at Reykjavik University and became a part of the Aperio system I got very interested in computer science too.

Students in the Aperio system are required to do independent studies and I really wanted my study to combine my fields of interest, that is, combining biology with mathematics and computer science. When I was deciding what I could do, there was one question stuck on my mind and I really wanted to know the answer too, at least to some extent. That question was: "How does the body know how to become a body?" In other words, what rules does the body follow during its development.

Next step was to choose an organ or at least a smaller system than the whole human body, because it is very obvious that it is too large. I decided that my project would be about the heart and its development, since the human heart and its function has always facinated me.

2.2 Related Work

Cellular automata (CAs) are good for simulating complex evolving systems. CAs have been used to model various systems and interactions for a long time, e.g biological systems, chemical system, traffic and social interactions. Some biological systems have been difficult to model, but in

recent years the use of cellular automata for biological systems has been increasing, where they have given good results.

Scientists have not yet succeeded in simulating the development of the heart. The standard way to model such biological system is using partial differential equations (PDEs). In the case of the heart, this is not possible, since PDEs require a detailed knowledge of physical terms that are not know for the heart development, e.g. the physical terms for the heart lopping are not known.

Despite of the standard way of using PDEs for simulating the biological system, some scientists have tried to use CAs for modeling and succeeded. Very few have tried to simulate the morphogenesis of systems in the human body but nevertheless, scientists have succeeded in creating a 2D model of vessel morphogenesis and vessel branching. There is presented a CA model, but the algorithm consists of a list of simple rules describing the essential biophysical features. The rule set for vessel morphogenesis in 2D consists of nine simple rules. (M. Markus, Böhm, and Schmick 1999)

Many other biological systems have been simulated, but they are often not as complicated as the heart development. The main focus of the scientists is examining the spatial and temporal pattern formation. These systems are e.g. reaction – diffusion systems, shell pattern formation, self-organization of ant trails and fibroblasts aggregation. (Ermentrout and Edelstein-Keshet 1993)

3 The Human Heart

The heart is the first functioning organ in the human body. From a zygote and until the embryo is about 3 weeks old the embryo gets oxygen and nutrients by simple diffusion. In week 3, the oxygen cannot reach all the embryo's cells by diffusion and therefore the development of the cardiovascular system begins. We will start by giving a short summarize about the heart development, then we will talk about feedback loops, cell signaling and different kinds of cell signaling in the heart.

3.1 The Heart Devlopment

Embryonic heart development takes about 3 weeks. The cardiogenesis involves growth, physical properties such as remodeling and morphogenesis, all self-organizing processes. There are five stages to heart development, namely cardiomyocyte determination and specification, formation of two primordial epithelial tubes and their fusion, heart looping, heart chamber formation and septation and valve formation. The first stage includes a specification and differentiation of the cardiomyocytes. This happens on day 17, when angiogenic cell clusters start to from in the yolk sac wall. These clusters lie in a horseshoe shape on the cardiogenic plate. This cardiogenic region is a special region of splanchnopleuric mesoderm, where all the vascular system is derived from. The day later they coalesce to form right end left endocardial tubes that are connected at the top. This is a primitive vascular network. It then extends towards and anastamose with the developing embryonic vasculature establishing a primitive circulatory system. The heart is derived from this primitive vascular system. On day 19 the tubes begin to develop from the cardiogenic region to a midline position in a region that will later become the thoracic region. This development happens because of embryo folding. Once the endocardial tubes are in this midline position they start to

fuse together and form a primitive heart tube. (Bartman and Hove 2005)The next stage is the most complex stage where the linear heart tube is transformed into an asymmetrical heart loop with a sequence of bending and twisting actions.(Abdulla et al. 2010) Many attempts have been made to quantify the stresses and strains within the developing heart. Potential mechanisms underlying looping are, for example, expansion of the cardiac jelly , differential growth of the heart tube and shortening of the dorsal mesocardium due to residual stress. Non of these hypothesis have been proved because they aren't consistent with the experimental data. Most of the previous researches that have been done on the heart looping indicate that the bending portion of the linear heart tube into the so-called C-shape is controlled by forces that are intrinsic to the heart tube itself. Lately there have been some suggestions that the winding part of the C-shape may be externally influenced by forces that act on the heart tube from the motion of adjacent tissues.(Bartman and Hove 2005) In the looping process distinct compartments become visible, i.e. the outflow tract, embryonic right ventricle, embryonic left ventricle and sinus venous. These compartments are key structures in the developing heart and are normally indicated as segments of the heart tube but cannot be distinguished until after the heart looping. The entire linear heart tube before the heart chamber formation is characterize by a low density of gap-junctions, but after the looping and the chamber formation the density of gap junctions becomes much higher, depending more on juxtacrine signaling.(Christoffels, Habets, et al. 2000) During the transformation of the linear heart tube into a four-chambered structure, the right atrium becomes directly connected to the right ventricle and the left ventricle to the outflow tract. This remodeling would require trans-differentiation of the left-ventricular segment. The formation of the chamber myocardium is a two-step process.(A Moorman et al. 2003) First there is the establishment of the “primary” transcriptional program within the cardiac crescent and linear heart tube, which is followed by the development of the heart chambers. When the chambers have formed the septation begins and the valve formation. (Anderson, Webb, et al. 2003)

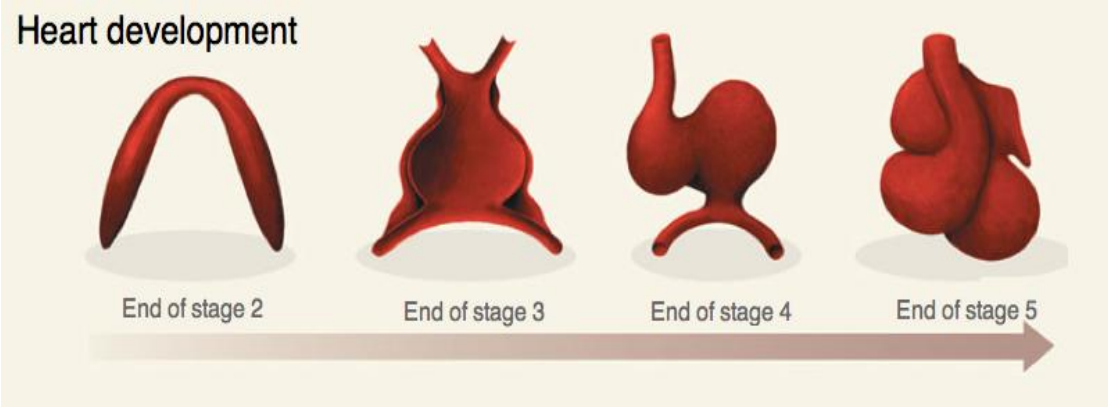

Figure 3: The Heart Development

The first stage is mostly processes that happen inside of the cells and we are not going to simulate what happens inside of the cells, so the starting point of the simulation is the second stage of the heart development. In the second stage clusters of angiogenic cells that lie in a horseshoe shape on the cardiogenic plate coalesce to form right and left endocardial tubes. This is the origin of the heart tube and will be the starting point of the simulation. We will now discuss feedback loop and different kinds of cell signaling in the heart.

