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Executable Modeling of Morphogenesis: A Turing-Inspired Approach

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Abstract. In his pioneering 1952 paper, "The chemical basis of morphogenesis", Alan Turing introduced, perhaps for the first time, a model of the morphogenesis of embryo development. Central to his theory is the concept of cells with chemical entities that interact with morphogens to drive embryonic development through changes in what he termed 'the state of the system'. Turing's concepts have inspired many mathematical and computational models proposed since then. Here we discuss the way Turing's ideas inspired our approach to the state-based modeling of morphogenesis, which results in a fully executable program for the interactions between chemical entities and morphogens. As a representative example we describe our modeling of pancreatic organogenesis, a complex developmental process that develops from a flat sheet of cells into a 3D cauliflower-like shape. We show how we constructed the model and tested the relations between morphogens and cells, and illustrate the analysis of the model against experimental data. Finally, we discuss a variant of the original Turing-Test for a machine's ability to demonstrate intelligence as a future means to validate computerized biological models, like the one presented here.

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1. Introduction

In his 1952 paper "The chemical basis of morphogenesis" [1, 2], Alan Turing introduced a mathematical model of the growing embryo. Turing considered three key factors that drive the development of an embryo: cell, morphogen, and 'the state of the system'. Cells in Turing's theory are elements that are mostly characterized by their chemical properties. Turing recognized that the characteristic action of genes is presumably chemical and thus the chemical basis is the most significant element in cellular activity. Turing termed morphogens the substances that chemically react with the cells to produce form, and defined the way the 'state of the system' at each step emanates from the state it was in a short moment earlier.

Many other mathematical models of embryological pattern formation have been developed since Turing's seminal work [3-5]. A different perspective of Turing's theory focuses on *behaviorally implemented pattern forming processes*, whereby patterns are created by computational agents that can take actions depending on their local space-time environment [6]. As in Turing's theory, pattern formation in these models involves interacting chemical substances (e.g., cell extrinsic ligands) and chemical-based moving entities (e.g., cells) [6-8].

In recent years, we have been developing a computational approach for the executable modeling of morphogenesis. As a representative example, we describe here our computational model of pancreatic organogenesis, first published in 2008 [9], a highly dynamic system that develops from a flat sheet of cells to obtain a complex cauliflower-like structure. As in many developmental systems, pancreatic organogenesis maintains interplay between chemical interactions that drive changes in individual cells over time and space. The approach has been further extended and applied to other developmental systems, namely, germline development in the *C. elegans* nematode [10] and neuronal migration in the rodent cortex [11].

To a large extent, the underlying principles of our approach were inspired by Turing's theory. Similar to Turing's work, cells in our model consist of chemical entities that interact with morphogens over time and space. Interactions between the cells and morphogens, as well as intercellular interactions between chemical entities, change the state of the system and drive the development. In our model, in contrast with Turing's definition, the state of the system changes after each chemical interaction of the cells.

To implement Turing's theory *in silico*, we specified executable behavior for objects in a biological system. As an execution (simulation) advances, instances of the model's elements are created and the behavior of the population emerges from concurrent execution of objects that have been endowed with identical specification, but containing probabilistic parts. The end result is an executable program that qualitatively simulates the dynamic behavior of the biological system over time. The simulations can then be executed under any set of circumstances from among those that are supported by the model's basic elements. The components of the simulation represent biological entities, such as cells, which react by various transformations to events involving neighboring components. By its executable nature, this approach is different from classical mathematical models, which usually formulate behavior of populations, rather than individual entities, using forms of differential equations.

We formalized the behavior of the biological entities using the visual formalism of *Statecharts* [12], which allow us to define the dynamics using a hierarchy of possibly orthogonal (concurrent) states with transitions, events, and conditions. Using the *Rhapsody* tool (`http://www.ibm.com/software/awdtools/rhapsody/`), statecharts can be compiled into a fully executable program and can be linked up with an animated front-end using the concept of *reactive animation* [13, 14], which is designed to combine reactivity with animation to enable their interplay at run-time.

We linked up our pancreas organogenesis model with an animated front-end that was built based on what is depicted in the literature. Each of the participating components is represented as a 3D element possessing attributes to represent change in location and behavior. For example, the cells are represented in the front-end as spheres; at run-time, an instance of a cell directs its corresponding animated sphere according to its active state. A differentiated cell might, for example, change its color to depict the new stage. As the simulation advances, the cells dynamically act in concert to drive the morphogenesis of the pancreas.

2. Biological Background: Pancreatic Organogenesis

In mice, pancreatic organogenesis initiates approximately at the 8th embryonic day, and is divided roughly into two transitions, primary and secondary [15]. During the primary transition, cells at the appropriate regions on the flat gut tube are specified as pancreatic and form a bud; during the secondary