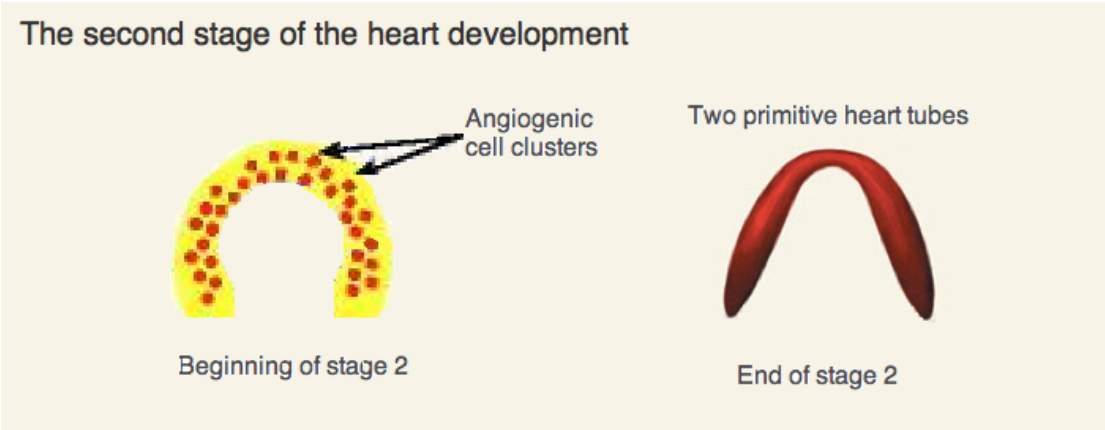

Figure 2: The second stage of the heart development

3.2 Feedback loops

A self-organizing system depends on positive and negative feedback loops, in our case cell signals. Therefore, the feedback loops are very important processes in the heart morphogenesis and are in fact key to finding some of the rules. These loops are signals that are looped back to control the system within itself and are divided into two groups, namely positive and negative feedback loops. The positive feedback loop is the type that is the most common in self-organizing system and processes, since the promote changes in a system, such as growth, morphogenesis and development of organs. The difference between these two types of loops is that in the positive feedback loop the system takes an initial change and then reinforces that change in the same direction, in other words called the snowballing effect, whereas in the negative feedback loop a positive change on the input signal will lead to a negative change in the output signal, which then leads to a smaller input signal.

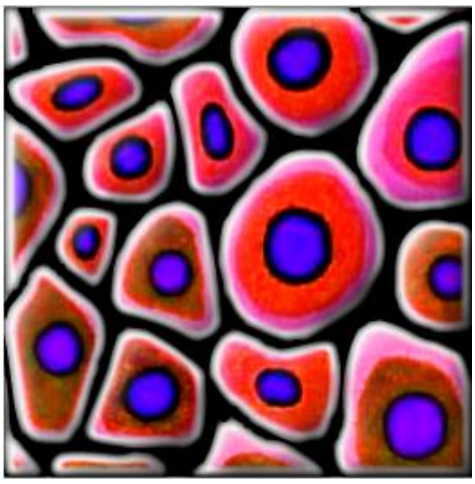

Figure 4: Cardiac Tissue

3.3 Cell signaling

The cell signals are the cells' communication system, but they use these signals to communicate with each other via direct contact (juxtacrine signaling), or over short distances (paracrine signaling). Now we will discuss some different cell signals.

3.3.1 Juxtacrine signaling

Some heart cells can form gap junctions that connect their cytoplasm to the cytoplasm of adjacent cells. A contact dependent signaling called the Notch

signaling mechanism is an example of juxtacrine signaling. This type of signaling requires a physical contact in order to communicate and is very important for embryonic development, since it allows a very precise control of cell differentiation.

3.3.2 Paracrine signaling

The paracrine signals target only cells in the vicinity of the emitting cell. During the past decade, developmental biologists have discovered that the induction of numerous organs is actually effected by a relatively small set of paracrine factors, since the embryo inherits a rather compact "tool kit" and uses many of the same proteins to construct various organs. Different paracrine factors operate over different distances, where some can diffuse over many cell diameters but some only work on their adjacent neighbors. Therefore a neighborhood of cell differs depending on the cell's signal. The paracrine signaling can be represented with feedback loops, where a cell sends out a molecule, that is either an activator or an inhibitor. The activator drives positive feedback and the inhibitor drives negative feedback. The molecule that the cell sends out could act on other cells within its neighborhood, but also on itself. That is called autocatalysis of a cell that leads to more positive output. There are two different interactions between these two kinds of signals, i.e. where the activator leads to the production of the inhibitor and where the inhibitor leads to the consumption of the activator.

3.3.2 Signals in heart development

The entire linear heart tube, up to the fourth stage of the development, is characterize by a low density of gap-junctions, which means that the cells depend more on paracrine signaling on the second stage than juxtacrine signaling. (Christoffels, Habets, et al. 2000)

The signal pathways that are known are bone morphogenetic proteins (BMPs), fibroblast growth factors (FGFs), *Wnts,* Hedgehog, members of the transforming growth factor-β (TGF-β**)** family of signaling molecules and Hippo signals.(Wagner og Siddiqui 2007) Out of these major families of signaling molecules are five major families of paracrine factors that act in the heart development. They are the family of fibroblast growth factors (FGF), the Hedgehog family, the *Wnt* family, Hippo family and the TGF-β superfamily. (Jones, Armes, and Smith 1996)

3.3.3 TGF-β signaling

The TGF-β superfamily consists of many molecules that are secreted peptide growth factors. The best known familymember is activin. This type of superfamily signaling is very imporant. The signals play very important role in the cells, where they regulate cell differentiation and cell growth and development. The signal is initiated at receptors in the cell membrane. Then series of reactions take place in the cytoplasm, but the signal finally travels to the cell nucleus, where the signal is translated. (Kingsley 1994)

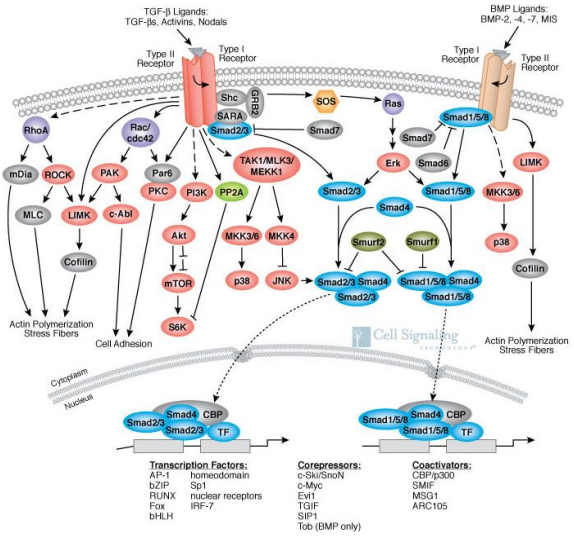

Figure 5: TGF-β signaling

3.3.4 *Wnt* signaling

This signaling network regulates many processes in development. It regulates the fate of the cell, that is, if it lives or dies, cell adhesion, cell growth, structural remodeling, cell polarity and morphology The network

consists of glycoproteins, *Wnt* family ligands, and the Frizzled and low-density lipoprotein (LDL) receptor-related protein LRP families of receptors. The pathway starts when a secreted glycoprotein binds to a receptor on the cell membrane. They are then transducted in two different ways: canonical and non-canonical way. We will not go into further details about this type of signaling right now.

(Logan and Nusse 2004)

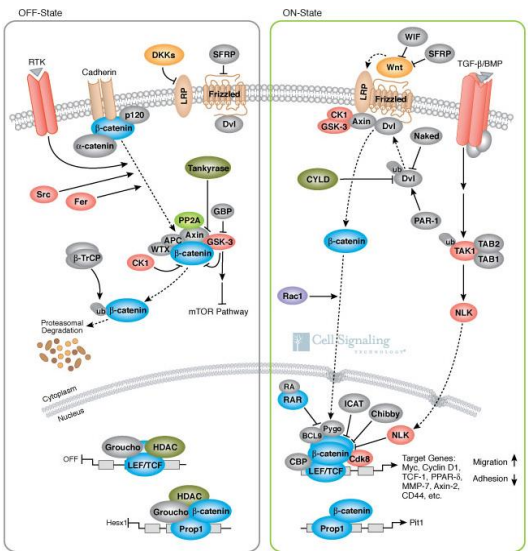

Figure 6: *Wnt* signaling

3.3.5 Hedgehog signaling

During the development the Hedgehog signaling pathway plays a critical role in time and position-dependent fashion. It regulates morphogenesis of varoius tissues and organs, including the heart. The Hedgehog signaling pathway is thought to be evolutionary preserved from flies to human. (Ingham and McMahon 2001)

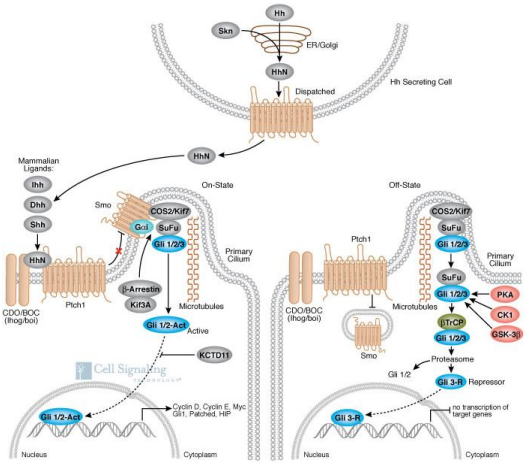

Figure 7: Hedgehog signaling

3.3.6 Notch signaling

The Notch signaling pathway mediates is a juxtacrine signal. Just like the Hedgehog signaling pathy it is evolutionary conserved in multicellular organism. This signaling pathway regulates the fate of the cells during the development in many organs, including the heart, and also in stem cells.[1] Humans, like other mammals, have four Notch proteins. The Notch signaling

pathway is active from the early stages of the heart development. It functions through lateral induction, that creates adjacent cell domains that have the same fate. The Notch activity contributes to the production of ligand with a positive feedback loop. As a result, the signaling occurs simultaneously in a developmental field. If there is a lack of Notch signaling, it will result in down regulation of ligand expression. That indicates the existence of a positive feedback loop.(Artavanis-Tsakonas, Rand, and Lake 1999)

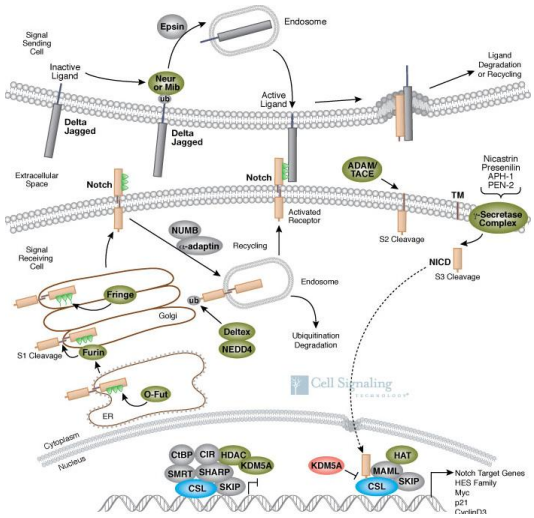

Figure 8: Notch signaling

3.3.7 Hippo signaling

The Hippo signaling pathway controls the cell proliferation, apoptosis and organ size. It is a response to changing cell density levels. If the cell density is low, the Hippo signaling pathway increases the expressions of genes that control cell growth and proliferation. When the cell density increases the pathway prevents the proteins, that increase the expressions of the genes, to enter the nucleus of the cell, so they can no longer increase the expression of genes that lead to increasing cell growth and proliferation. (J Huang, Wu, et al. 2005)

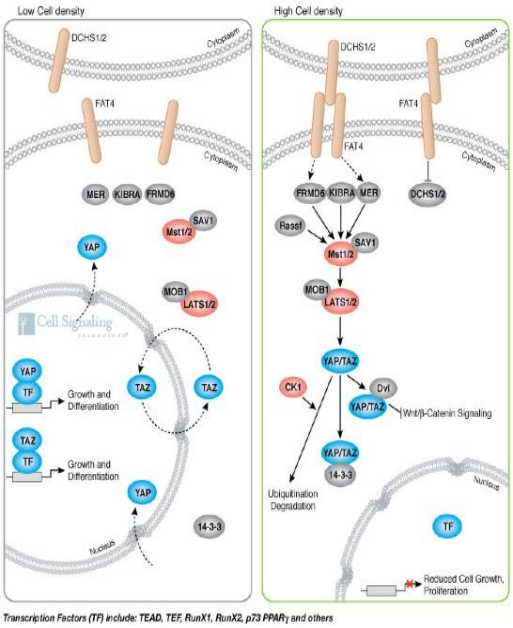

Figure 9: Hippo signaling

4 Cellular Automata (CAs)

We mentioned it in the introduction that the most common way to simulate a complex system is using PDEs. Our choice is to use CAs. When evaluating the upsides and the downsides of both alternatives, the CA approach is the better choice in our case. The reason is that we want to find some general rules that the heart uses during its development and because not all physical terms of the heart development are known, which would make the PDE model very hard or even impossible for us to compute. The reason why it is so hard that a detailed knowledge of algebraic formulas of the physical terms for the process is required and when the physical terms are not known, such as for the heart looping process, the algebraic formulas are also not known. In the end we will end up with a little less precise and understandable results, but the creation of the model, that is finding the rules, will be easier since we are dealing with simple rule set and not a set of differential equation. In addition, the computational time is shorter and the model requires a less storage. In other words, we can say that the CAs use their set of simple rules to mimic the physical laws of the system involved. Although the model is a little less precise, it shows the essential features of the system, instead of all interactions of the system, but most of the time the essential features are all we need in order to compare our results to experiments or to make predictions. (Toffoli 1984)

Now we will briefly discuss the features of CAs and how we can use them to simulate the development of the heart. First there is a short introduction on CAs, then we will talk about the dimensions of the model, what the correspondence between CA cells and biological cell components is, how many stages the model will have and translating cell signaling to rules. Finally we will introduce the CA software that will be used for the simulation.

4.1 About CAs

The first man to propose a system that uses simple rules to mimic processes in real life was a Hungarian mathematician, John von Neumann. His aim was to make a system that self-reproduced and he did that by creating a cellular automata machine called Von Neumann machine in 1945.

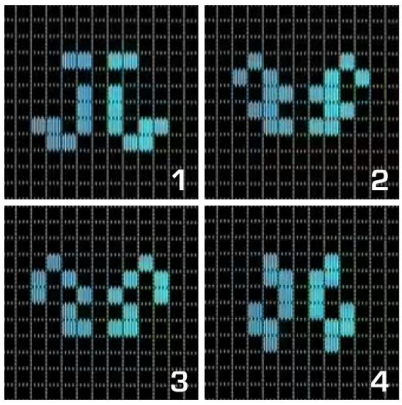

Figure 10: A snapshot of cycle from John Conway's Game of life

The best-known cellular automaton is John Conway's game of life. The game consists of cells that are discretely arrayed on a lattice and few simple mathematical rules. The cells follow the rules and can live, die or multiply.

Cellular automata (CAs) are dynamic systems that are discrete in several respects; they consist of a discrete spatial lattice of sites, they evolve in discrete time steps and each site has only a finite discrete set of possible values. CA models can be a 1D, 2D or a 3D model, depending on the system that is being simulated. The subunits of CA models, called cells, are discretely arrayed on a grid or lattice. Each cell is at any given time in one of a finite number of states. Moreover, each cell follows a set of simple rules, where the cell's state and is neighbors' state is taken into consideration. In most CAs the set of configuration that is generated contract with time and they are stable under small perturbations in initial conditions. They compute quickly and in parallel.