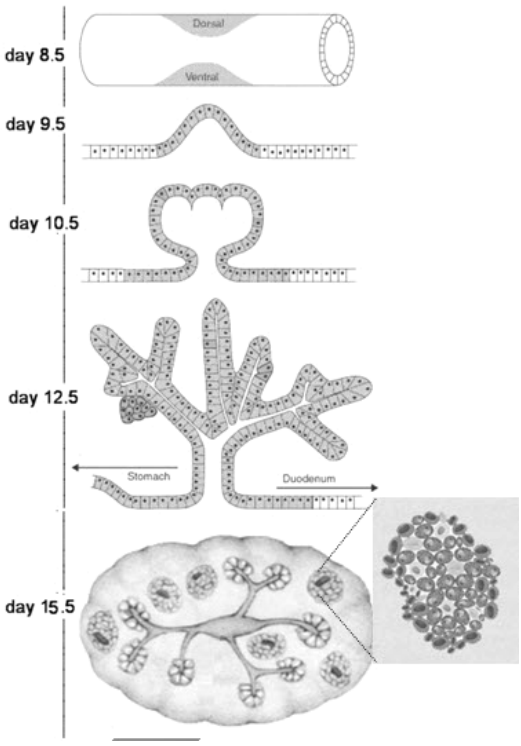

Figure 1. Illustration of pancreatic organogenesis (adapted from [20]): The process of pancreatic organogenesis in mice is roughly divided into two transitions, primary and secondary [15]. The *primary transition* starts with a budding process at the oriented region on the flat gut and ends roughly when the tissue is specified as pancreatic and develops the lobed structure (approximately embryonic day 8.5-12.5). The *secondary transition* lasts until natal and consists of terminal exocrine/endocrine differentiation, formation of the *islet of Langerhans* and maturation of the organ [16]. By the time of birth, the pancreas achieves its final pattern and increases the tissue mass in the following early post natal days.

transition, the bud evolves to form a lobulated structure (Figure 1). The organogenesis process terminates when endocrine cells aggregate to form many sphere-like endocrine tissues, termed the islets of Langerhans, which are embedded within the exocrine pancreas [16].

Molecular and morphogenetic mechanisms drive chemical interactions that act in concert to regulate the development of the organ. The molecular mechanisms regulate the differentiation and development of individual cells, whereas the morphogenic mechanisms gather the cells together to form a cauliflower-shaped organ. These processes do not occur independently but, rather, decisively affect each other. For example, the spatial location of a cell governs its chemical interactions and, vice versa, the state of differentiation of a cell influences its spatial location [16, 17]. The extracellular matrix (ECM) that surrounds the pancreatic tissue is essential to normal development. Experimental studies have shown that mice lacking a normal ECM failed to develop the organ [18-20].

An example of such a signaling process is pancreatic specification, which directs endodermal cells toward a pancreatic fate [18, 19]. Specification largely depends on two external signals from the notochord, activinβ and FGF2. These signals inhibit expression of proteins that repress the expression of the pancreatic marker, Pdx1. Hence, an endodermal cell will not commit to a pancreatic fate unless it receives both signals from the notochord.

3. Implementation of Turing's Theory

3.1. Chemical composition of a pancreatic cell as a computational agent

The chemical entities in our model (e.g., molecules and genes) are represented in the Cell object, which consists of three elements, the nucleus, the membrane and the cell itself. The nucleus operates as an internal signaling unit that expresses genes to drive cellular development, while the membrane acts as an external signaling unit that senses the environment and alerts the cell. The cell itself changes states in response to the various signals (Figure 2). Cells are considered to be the basic objects, and the progress of the simulation/execution relies very much on their behavior. An execution of the model is initiated with approximately 500 cells, which, with the aid of additional processes, proliferate and create new instances. A typical execution ends with around 10,000 objects.

The Membrane object handles interactions between the cell and its environment. In particular, it specifies the behavior of receptors, which are responsible for perceiving external signals. Each receptor in the membrane recognizes a specific molecule, which binds to it and activates signaling pathways that may regulate various mechanisms in the cell. To model the membrane, we defined each receptor as an independent component that can be either in state Unbound or in state Bound. The membrane also specifies more advanced behaviors, such as migration receptors, which sense the gradient of relevant factors in the cell's vicinity and acts accordingly.

The Nucleus object specifies the behavior of genes that regulate cell development. To model it, we took a simplistic approach, defining each gene as an independent component that can be either in state Expressed or in state Unexpressed. Some genes, even when expressed, can be non-active. The statecharts of these contain two additional states, Present and Active, within the state Expressed.

The Cell object itself describes the behavior of the various molecular mechanisms in a cell during its lifespan (e.g., differentiation, proliferation, death). We specified the mechanisms as independent components, which act concurrently at run-time to drive the cell's behavior. The Cell object also carries the spatial 3D coordinates of the cell, updating their values at run-time as the simulation progresses.

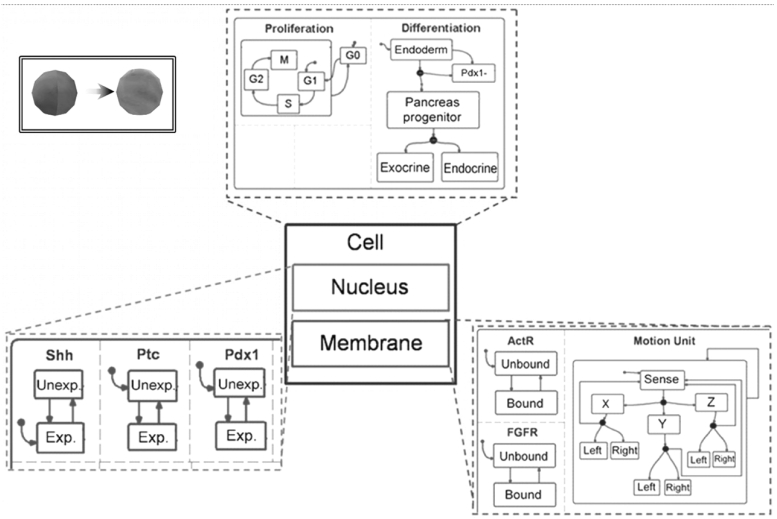

Figure 2. The model for a cell as an autonomous agent. The visualization is shown at the top left