Next we introduce the formal definition of cellular automata. A cellular automaton is a sequence, defined as a 4-tuple A= (L, S, ρ, ψ) where,

- L is a cell-space, usually a lattice,
- S is the set of states,
- ρ is the neighborhood relation,

- ψ is the local transition function,

where the state of the cell at the next step $s_{t+1} \in S$ is determined by the local transition function ψ, depending on both the actual state of the cell itself and on its neighbors' state. That is,

$$s_{t+1}(x) = \psi(s_t(x), \{s_t(y) : (x,y) \in \rho\}).$$

Each element x of the cell space has a time value $s_t \in S$ at a given t, where t is discrete.

The local transition function ψ, also called an update function, can be either deterministic or stochastic and applied synchornously or asynchronously. The local transisition function is the rule set.

There are two types of neighborhood relations that are most frequently used, namely, the Neumann neighborhood containing the four adjacent cells (North, East, West and South) and the Moorian neighborhood consisting of eight adjacent cells (including Norh-East, South-East, South-West and North-West as well). (Rácz and Bulla n.d.)

Different CAs exists, but there are three types of CA models: deterministic or "Eulerian" automata, "lattice" gas" models and "solidification" models. In Eulerian automata the lattice is fixed and each lattice point has a state that is linked with it. The state at the next time step is only determined from the neighbors and the current state of the cell. This type most closely resembles evolution equations. This is the type of CA model that is used in this research. The second type of CAs, the lattice gas models are also called particle systems we have a discrete spatial grid and particles that move on that grid and interact in some way that the rules say. These systems are normally driven by random events. The third type, "solidification" models are like lattice gas model, except from that when a particle is in a "bound" state it cannot move or disappear ever again.(Ermentrout and Edelstein-Keshet 1993)

4.2 Using CAs to model the heart

Cellular automata (CAs) are a relevant option for the present work because they are relatively simple and can show the essential properties of self-organization, such as the feedback loops. The short-term goal is to find the rules that the heart follows during the second stage of its development, and since CAs are built by formulating a rule set for each CA subunit, it makes it possible for us to use CAs for modeling.

4.2.1 Dimension of the model

CAs can be in different dimensions. A simulation of the development of the heart in 2D would only show us the cross-section of the heart. In the beginning of the heart development two tubes are formed that later combine to form a single heart tube. This heart tube then coils up in a handle, called the bulboventricular loop. In real life these two tubes are hollow and their walls fuse together to make a single hollow tube. A simulation of this process in 2D would give us filled tubes (lines) that combine to make a single filled tube (line). The simulation of the bulboventricular loop in 2D would be the single filled tube that shortens and gets thicker and in the end would look similar to a filled circle, but in fact it is a hollow tube is coiling and thereby forming a coil spring. As we see, the rule set for creating a hollow tube would be totally different from creating a single filled line that has the same diameter as the tube. Also, we would have a completely different rule set for the "coil" in 2D then in real life, since it is not even a coil when simulating in 2D. From this we can draw the conclusion that a 2D model would never give us a realistic result, since it would give us a totally different rule sets.

The complexity of the heart development is great; it includes a lot of morphogens and other molecules and a lot of other interactions between cells.

Therefore, starting from beginning of making the model in 3D is very complex and obviously its level of complexity is a lot higher than in a 2D model.

For the first step of the process, that is the formation of the two tubes and their fusion to the single heart tube, the 2D simulations looks almost the same as if you were watching it in real life and not viewing the inner structure. The rules for the attraction of the tubes and their fusion together in 2D could be used to derive similar rules for the 3D model.

Thus, we will start by creating the model in 2D, since the complexity of the system is a lot simpler in 2D than in 3D. In the future the 2D model can then be used to derive the rules for the 3D model.

4.2.2 Correspondence between CA cells and biological cells

The heart is a multi-cellular organ, made from millions or even billions of cells. If one CA cell would represent a single cell then the model would be built out of billions of CA cells, which make is too large to view while it is being computed. That would happen because in CAs the cells are larger than biological cells. One the other hand, the self-organization is driven by many interactions between the components of the system, such as the feedback loops of the cell signals that the cell sends to another cell or the interaction between two adjacent cells through physical contact. Therefore, it is important that the model has these features. If one CA cell would represent a part of a cell, the model would be many times bigger than the model where one CA cell represents a cell, thus, the model would be way to large when computing. Also, it would be to detailed since we do not want to show interactions within the cell. If one CA cell would represent many cells or even a tissue, we would lose the essential features of the self-organization, that is, communication between cells using cell signals. In this case we would only represent communication between tissues, but not show the communication between each cell within the tissue.

The levels of organization tell us that organs are made of many tissues that again are made of many cells. To display the essential properties of self-organization, such the feedback loops, but on the same time not having to large model to build, it is possible to let the model be a multi-scale model, where each subunit of the CA model on the highest level represents a single tissue, but within each subunit that represents a tissue there is a lower level. On that level each cellular automata subunit represents a single cell, and within the tissue subunit, there are many cellular subunits - just like in real life. The molecular level is then beneath the cellular level, where we can represent the paracrine cell signaling, where the cell sends a signal in form of a molecule that either acts back on the cell itself via autocatalysis, or acts on another cell.

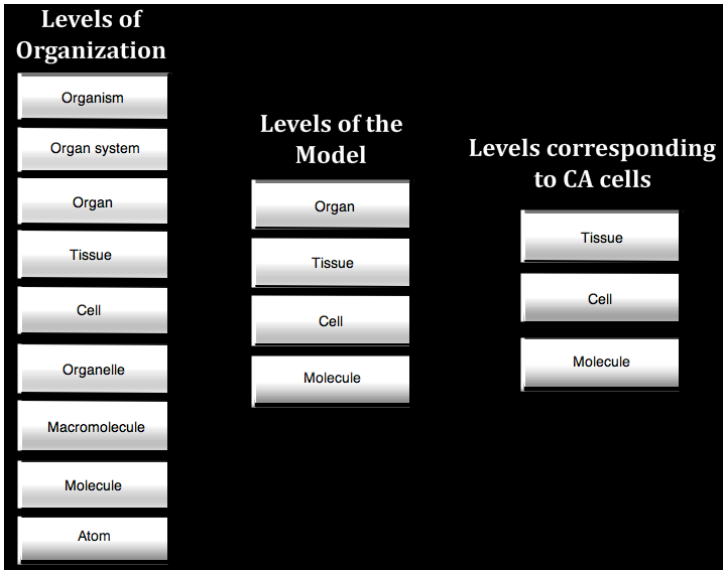

Figure 11: The levels

The levels of organization in the human body are nine: atom, molecule, macromolecule, organelle, cell, tissue, organ, organ system and organism. It is important to note that when we use the term that the model will be on three levels, it means that there is correspondence between three of the levels of organization and CA cells. Nevertheless, the model will show us more than those three levels, because it will show the heart, which means that the model will show us the molecular level, cell level, tissue level and the organ level.

Thus, the model will be a multi-scale model to preserve the essential features of self-organization without having to large model to view. In the future the model will have three levels, where on the highest level each CA cell represents a tissue, on the next level below that each CA cell represents a single cell and on the lowest level each CA cell represents a molecule sent from the cell. In the beginning the model will only be on two of these levels, i.e. the molecular level and the cellular level.

4.2.3 The stages of the model

A CA model can have different stages, where in each stage we have a different rule set. Lets review briefly the stages of the heart development and from that decide how many stages the model should have.

Lets review what was said about the stages of the heart development in the chapter about the heart. The heart development can be divided into 5 stages, namely cardiomyocyte determination and specification, formation of two primordial epithelial tubes and their fusion, heart looping, heart chamber formation and septation and valve formation. Moreover, one of these stages, the heart chamber formation, can be divided into two processes. First there is the establishment of the "primary" transcriptional program within the cardiac crescent and linear heart tube and then the development of the heart chambers starts. All these stages are very different, which means that the cells follow different rules within each stage. The specification and differentiation of the cardiomyocytes will not be simulated, since we are not going to simulate all the inner interactions of each cell. Thus, the first stage of the simulation is when the angiogenic cell clusters fuse and then form two primordial epithelial tubes, so the first step in simulating the heart is to make a rule set for this stage. For the simulation of the second stage we need to make a rule set that makes the two separate primordial epithelial tubes from the previous stage combine to from a single tube etc. Thereby, we need to develop a different rule set for each stage of the heart development.