3.2. Specifications of morphogens in the extra-cellular matrix

The morphogens in the surrounding environment were modeled as a 3D computational grid that overlies the position of the cells. The grid contains data regarding the position of the cell and relevant tissue. Thus, for example, the grid indicates the location of the notochord tissue according to where the model positions it. A similar approach was employed to define the position of the Aorta, the Mesenchyme and the Blood vessels (Figure 3, top). Four more grids indicate the concentrations of morphogens to direct the proliferation, differentiation and motility of cells. These are ActivinBeta and FGF2, which promote the specification of endodermal gut cells as pancreatic, and FGF10 and BMP4, which regulate cell proliferation.

The model updates the concentrations of the factors in the ECM grid cubes as the source tissue develops. For example, the notochord secretes several factors in the extracellular space. Accordingly, in our model the notochord object regulates concentrations of relevant factors in the ECM grid next to its specified location. The animated front-end visualizes these tissues; for example, the mesenchyme is represented by a tissue-like space that changes its color when the aorta is present. A long tube, representing the endodermal gut, lies at the center of the ECM. The notochord, when it exists, is represented by a transparent green tube that lies above the gut. The behavior of the gut is outside the scope of the model and serves solely for visualization purposes (Figure 3, middle).

We assumed that there is no direct interaction between these objects but, rather, that the interaction is carried out indirectly through the ECM object. For example, the notochord may interact with the ECM but cannot interact directly with the mesenchyme (Figure 3, bottom). To be faithful to the biology, we also prevented direct interaction between tissues and cells. Cells interact indirectly with the tissues when they sense concentrations of factors in the ECM that were previously produced by a tissue.

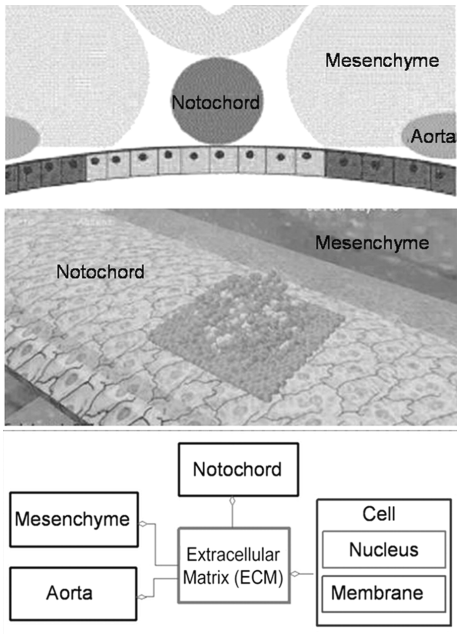

Figure 3. Modeling the extracellular space: illustration of the extracellular space (top), the 3D animated front end (middle), and the interaction scheme between the objects (bottom).

4. Model Execution

4.1. Development of pancreatic organogenesis from chemical interactions between cells and morphogens

As in the Turing's theory, the development of our model progresses through 'changes in the system state'. The changes of state in the model are a result of the activity of the chemical entities in the cells. Once the model is executed, instances of the Cell object are created and appear in the front-end as a sheet of red spheres at the proper location on the flat endodermal Gut. Once a Cell instance is created, one state in each concurrent component of its statechart is set to be the active state. At this point, the Cells are uniform and their active states are set to the initial states (designated by a stubbed arrow in the statechart). In parallel, the environment is initiated and defines the initial concentrations of factors in the extracellular space. As the simulation advances, cells respond to various events (e.g., the concentration of factors in their close vicinity) by changing their active states accordingly. Hence, the cell sheet loses uniformity at a very early stage of the simulation.

As the simulation advances, cells differentiate, proliferate and move, in response to various signals. These processes are driven by many extracellular events (e.g., from the membrane) and intra-cellular events (e.g., from the nucleus). The events, in turn, change the active states in orthogonal pieces of the statechart specification, thus moving through the various stages of the cell's life cycle. The cells as a population act in concert to drive the simulation by promoting various decisions in individual cells.

4.2. A detailed example of chemical interactions between morphogen and cells

To elucidate the way we have implemented Turing's concepts, this section discusses in some detail how the model handles the pancreatic specification process by which endodermal cells commit to a pancreatic fate. This process implicates the two morphogens mentioned earlier, FGF2 and ActivinBeta, which are secreted by the notochord tissue. The morphogens bind to receptors on the membrane of the cells and trigger a signaling process that eventually leads to the expression of the pancreatic differentiation marker PDX1. Figure 4 provides an illustration of the process as it appears in one of the related papers [19]. In this process, the notochord, a tissue that lies above the endodermal gut, secretes the FGF2 and Activinβ. When a cell comes in contact with these two factors, the corresponding receptors bind to them and initiate a chain reaction of activities. Eventually, the cell activates the pancreatic marker Pdx1, and is specified as a pancreatic cell. In parallel, the cell proliferates and migrates.