Thus, we will make one rule set to simulate start with. That should represent the rules that the heart follows during the second stage of the heart development. Later, one rule set for each stage of the heart development will be made, except for the fourth stage where two sets will be made.

4.2.4 Translation of cell signals into rules

Now we will briefly discuss how we can find the rules for the simulation of the evolution of the heart.

A CA model has rule sets to follow. When simulating morphogenesis and the evolution of the heart these rules are the communication between the heart cells. We disucssed it in the heart section that the cell uses different types of signaling, i.e. the paracrine signaling and the juxtacrine signaling. Within each type we have different signals, e.g. in paracrine signaling the signal can be either activating or inhibiting. Therefore, the rule sets will consist of different rules that represent different kinds of cell signals – but like we know there are many interactions in the cell, so we need to find a way to sort these signals into different groups and find a good way to derive the rules. We will discuss our findings on that later in the result section

In section 4.3 the simplifications of the model are discussed. There we mention that we will divide molecules into two. In the cell signaling section we discuss different types of paracrine signaling: Hedgehog, *Wnt*, and TGF-β signaling. As a continued work from this research these processes need to be studied in details and try to group them into activating or inhibiting signaling pathways or try to divide some of them into two if they can be both inhibiting or activating.

4.3 Simplifications of the model

Eventhough many simplifications might not be biologically realistic, they are methodologically justified as initial step. In addition to being a 2D model at the beginning we will start with some other simplifications.

The majority of the heart consists of seven types of cells: cardiomyocytes, pacemaker cells, Purkinje cells, smooth muscle cells, fibroblasts, endothelial cells and adipocites. The first four types are excitable cells that generate action potentials in the heart, but the remaining three types are called non-excitable cells. For simplifications we only have one cell type on the level where we have correspondence between CA cells and biological cells. Since we are only have one type of cells and are not making any difference between excitable cells and non-excitable cell we will not simulate the heart beat.

During the heart development many molecules are signaling molecules. Since the activator leads to the production of the inhibitor and the inhibitor leads to the consumption of the activatior, for simplification we will have two types of molecules – activating molecule and inhibiting molecule.

To start with, the simulation we will only have two levels, that is, molecular level and cellular level.

4.4 Cellular automata software

The model will be built on a cellular automata software system for emergence research called Vélaldin. This software is open source and will be adapted- and extended to suit our needs in simulating self-organization of the heart. Vélaldin is designed with an emphasis on flexibility of cells, enabling the user to define and implement its features, i.e. the model can be layered, it can either be a 1D, 2D or a 3D model and the rules and states of cells are

determined by the developer.

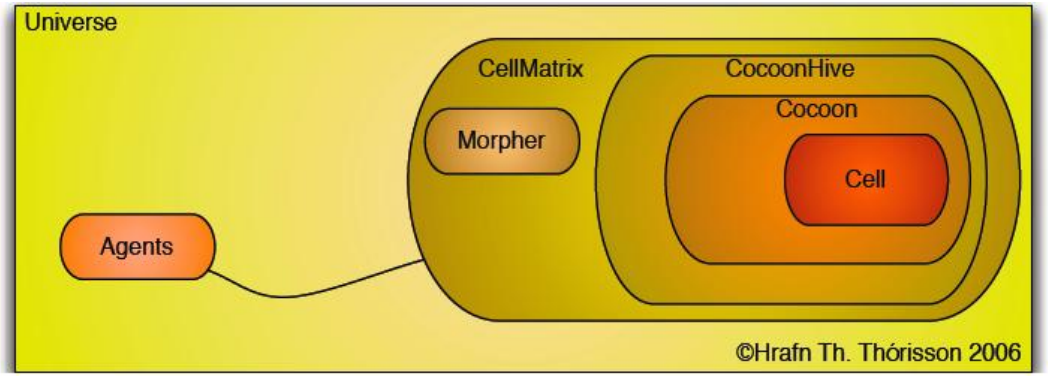

Figure 12: Vélaldin

The cells in Vélaldin form a matrix to allow neighbor-checks. At any given time, each cell is stored within a *Cocoon* object. The linked *Cocoons* are called *CocoonHive*, but they are wrapper-objects for primitive arrays of *Cocoons*. The *Cocoons* give the system the ability to update and go to the next stage without having to loop through thousands of neighbor-checks. To increase the updating-possibilities of a *Cellmatrix, Universe* uses an object called *Morpher* that parses the list of *Cocoons* and initiate morphing of cells.

5 Simulation of the Heart Development using CAs

In this section we will discuss the results of the project. The research questions we are going to answer in this chapter are:

- The neighborhood size of the CA cells.
- The size of the rule set.
- Deriving the rules
- If different rule sets can simulate the stage.
- The effect small changes in the rule set will have on the simulation.

5.1 Neighborhood size of the CA cells

We will now discuss the results on how we will determine the neighborhood size of a CA cell, and the difference of the neighborhood relations when we have different levels.

We mentioned in the method section that the model has three different levels, that is, there is correspondence between a single CA cell and tissue, single CA cell and a biological cell and a CA cell and a molecule. These levels are not layers. Beneath the highest level, i.e. the tissue level, there is the cell level and beneath the cell level there is the molecular level. This means that rules on the molecular level will have impact on the cell level and the rules on the cell level will have impact on the molecular level. This also holds for the cell level and the tissue level, that is, the rules on both levels will have impact on the other level.

The molecular level is for the paracrine signaling. We discussed it in the

section about the cell signals, that there is a difference in diffusion length between families of molecules. This means that some families of molecules have only effect on their nearest neighbors, while some of them can have impact on cells maybe two or three cells away. With all these different molecules and cells, it is obvious that we can't have different neighborhoods for different types of molecules.

On the cell level there is juxtacrine signaling, a contact dependent signaling - where the cells communicate with each other by using gap junctions. This means that the cells can only communicate with their nearest neighbors by using this kind of signaling.

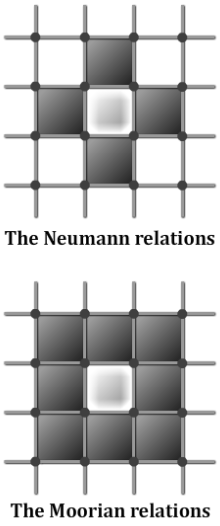

Figure 13: Neighborhood relations in CAs

Since we have several types of molecules that diffuse over different lengths and two types of signaling we need to determine the neighborhood size of the CA cell on the molecular level and on the cell level, separately.

On the cell level we want to derive rules for juxtacrine signaling, where the cell sends signals to their closest neighbors. As we talked about in the section about CAs, there are two types of neighborhood relation that are frequently used, namely, the Neumann neighborhood containing the four adjacent cells (North, East, West and South) and the Moorian neighborhood consisting of eight adjacent cells (including Norh-East, South-East, South-West and North-West as well).

5.1.1 Neighborhood size on the cellular level

We will use the Moorian neighborhood relation for the CA cells on the

cellular level. On the cell level each CA cell represents a single cardiac cell. The cardiac cells are all connected and can have impact on their neighbors through contact. The neighborhood of cells in real life is more like Moorian neighborhood. See figure. Cells are not squares with four sides, and most of the time have more neighbors. Therefore we choose the Moorian neighborhood relation, since it is more like in real life than the Neumann neighborhood.

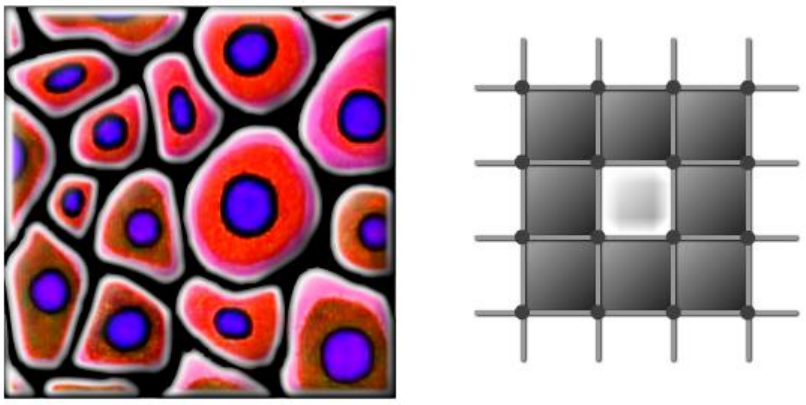

Figure 14: The neighborhood relations in a tissue and a CA model

5.1.2 Neighborhood size on the molecular level

As mentioned above, on the molecular level we have many types of molecules that diffuse over different lengths. Diffusion of molecules is the spread of molecules to a region of higher concentration to regions of lower concentration, so in the end there will be the same concentration of the molecule on the regions. In other words, the concentration of the molecule is averaged over the regions, so that there is the same concentration of the molecule in the regions.

To solve the problem with different diffusion lengths of molecules, we will use different approach. The neighborhood relation for the CA cells on the molecular level will be the Moorian relation, but since we have different types of molecules diffusing over different cell lengths, we will solve that problem

by using rules that represent the diffusion of the molecules. These rules average the molecule over an area, which hasn't been determined yet.

In the section about the simplifications we said that we have two types of molecules, activating and inhibiting. Because of the interaction between the two types of molecules we can't take the average of the concentration of both types, but we need to do it separately. Therefore, we will need two rules, one that represents the diffusion of the activating molecules and one that represents the diffusion of the inhibiting molecules.

Thus, the neighborhood relation on the cell level will be the frequently used Moorian relation, where each cell has eight neighbors. On the molecular level we will use the same neighborhood relation, i.e. the Moorian relations, in addition to rules that average the molecules over some area.

5.2 Size of the rule set

The size of the rule sets depends on which stage of the heart development we are simulating. Despite of this, there is always a minimum number of rules that need to be in each set. We will now discuss what rules we always need to have when we simulate the heart development.

Above we discussed cell signaling – both paracrine and juxtacrine signaling. Cell signals are what drive the heart development. We know that paracrine signaling are driven by negative and positive feedback loops. We also know that juxtacrine signaling is a contact-dependent signal. In the paracrine signaling we know that different types of molecules, that diffuse over different cell lengths and have different impact on the cell it acts on, i.e. it can be activating or inhibiting. We will divide the molecules into two different types of molecules, namely, activators and inhibitors.

In the section about cell signaling, it is said that the there are two types interactions between activating and inhibiting molecules and when the concentration of inhibitor is low enough autocatalysis can occur. These interactions all need to be interpret into rules, along with the juxtacrine signaling.

5.2.1 Rule representing autocatalysis

Autocatalysis occurs when a molecule that is sent to the extracellular matrix by a cell has an impact on the cell itself again. The molecule is an activator and has positive effect on the cell. This type of cell signaling only happens when the amount of inhibitor is low enough.

5.2.2 Rules representing interactions between activator and inhibitor

The feedback loops are a large part of the heart development and therefore they need to be represented by the model. Thus, the model needs to have rules that describe the feedback loops.

There are two different interactions between an activating molecule and an inhibiting molecule, i.e. the activating molecule can lead to the production of the inhibiting molecule and the consumption of activating molecule as a result of the inhibiting molecule. It is very important that the model represents these two interactions, because otherwise the model will not represent the feedback loops.

5.2.3 Rules representing the diffusion of the molecules

In the section about the neighborhood size of the CA cells, we mentioned that we will use rules for the diffusion of the molecules. These rules

average the molecule over a circle with the radius *r*, where *r* is the cell edge length of the molecule. We will use two rules to represent the diffusion of the molecules, one that represents the diffusion of the activating molecules and one that represent the diffusion of the inhibiting molecules.

5.2.4 Rules representing the decay of the molecules

When using the rules for the diffusion of the molecules, we need to consider the decay of molecules in the extracellular fluid. All molecules degrade in the extracellular matrix, both activating and inhibiting molecules. The time of decay depends on the molecule - but there are two main types of decay, i.e. linear decay and exponential decay, but we need to consider both types.

Since we have two types of molecules, we need to derive two rules for the degradation, one for the activating molecules and one for the inhibiting molecule.

5.2.5 Rule representing the Notch signal

The Notch and the juxtacrine signaling also need to be represented, since the Notch signaling is a very important type of signaling in the embryo and heart development. As said before, the Notch signaling depends on physical contact of two or more adjacent cells. The Notch signal controls cell differentiation through physical contact. Since a physical contact is required for the Notch and other juxtacrine signals the rules for these signals can be on the cellular level.

5.2.6 Rules representing other juxtacrine signals

The Notch signal is like said before very important signal in the heart

development and controls cell differentiation. Nevertheless, there are other juxtacrine signals that act in the heart development. These signals control for example if the cell dies or continues to live. Since a physical contact is required both for the Notch and other juxtacrine signals the rules for these other juxtacrine signals will be on the cellular level.

The rules on the cellular level will take into account the amount of adjacent populated cells at the time *t* and from the information about the amount of neighbors, the cell will either differentiate, do nothing or die.

This means that the rule sets must consists of rules that represent all these cell signaling pathways. Out of these nine interactions there are seven rules that are for the molecular level. The other two are for the cellular level. We need to keep in mind that this is not a complete list of all the cell signals that are active during the heart development, which means we cannot determine at this moment the accurate size of the rule set.

5.3 Deriving rules

In this section we will discuss how we can derive the rules from the cell signals in an efficient way, so that we actually generate the rules that the heart follows.

5.3.1 How do we generate rules?

Lets start by review the formal definition of cellular automata. A cellular automata is a sequence, defined as a 4-tuple A= (L, S, ρ, ψ) where,

- L is a cell-space, usually a lattice,
- S is the set of states,
- ρ is the neighborhood relation,

- ψ is the local transition function (rule set),

where the state of the cell at the next step $s_{t+1} \in S$ is determined by the local transition function ψ, depending on both the actual state of the cell itself and on its neighbors' state. That is,

$$s_{t+1}(x) = \psi(s_t(x), \{s_t(y) : (x,y) \in \rho\}).$$

From this we see that the rules control state changes. Moreover, each CA has a rule set to follow, so the rules control the changes of the cells. The CA model should mirror the heart development and what actually goes on during the development. Therefore, we need to find a way to generate the rules for the CA model. Rules that we make up might work and gives us the same result, but how do we know they mirror the biology like we want them to do.

5.3.2 Where do we get the rules from?

The problem is: where do we get the rules? How can we generate rules so we know that they mirror the biology?

We cannot make the rules up. If we would make them up we cannot confirm that they are mirroring the biology and therefore might not be the rules that the heart follows during the heart development.

The rules cannot be found in the biological literature. If they could be found there, this project would of course be a waist of time because the aim is to find the rules that the heart follows, because they are unknown.

We need to find an efficient way to generate rules, so we know that they mirror biology and the actual rules that the heart follows during its development. Now we will discuss they approach we want to use for deriving the rules.