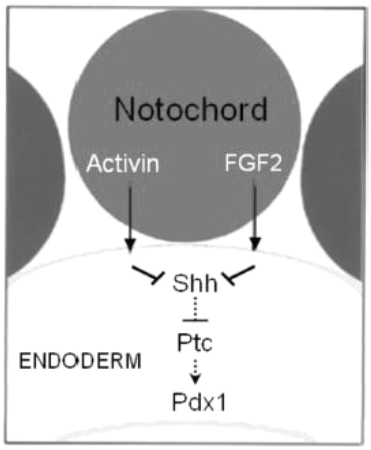

Figure 4. Illustration of the interactions between morphogens and cells in the pancreatic specification process (adapted from [19])

In our model, we assumed that the concentrations of the two morphogens gradually decrease from the central position of the notochord to the gut tube (see Figure 3). In the cell design, two statechart components of the Membrane element (Figure 2 Left) were designed to represent the FGF2R and ActivinR receptors. Accordingly, the ActivinR receptor is represented by two states, Unbound and Bound, and two transitions between them. The transition actbeta>actbetaTH goes from state Unbound to state Bound, and the transition actBeta⇐actTH goes in the opposite direction. At runtime, the Membrane continuously senses the factors in its vicinity until it determines that the concentration of ActivinR is above a predefined threshold. This causes the transition to become enabled and the active state moves from Unbound to Bound. When the opposite occurs, the other transition is enabled, and the active state moves accordingly. The FGF2R receptor is implemented similarly as another independent component.

The genes are implemented in a similar manner, to specify their behavior in the nucleus object. Three key genes, SHH, Ptc and Pdx1, are implicated in the signaling pathway that drives pancreatic specification. Each of these defines an independent component, which can be either in state Expressed or in state Unexpressed. The transition expSHH is defined from Unexpressed to Expressed and represents expression of the SHH gene. The SHH gene can be shut down, and thus the reverse direction

defines the repSHH transition representing repression of the SHH gene. The other two genes, Ptc and Pdx1, are formalized in a similar way.

The Differentiation component of the cell defines states for developmental stages in pancreatic development (e.g., pancreatic progenitor). Therefore, the transitions that are defined between the states describe the necessary conditions for the developmental progress. For example, the IS_IN(Pdx1EXP) guard is activated when the active state of the Pdx1 gene in the nucleus is set to Expressed (i.e., this cell expresses the pancreatic marker). Orthogonally to this, the Proliferation component defines a state for each stage of the cell cycle and the appropriate transitions between them (e.g., the transition evS goes from state G1 to state S). Similarly, the transition evM leads state G2 to state M, which defines the end of the proliferation process. Moreover, state M holds the duplication instructions of the Cell, namely how to create a new identical instance of a cell. The transition exitCC leads from the proliferation stages (i.e., G1, S, G2, and M) to the resting state G0.

Once a Cell instance is created, the initial state in each component (designated by a stubbed arrow) is set to the active state. As the simulation advances, the cell responds to various events by changing its active states accordingly. When a Cell object senses that the concentration of acitivinBeta goes above the predefined threshold, its Membrane enables the transition actBeta>actBetaTH and the active state of the ActR component moves from state Unbound to state Bound. A similar scenario moves the active state of the FGFR component to state Bound when the concentration of FGF2 gets to be above a certain threshold. When the active states of FGFR and ActR are set to Bound, the repSHH event is generated and the active state of the SHH gene in the nucleus becomes Unexpressed. Consequently, the event expPtc is generated, and the active state of Ptc becomes Expressed. In turn, a chain of events is initiated, and eventually the expPdx1 event is generated and the active state of Pdx1 moves to Expressed (i.e., this instance of Cell expresses the pancreatic marker). Consequently, the IS_IN(Pdx1_EXP) transition in the Differentiation component is enabled, and the system transitions from state Endoderm to state Pancreas progenitor. Accordingly, the corresponding animated sphere for the cell changes color from red to green, indicating that pancreatic specification has been accomplished.

5. Model Testing and Analysis

5.1. Comparison of the model against experimental knowledge

To test that the setup of the chemical composition and the morphogens in our model generates simulations that conform to the biological knowledge, we compared the output against illustrations and histology of the pancreas. We found that the general structure emerged in the simulation recapitulates key features of pancreatic development. As in the biological data, the simulation developed a pancreatic bud from the initial flat tissue at the early stages. Later on the structure displayed a 'mushroom'-like structure that, eventually, results with a lobulated 'cauliflower'-like structure similar to the genuine structure of the organ (figure 5 and clips in `www.wisdom.weizmann.ac.il/ yaki/organogenesis/`). Furthermore, a cross-section of the simulation at a time corresponds to embryonic day 10 was comparable with a histological cut of the pancreas approximately at a similar time point (Figure 6). Both showed an empty bud whose cell population consists mostly of pancreatic cells. Interestingly, the minority of the cells, although not explicitly programmed to do so, remained unspecified. These cells experienced abnormal cellular-morphogen interactions and did not express the pancreatic marker as the majority of the population.

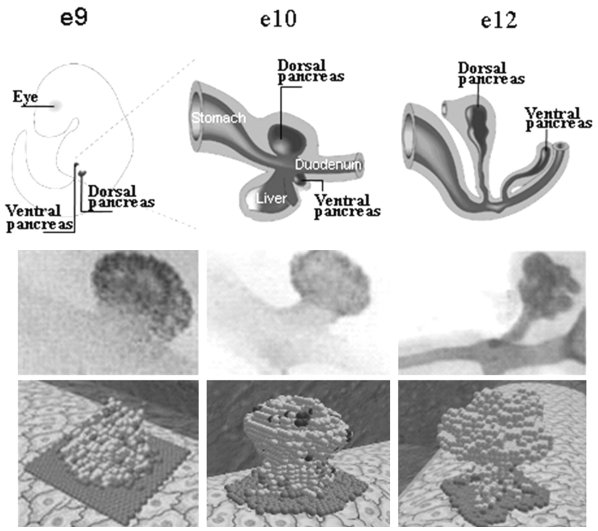

Figure 5. Comparison of illustration (top) and histology (middle) against the the emergent structure in the pancreas model (bottom) at three different stages of development (reproduced with permission from [21]).

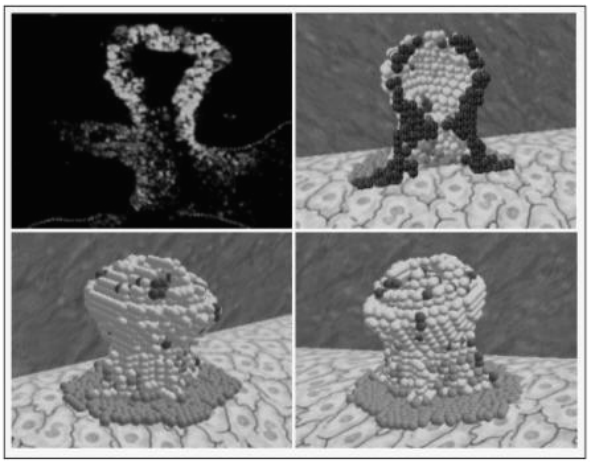

Figure 6. Histological cross-section image vs. the simulation at embryonic day 10. Notice the emerging Pdx1-negative red clusters in the simulation (dark red) (reproduced with permission from [16]).

Analysis of this phenomenon revealed that the unspecified population shares similar characteristics with a largely unexplained phenomenon observed in vivo termed *primary transition cells*. Similar to the in-vivo primary transition clusters, the unspecified in-silico population does not express the pancreatic marker and aggregate in clusters at the top of the bud. Further analysis revealed that the unspecified pancreatic cells in the simulation achieved a maximum approximately at embryonic day 10 displaying an average of 4% of the population (Figure 7, left). We found that the frequency of primary transition cells in vivo in the confocal histology in Figure 7, right is approximately 6% in qualitative agreement with the observed frequency in the model.

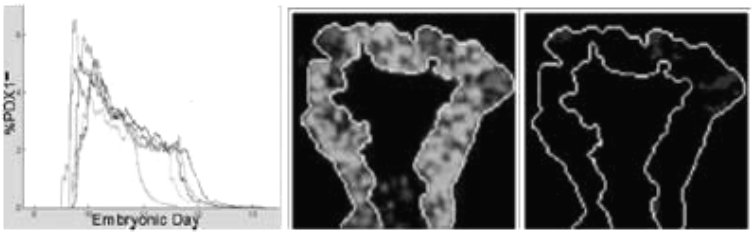

Figure 7. Analysis of the unspecified clusters at day 10. Left: unspecified cells as function of time. Right: the domain of primary transition cells in the pancreatic bud.

5.2. Relations between the chemical composition and morphogens in the model

One way to test the relations between model's design and the tissue formation in our model is by reducing morphogen concentrations in the environment. This is done by disabling the elements in the extracellular matrix that secrete factors into the environment. This in-silico analysis simulates in-vivo experiment in which a specific tissue is ablated from the organism. In the spirit of Turing's theory, this setup changes the morphogens in the environment keeping the chemical cellular composition in place. Consequently, the 'the state of the system' under the altered cell composition over time is different than under the original setup.

In one of the in-silico experiments, we disabled the notochord that largely mediates pancreatic specification. The altered setup lacks two essential morphogens that are essential for normal development of pancreatic cells. Thus, when the model is executed, the cells do not sense the essential morphogens and thus the chemical composition does not trigger the required signaling pathways for proper development. This results with unspecified cells that gathered to form the initial bud but failed to develop the mature lobulated organ (Figure 8). This outcome is consistent with similar in vivo findings that revealed an undifferentiated bud in mice whose notochord was ablated (Figure 8).