5.3.3 Generating rules

We said earlier that the rules represent the cell signals in the development of the heart. This means that we need to derive rules that interpret the cell signals. In the section above about the size of the rule sets, we talked about different rules that need to be in the each rule set. These rules represent

- Autocatalysis
- The interactions between activator and inhibitor
- The diffusion of the molecules
- The degradation of molecules
- The Notch signal
- Other juxtacrine signals

We need to derive the rules for each level in two parts, depending on if it is a paracrine signaling or juxtacrine signaling, but like we said earlier, the paracrine signaling uses molecules, activating or inhibiting, and the juxtacrine signaling is a contact-depending signaling.

In systems biology process algebra and finite state machines (FSM) have been used to formally and quantitatively reason about biological systems. That has been done to understand them, and predict behavior. (Ciocchetta og Hillston 2008)

Since we cannot make the rules up and we cannot find them in the literature, we need a specific way to derive the rules. Cell signaling drives the heart development and rules causes the CA model to change states and evolve, therefore the rules will represent the cell signaling. The cell signaling includes feedback loops and many interactions between molecules and cells. Using FSM of the cell signaling on each level makes us able to translate the cell signals into rules. Using this approach we know that the rule set is grounding the cell signaling, so the CA model would mirror the biology.

This means that when we want to derive the rules for the paracrine signaling we need to make finite state machine (FSM) of the cell signaling processes. From the FSM we will derive simple rules for the paracrine signaling. These simple rules will in fact tell us what the finite automata does under some given circumstances. These rules that are derived from the FSM represent for the cell signaling on the molecular level. This means that this FSM must show us autocatalysis, the interactions between activating and inhibiting molecules and diffusion and decay of both activating and inhibiting molecules.

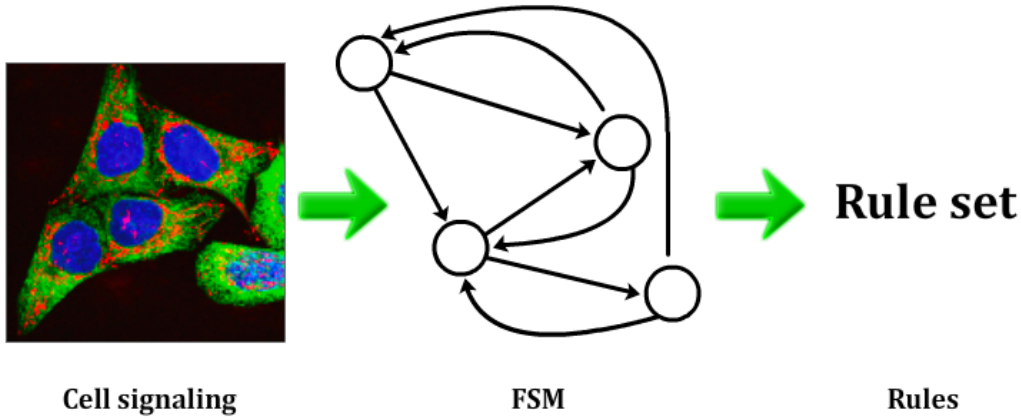

Figure 15: The process for generating the rules

When deriving the rules for the juxtacrine signaling on the cell level, we will make another FSM that shows us the juxtacrine signaling. That FSM is simpler than the FSM for the molecular level, since this type of signaling depends on contact, so the cell will either live, die or differentiate depending on how many neighbors it has. The FSM for the signaling on the cell level is simpler than the FSM for signaling on the molecular, because juxtacrine signaling is relatively simple compared to paracrine signaling.

Thus, the rules that represent the cell signaling that drives the development of the heart are derived in two separate parts, but both use FSMs

as an intermediate, to help us simplify the cell signaling processes so we can be able to derive rules from them.

5.4 Can different rule sets simulate each state?

We will now briefly discuss if different rule sets can be used to simulate the same stage.

By using FSMs to show how the cell signals function and derive simple rules from the FSMs, we can be sure that these are actually the rules that the heart follows during the fetal development. The reason is that the rules represent the signals that the cells use to communicate and because of these communications the heart develops. We can therefore be quite sure that the heart uses these rules during the fetal development and thereby having a rule set that should function for the simulation of the heart.

On the other hand, we can never be completely sure that there is no other rule set that can give us the same results as the rule set that we derive for each state of the heart development. That set would probably not represent the actual cell signals in the heart development, like the set derived by using finite automata for the cell signals, and is therefore not a set of rules we are interested in.

Thus, we cannot be fully sure of that a different rule set can simulate the same stage, but we can be sure that our rule set is a valid rule set and from that rules we can determine the rules that the heart follows during fetal development.

5.5 The effect that a little change in the rule set will have on the model.

We will now discuss the effect that a little change in the rule set will have on the model.

If we derive the rules by using FSMs of the cell signaling processes we don't need to worry about changes in the rule set. The FSMs are derived form the cell signaling processes and the rules are derived from the FSM, so the rules in the rules sets must all be essential, like the cell signaling processes must be essential for the development.

An extra rule would have great impact on the model, since it would change how the model of the heart evolves. An extra rule would mean extra cell signaling, which could be interpret as a birth defect or the end product would not look like heart at all. On the same hand, if we skip some rule from the rule set, it means skipping an essential cell signal process in the heart – which would make the CA model evolve in a different way and could be interpret either as a birth defect or the resulting model would be something else than a heart.

Thus, by using the FSMs as intermediate step we don't have to worry about having extra rules in the set, because the FSMs should show all the essential cell signaling processes for the heart development, and therefore we don't have to worry about having any extra rules. If there would be some additional rules or some rules missing the model would not be mirroring the biology, and the model would not look like the heart development does in real life.

6 Conclusion

The neighborhood size of CA cells depends if we are on the cell level or the molecular level. The neighborhood relations for both levels are the Moorian neighborhood relations, but on the molecular level we will also have additional rules for the diffusion of molecules, where the consentration of each type of molecule is averaged over some circular area. The additional rules are needed because different molecules diffuse over different lengths.

The heart development is driven by cell signaling, and the evolution of the CA model is driven by the rule set that control the changing of states. Therefore, the rules must mirror the cell signaling. In the beginning we use only two types of molecules, activating and inhibiting. That is done to simplify the model. The interactions between the molecules and the impact that they have on themselves and each other need to be represented, along with the diffusion rules and the decay of molecules in the ECF. The contact – dependent signaling also needs to be represented. Therefore, each rule set for each stage of the heart development must, at least, contain rules for the nine signaling processes and interactions.

We want to derive rule sets that mirror the cell signaling and we don't have to worry about if we have the right rule set or if we have any rules that are not necessary for the model to function and evolve in the right way. By generating FSMs that simplifies the cell signaling processes and deriving the rules from the FSMs, we can be sure that the rules mirror the biology which is the most important thing. The FSMs should include all the signaling processes needed for the heart to develop, so we don't have to worry about having to many rules that don't do nothing for the model.

7 Future Work

The next step is to dig deeper into the cell signaling processes that function during the second stage of the development, which will be the starting point of the simulation. We want to how they can be simplified, so we can be able to use them for the FSMs. The paracrine processes need to be divided into activating and inhibiting signaling processes. When that has been done, an FSM for each of the level – both the cell level and the molecular level, has to be made and the rule sets will be derived from the FSMs. When some rules have been generated by using the FSM intermediate, we can start implementing the model.

Acknowledgement

I would like to thank Kristinn R. Thórisson, my supervisor for his help, guidance and support throughout while I have been working on this research. I would also like to thank Gunnar Steinn Valgarðsson for helping me making some of the figures and Kristín Rós Ásgeirsdóttir for proofreading this thesis.

Terminology

Anastamosis

The connection of normally separate parts or spaces so they intercommunicate.

Angiogenesis

The process of developing new blood vessels.

Apoptosis

Programmed cell death as signalled by the nuclei in normally functioning human and animal cells when age or state of cell health and condition dictates.

Cardiogenesis

The development of the heart in the embryo

Cardiomyocyte

The cell of a tissue in a heart muscle.