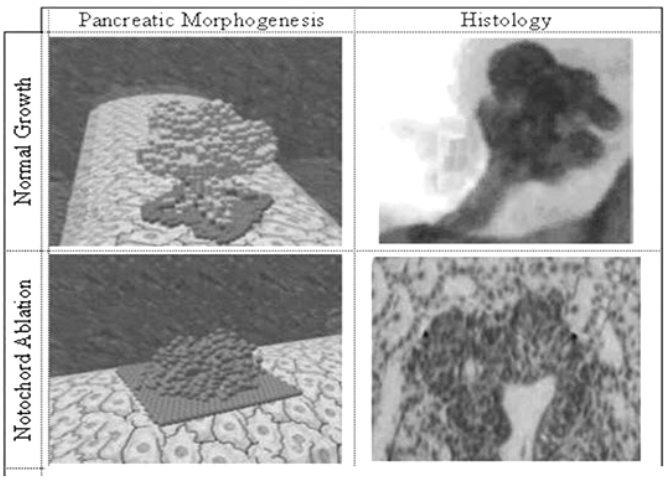

Figure 8. Relations between the morphogens and tissue formation: normal pancreas (top) and notochord ablation (bottom).

Another, somewhat complementary way to study relations between the model design and the tissue formation in the model is through modified chemical composition of the pancreatic cell. The altered composition changes the way cells react to the signals in the environment and may lead to a distinct behavior of the system. In the spirit of Turing's theory, this trial changes the cell composition keeping the same morphogen layout.

To illustrate this setup, we specified an extreme case, in in which we shuffled the expression of genes in the nucleus in a way that retains the same chemical characteristics as the original nucleus (in a way similar to that done in the Erdos-Renyi algorithm[22]). This design mimics a scenario whereby the chemical composition is determined randomly and chemical entities are not positioned properly on the signaling pathway (an example of such shuffle is given in Figure 5, top). Consequently, the interactions between cells and morphogens cannot trigger normal pancreatic development. Specifically, the design interferes with the sequence of chemical interactions that lead to the expression of the Pdx1 gene.

The modified chemical composition cannot follow the normal development gene linage. Rather, they do not respond to the morphogens at their environment and remain unspecified. Thus, the simulation does not reflect the development of the normal structure of the pancreas. At the early stages the simulation displays normal development with a preliminary proliferation of the tissue, but as time progresses the normal formation of the pancreatic bud is blocked. The modified chemical compositions result in mutated cells that form a flat tissue of unspecified cells close to the gut endoderm (Figure 5 bottom). These results verify that the specific chemical composition in the model is essential for proper development of pancreatic organogenesis.

6. Verifying Computational Models [recap from [23]]

We have presented some of the techniques we utilized to analyze the output of the pancreas model. However, the long-run perspective of computerized models raises the challenge of verifying that the output of the models fully concurs with the experimental knowledge. Here, we reintroduce an idea of the third-listed author, who suggested a variant of the well-known *Turing test* from 1950 [24], as a means to validate the authenticity and completeness of computerized biological models. Below, we reproduced verbatim the relevant sections from his paper "A Turing-like test for biological modeling" [23],

> *"The original concept was proposed by Alan Turing in 1950 as an 'imitation game' for determining whether a computer is intelligent. In this test, a human interrogator, the candidate computer and another human are put in separate rooms, connected electronically. Alice, the interrogator, doesn't know which is the human and which the computer, and has a fixed amount of time to determine their correct identities by addressing questions to them. The computer has to make its best effort to deceive Alice, giving the impression of being human, and is said to pass the Turing test if after the allotted time Alice doesn't know which is which. Succeeding by guessing is avoided by administering the test several times.*
>
> *"If we were to apply the idea in Turing's paper to validate biological models, what types of modifications to the original test would we have to implement? First, to prevent us from using our senses to tell human from computer, Turing employed separate rooms and electronic means for communication. In our version, we are not simulating intelligence but development and behavior. Consequently, our 'protection buffer' will have to be quite more*

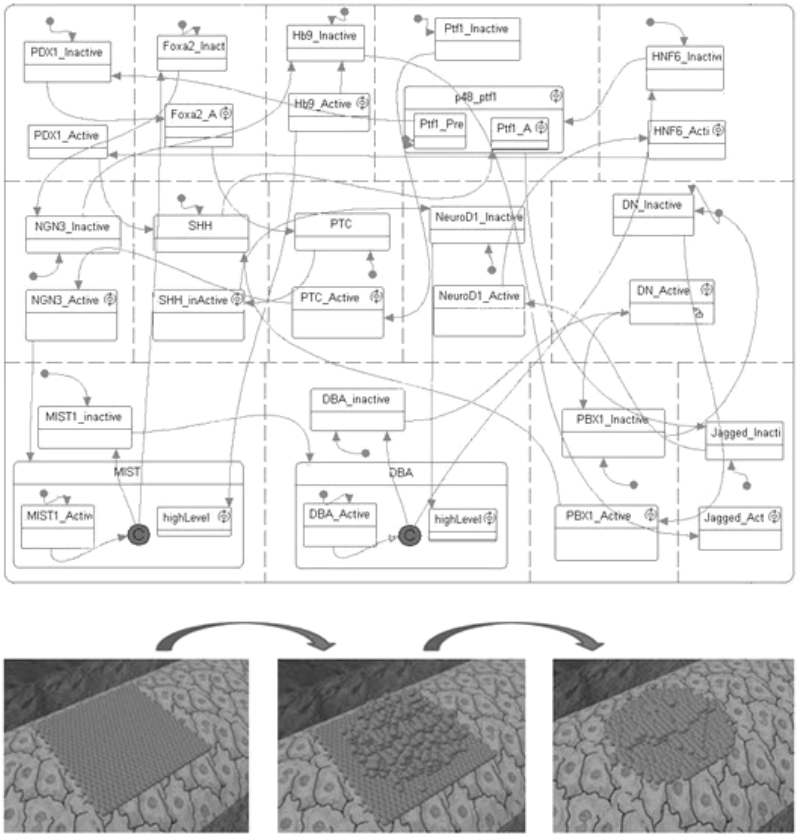

Figure 9. Relationship between the chemical composition and tissue formation: Top: A randomized gene expression in the statechart of the nucleus. Bottom: Snapshots of the emerging structure from the randomized model at three time points.