Cell growth

The increase in cytoplasmic volume, as in cell development and cell reproduction or the increase in size or population of cells, as in mitosis.

Cytoplasm

The part of the cell between the cell membrane and the nuclear envelope. It is the jelly-like substance in a cell that contains the cytosol, organelles, and inclusions, but not including the nucleus.

Diffrentiation

The normal process by which a less specialized cell develops or matures to become more distinct in form and function.

Diffusion

A type of passive transport, therefore, it is a net movement of molecules along a concentration gradient.

Extracellular matrix

Any material produced by cells and secreted into the surrounding medium, but usually applied to the non-cellular portion of animal tissues.

Gap junctions

Connections between cells, which allow passage of small molecules and electric current.

Ligand

A molecule, ion or atom bonded to the central metal atom of a coordination compound. Also, any substance (e.g. hormone, drug, functional group, etc.) that binds specifically and reversibly to another chemical entity to form a larger complex.

Morphogenesis

Differentiation and growth of the structure of an organism (or a part of an organism). The development of form; the overall consequence of determination, differentiation, and growth.

Morphogens

Diffusible substance that carries information relating, for example: to position in the embryo and thus determines the differentiation that cells perceiving this information will undergo.

Myocardium

A term used to describe the middle layer of the heart wall (heart muscle).

Proliferation

Increase in cell number by division.

Yolk sac

One of the set of extra embryonic membranes, growing out from the gut over the yolk surface, in birds formed from the splanchnopleure, an outer layer of splanchnic mesoderm and an inner layer of endoderm.

Zygote

A cell in diploid state following fertilization or union of haploid male sex cell (e.g. sperm) and haploid female sex cell (e.g. ovum).

References

Abdulla, T, R Imms, J M Schleich, and R Summers. 2010. “Multiscale information modelling for heart morphogenesis.” *Journal of Physics: Conference Series* 238:012062.

Anderson, Robert H, Sandra Webb, Nigel A Brown, Wouter Lamers, and Antoon Moorman. 2003. “Development of the heart: (2) Septation of the atriums and ventricles.” *Heart* 89:949-958.

Artavanis-Tsakonas, Spyros, Matthew D. Rand, and Robert J. Lake. 1999. “Notch Signaling: Cell Fate Control and Signal Integration in Development.” *Science* 284:770 -776. (Accessed April 27, 2011).

Bartman, Thomas, and Jay Hove. 2005. “Mechanics and function in heart morphogenesis.” *Developmental Dynamics* 233:373-381.

Camazine, Scott. 2003. *Self-organization in biological systems*. Princeton University Press.

Christoffels, Vincent M., Petra E. M. H. Habets, et al. 2000. “Chamber Formation and Morphogenesis in the Developing Mammalian Heart.” *Developmental Biology* 223:266-278.

Cickovski, T. M, Chengbang Huang, et al. 2005. “A framework for three-dimensional simulation of morphogenesis.” *Computational Biology and Bioinformatics, IEEE/ACM Transactions on* 2:273-288. (Accessed April 27, 2011).

Ciocchetta, Federica, and Jane Hillston. 2008. “Process Algebras in Systems Biology.” Pp. 265-312 in *Formal Methods for Computational Systems Biology*, vol. 5016, edited by Marco Bernardo, Pierpaolo Degano, and Gianluigi Zavattaro. Berlin, Heidelberg: Springer Berlin Heidelberg http://www.springerlink.com/content/a131181225076603/ (Accessed May 13, 2011).

Costa, M. Margarida, and Jorge Sim\ ao. 2008. “Mathematical analysis of branching morphogenesis.” P. 100–107 in *Proceedings of the 2nd WSEAS international conference on Computational chemistry*. Stevens Point, Wisconsin, USA: World Scientific and Engineering Academy and Society (WSEAS) http://portal.acm.org/citation.cfm?id=1576571.1576590 (Accessed April 27, 2011).

Ermentrout, G. B., and L. Edelstein-Keshet. 1993. “Cellular automata approaches to biological modeling.” *Journal of Theoretical Biology* 160:97-97.

Gilbert, Scott F., and Susan R. Singer. 2010. *Developmental Biology*. Sinauer Associates, Incorporated.

Huang, Jianbin, Shian Wu, Jose Barrera, Krista Matthews, and Duojia Pan. 2005. “The Hippo Signaling Pathway Coordinately Regulates Cell Proliferation and Apoptosis by Inactivating Yorkie, the Drosophila Homolog of YAP.” *Cell* 122:421-434. (Accessed May 4, 2011).

Ingham, Philip W., and Andrew P. McMahon. 2001. “Hedgehog signaling in animal development: paradigms and principles.” *Genes & Development* 15:3059 -3087. (Accessed May 4, 2011).

Izaguirre, JA et al. 2004. “CompuCell, a multi-model framework for simulation of morphogenesis.” *Bioinformatics* 20:1129.

Jones, C. M., N. Armes, and J. C. Smith. 1996. “Signalling by TGF-[beta] family members: short-range effects of Xnr-2 and BMP-4 contrast with the long-range effects of activin.” *Current Biology* 6:1468-1475.

Kingsley, D M. 1994. “The TGF-beta superfamily: new members, new receptors, and new genetic tests of function in different organisms.” *Genes & Development* 8:133-146.

Kusch, Ingo, and Mario Markus. 1996. “Mollusc Shell Pigmentation: Cellular Automaton Simulations and Evidence for Undecidability.” *Journal of Theoretical Biology* 178:333-340.

Logan, Catriona Y., and Roel Nusse. 2004. “THE WNT SIGNALING PATHWAY IN DEVELOPMENT AND DISEASE.” *Annual Review of Cell and Developmental Biology* 20:781-810. (Accessed May 4, 2011).

Markus, M., D. Böhm, and M. Schmick. 1999. “Simulation of vessel morphogenesis using cellular automata.” *Mathematical biosciences* 156:191-206.

Moorman, Antoon, Sandra Webb, Nigel A Brown, Wouter Lamers, and Robert H Anderson. 2003. “DEVELOPMENT OF THE HEART: (1) FORMATION OF THE CARDIAC CHAMBERS AND ARTERIAL TRUNKS.” *Heart* 89:806-814.

Ramasubramanian, Ashok et al. 2008. “On Modeling Morphogenesis of the Looping Heart Following Mechanical Perturbations.” *Journal of Biomechanical Engineering* 130:061018-11.

Rácz, É V. P., and M. Bulla. n.d. “Cellular Automata Models of Environmental Processes.” Pp. 109-119 in.

Reilly, Karlyne M., and Douglas A. Melton. 1996. “Short-Range Signaling by Candidate Morphogens of the TGF[beta] Family and Evidence for a Relay Mechanism of Induction.” *Cell* 86:743-754.

Runyan, R B et al. 1990. “Signal transduction of a tissue interaction during embryonic heart development.” *Cell Regulation* 1:301-313.

Taber, L. A., and R. Perucchio. 2000. “Modeling heart development.” *Journal of elasticity* 61:165-197.

Taber, Larry A. 1995. “Biomechanics of Growth, Remodeling, and Morphogenesis.” *Applied Mechanics Reviews* 48:487-545.

Toffoli, Tommaso. 1984. “Cellular automata as an alternative to (rather than an approximation of) differential equations in modeling physics.” *Physica D: Nonlinear Phenomena* 10:117-127.

Wagner, Michael, and M. A. Q. Siddiqui. 2007. “Signal Transduction in Early Heart Development (I): Cardiogenic Induction and Heart Tube Formation.” *Exp. Biol. Med.* 232:852-865. (Accessed April 27, 2011).

Wolfram, Stephen. 2002. *A new kind of science*. Wolfram Media.

Wolfram, Stephen. 1986. *Theory and applications of cellular automata: including selected papers, 1983-1986*. World Scientific.