complex-intelligent, in fact! It would have to limit the interrogation to be purely behavioral and to incorporate means for disguising the fact that the model is not an actual living entity. These would have to include neutral communication methods and similar-looking front-ends, as in Turing's original test, but also means for emulating the limitations of actual experimentation. A query requiring three weeks in a laboratory on the real thing would have to elicit a similarly realistic delay from the simulating model. Moreover, queries that cannot be addressed for real at all must be left unanswered by the model too, even though the main reason for building models in the first place is to generate predictive and work-provoking responses even to those.

"Second, our test is perpetually dynamic, in the good sprit of Popper's philosophy of science. A computer passing the Turing test can be labeled intelligent once and for all because, even if we take into account the variability of intelligence among average humans, we don't expect the nature and scope of intelligence to change much over the years. In contrast, a model of a worm or a fly that passes our test can only be certified valid or complete for the

present time. New research will repeatedly refute that completeness, and the model simulating other kinds of natural systems will have to be continuously strengthened to keep up with the advancement of science. The protection buffer will also have to change as advances are made in laboratory technology (but, interestingly, it will have to be made weaker, since probing the model and probing the real thing will become closer).

"Third, our interrogators can't simply be any humans of average intelligence. Both they, and the buffer people responsible for 'running' the real organism and providing its responses to probes, would have to be experts on the subject matter of the model, appropriately knowledgeable about its coverage and levels of detail.

"Clearly, this modified test is not without its problems, and is not proposed here for immediate consideration in practice. Still, it could serve as an ultimate kind of certification for the success of what appears to be a worthy long-term research effort. Variations of the idea are also applicable to efforts aimed at modeling and simulating other kinds of natural systems."

7. Discussion

Turing's pioneering work has led to an increasing interest of the scientific community in applying his ideas for modeling morphogenesis. Turing's method has proven beneficial in modeling patterns in numerous biological tissues from diverse organisms. These include zebrafish pigment cells[25], cellular self-organization [26], iodate-sulfite-thiosulfate [27] and zebra skin pattern [28]. Possible future directions of Turing's theory were described in [29], and a possible alternative was suggested in [30]. In parallel, various computer science techniques have been adjusted to biological modeling and are applied to model tissue morphogenesis in the spirit of Turing's theory. Among these are cellular automata [31], hybrid automata [32], stochastic simulation and the PI-calculus [33].

In this paper we described our approach to modeling morphogenesis. It is a computational approach that results in a fully executable program for the chemical interactions between cells and morphogens as well as the intercellular regulation within the cells. Using a model of pancreatic organogenesis as a representative example, we elucidated how the approach can extend Turing's concepts of morphogenesis to allow us to go beyond the simple reaction-diffusion models, which often fail to take into account the detailed behavior of a large number of interacting agents. We believe that this extension of Turing's theory constitutes a biologically-plausible method for the way interacting agents perform pattern-forming tasks. Indeed, the emerging structure in the representative example is in agreement with experimental observations and revealed properties that were not explicitly programmed into the model.

Using the language of statecharts and the reactive animation concept, we implemented the behaviors of agents as basic pattern-forming entities. The collective chemical interactions of the numerous cells with the morphogens change the state of the pancreatic systems and drive the organogenesis throughout the simulation period. We further verified that the sequence of chemical interactions is specific and cannot be replaced with a random sequence. We briefly described how the output of the model can be compared with biological data, and how unforeseen properties emerge from the simulation at run time.

A future research direction along the lines presented in this paper would be to try to fully understand the relations between the original equations of Turing and the behavior of computational agents (see related discussion in [6]). This direction could help in developing an automated system that enables the translation of Turing's mathematically-based models to computational ones, and vice versa. It would

also provide means to connect mathematical work with computational models and to make experiments showing the actual in silico implementation of mathematical pattern-forming mechanisms.

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References

[1] Turing, A.M.: *The chemical basis of morphogenesis*. Philosophical Transactions of the Royal Society of London. B 1952. **327**: p. 37-72.

[2] Turing, A.M.: *The chemical basis of morphogenesis*. 1953. Bulletin of mathematical biology, 1990. **52**(1-2): p. 153-97; discussion 119-52.

[3] Caicedo-Carvajal, C.E. and T. Shinbrot: *In silico zebrafish pattern formation*. Developmental biology, 2008. **315**(2): p. 397-403.

[4] Shinbrot, T., et al.: *Cellular morphogenesis in silico*. Biophysical journal, 2009. **97**(4): p. 958-67.

[5] Swindale, N.V.: *A model for the formation of ocular dominance stripes*. Proceedings of the Royal Society of London. Series B, Containing papers of a Biological character. Royal Society, 1980. **208**(1171): p. 243-64.

[6] Bonabeau, E.: *From classical models of morphogenesis to agent-based models of pattern formation*. Artificial life, 1997. **3**(3): p. 191-211.

[7] Smith, B.J. and D.P. Gaver, 3rd, *Agent-based simulations of complex droplet pattern formation in a two-branch microfluidic network*. Lab on a chip, 2010. **10**(3): p. 303-12.

[8] Fortuna, S. and A. Troisi: *An artificial intelligence approach for modeling molecular self-assembly: agent-based simulations of rigid molecules*. The journal of physical chemistry. B, 2009. **113**(29): p. 9877-85.

[9] Setty, Y., et al.: *Four-dimensional realistic modeling of pancreatic organogenesis*. Proceedings of the National Academy of Sciences of the United States of America, 2008. **105**(51): p. 20374-9.

[10] Setty, Y., et al.: *A model of stem cell population dynamics: in-silico analysis and in-vivo validation*. Development, 2011: p. In Press.

[11] Setty, Y., et al.: *How neurons migrate: a dynamic in-silico model of neuronal migration in the developing cortex*. BMC systems biology, 2011. Under Review.

[12] Harel, D.: *Statecharts: A visual formalism for complex systems*. Sci. Comput. Program., 1987. **8**(3): p. 231-274.

[13] Efroni, S., H. David, and C.I. R.: *Reactive Animation: Realistic Modeling of Complex Dynamic Systems*. Computer, 2005. **38**(1): p. 38-47.

[14] Harel, D. and Y. Setty: *Generic Reactive Animation: Realistic Modeling of Complex Natural Systems*, in Proceedings of the 1st international workshop on Formal Methods in Systems Biology 2008, Springer-Verlag: Cambridge, UK. p. 1-16.

[15] Pictet, R.L., et al.: *An ultrastructural analysis of the developing embryonic pancreas*. Developmental biology, 1972. **29**(4): p. 436-67.

[16] Jensen, J.: *Gene regulatory factors in pancreatic development*. Developmental dynamics : an official publication of the American Association of Anatomists, 2004. **229**(1): p. 176-200.

[17] Slack, J.M.: *Developmental biology of the pancreas*. Development, 1995. **121**(6): p. 1569-80.

[18] Kim, S.K., M. Hebrok, and D.A. Melton: *Notochord to endoderm signaling is required for pancreas development*. Development, 1997. **124**(21): p. 4243-52.

[19] Kim, S.K. and M. Hebrok: *Intercellular signals regulating pancreas development and function*. Genes & development, 2001. **15**(2): p. 111-27.

[20] Kim, S.K. and R.J. MacDonald: *Signaling and transcriptional control of pancreatic organogenesis*. Current opinion in genetics & development, 2002. **12**(5): p. 540-7.

[21] Edlund, H.: *Pancreatic organogenesis–developmental mechanisms and implications for therapy*. Nature reviews. Genetics, 2002. **3**(7): p. 524-32.

[22] Bollobas, B.: *Random Graphs*. Cambridge University Press, 2001.

[23] Harel, D.: *A grand challenge: full reactive modeling of a multi-cellular animal*, in Proceedings of the 6th international conference on Hybrid systems: computation and control 2003, Springer-Verlag: Prague, Czech Republic. p. 2-2.

[24] Turing, A.M.: *Computing Machinery and Intelligence*. Mind 1950. **236**: p. 433-460.

[25] Nakamasu, A., et al.: *Interactions between zebrafish pigment cells responsible for the generation of Turing patterns*. Proceedings of the National Academy of Sciences of the United States of America, 2009. **106**(21): p. 8429-34.

[26] Klika, V., et al.: *The Influence of Receptor-Mediated Interactions on Reaction-Diffusion Mechanisms of Cellular Self-organisation*. Bulletin of mathematical biology, 2011.

[27] Liu, H., et al.: *Pattern formation in the iodate-sulfite-thiosulfate reaction-diffusion system*. Physical chemistry chemical physics : PCCP, 2011.

[28] Gravn, C.P. and R. Lahoz-beltra: *Evolving morphogenetic fields in the zebra skin pattern based on Turing's morphogen hypothesis* Int. J. Appl. Math. Comput. Sci., 2004. **14**(3): p. 351-361.

[29] Howard, J., S.W. Grill, and J.S. Bois: *Turing's next steps: the mechanochemical basis of morphogenesis*. Nature reviews. Molecular cell biology, 2011. **12**(6): p. 392-8.

[30] Harris, A.K., D. Stopak, and P. Warner: *Generation of spatially periodic patterns by a mechanical instability: a mechanical alternative to the Turing model*. Journal of embryology and experimental morphology, 1984. **80**: p. 1-20.

[31] Boldea, C. and C. Boboila: *Pattern generation using an ultra-discrete cellular automata model for thomas-murray reaction-diffusion system*, in Proceedings of the 2nd conference on European computing conference2008, World Scientific and Engineering Academy and Society (WSEAS): Malta. p. 458-462.

[32] Ghosh, R. and C. Tomlin: *Symbolic reachable set computation of piecewise affine hybrid automata and its application to biological modeling: Delta-Notch protein signaling*. IEE Transactions on Systems Biology, 2004. **1**: p. 170-183.

[33] Priami, C.: *Algorithmic systems biology*. Commun. ACM, 2009. **52**(5): p. 80-88.