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Multi-scale computational modeling of developmental biology

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ABSTRACT

Motivation: Normal development of multicellular organisms is regulated by a highly complex process in which a set of precursor cells proliferate, differentiate and move, forming over time a functioning tissue. To handle their complexity, developmental systems can be studied over distinct scales. The dynamics of each scale is determined by the collective activity of entities at the scale below it.

Results: I describe a multi-scale computational approach for modeling developmental systems and detail the methodology through a synthetic example of a developmental system that retains key features of real developmental systems. I discuss the simulation of the system as it emerges from cross-scale and intra-scale interactions and describe how an *in silico* study can be carried out by modifying these interactions in a way that mimics *in vivo* experiments. I highlight biological features of the results through a comparison with findings in *Caenorhabditis elegans* germline development and finally discuss about the applications of the approach in real developmental systems and propose future extensions.

Availability and implementation: The source code of the model of the synthetic developmental system can be found in www.wisdom.weizmann.ac.il/~yaki/MultiScaleModel.

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Supplementary information: Supplementary data are available at *Bioinformatics* online.

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1 INTRODUCTION

Many developmental systems in multicellular organisms are regulated by a small set of precursor cells that proliferate, differentiate and move to form a functioning tissue (He *et al*., 2009; Laird *et al*., 2008; Robert, 2004). Developmental systems embody a highly dynamic and complex process, which advances in numerous individual cells simultaneously. Molecular interactions over a complex regulatory network drive the development by orchestrating cell-extrinsic cues and cell-intrinsic regulation (Dada and Mendes, 2011). However, due to hurdles in *in vivo* experimentations, data in developmental biology are largely static, and the granularity of knowledge about timing and function of cellular mechanisms remains coarse (Dada and Mendes, 2011).

Analysis and study of developmental systems can be done over distinct scales: organism, tissue, cell, molecule, gene, etc. (Cohen and Harel, 2007). These scales are not merely a matter of zooming in and out. Rather, scales introduce the concept of emergent behavior: the behavior at a specific scale that is not explicitly expressed in any of the elements at the lower scale and results from their collective activity (Cohen and Harel, 2007). The activity of a tissue, for example, emerges from the collective activity of the individual cells that comprise it. Similarly, cell activity emerges from the collective molecular interactions over the regulatory network.

Three hierarchical scales, molecular, cellular and tissue, are important in the analysis of developmental systems (Dada and Mendes, 2011; Setty *et al*., 2008). At the molecular scale, an intracellular regulatory network controls molecular mechanisms such as gene expression, receptor activity and protein degradation. Above the molecular scale is the cellular scale where each individual cell decides on its next developmental step, i.e. proliferation, fate determination and motility. Further above is the tissue level where the cell population acts in concert to develop its anatomy and function. Activity at each scale drives behaviors at the above and below scales. For instance, interactions in the regulatory network at the molecular scale determine, at the cellular scale, individual cell decision-making. These, in turn, collectively stimulate anatomical formation at the tissue scale. Conversely, the position of a cell in the tissue determines which cell-extrinsic cues it detects and the decisions it makes. A change in elements at one scale may significantly affect the activity at other scales, resulting in an altered development.

Multi-scale modeling is gaining increased focus in computational biology (Edelman *et al*., 2010; Maus *et al*., 2011; Meier-Schellersheim *et al*., 2009; Palsson, 2000). It served to study various biological systems, including the electrical activity of the heart (Noble, 2002), the function of the lungs (Tawhai and Bates, 2011) and the multi-physical phenomena that govern cortical bone behavior (Lemaire *et al*., 2011). However, most of these studies focus on function and physiology of the adult tissue and often do not handle dynamic aspects of the development.

The approach presented here tackles dynamics and emergence in the process of development. The approach models developmental systems by structuring known biological information in designated elements in each scale: (i) molecular mechanisms implicated in the regulatory network are formalized at the molecular scale, (ii) proliferation, fate determination and motility are defined as decision-making at the cellular scale and (iii) anatomic constraints and cell-extrinsic cues are defined at the tissue scale in a grid that overlays the structure. Cross-scale interactions are modeled as events that emanate from element in one scale and are translated to events at the other scales. The collective intra- and cross-scale events

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drive the simulation over time yielding a dynamic representation of the process.

To illustrate the underlying principles of the approach, I discuss a model of a synthetic developmental system. The synthetic system is intended to simplify the complexity of developmental systems by abstracting key elements in the process. Thus, it generalizes common mechanisms in many developmental and stem-cell systems (Robert, 2004; Yener *et al*., 2008). A biological system that particularly illustrates the reality of the synthetic developmental system is the *Caenorhabditis elegans* germline development (Hubbard and Greenstein, 2000). This developmental system was modeled and analyzed using the presented approach and provided insights and predictions that were validated in a subsequent *in vivo* study (Setty *et al*., 2012).

In this article, I describe the general framework and the way in which each scale of the system is formalized. I then describe how the overall dynamic development emerges over time from cross-scale activity. I highlight the concept of emergence and its importance by showing how a directed mutation in the regulatory network results in a distinct tissue pattern. Although simplified, the synthetic system retained features of more complex systems. In particular, it is aligned with some experimental finding in the *C. elegans* germline development. Finally, I discuss applications of this approach in distinct systems from evolutionarily diverse organisms. I further discuss future directions, extensions and possible applications of the approach.

2 SYSTEM AND METHODS

2.1 Reactive model

Computational techniques (e.g. Petri Nets, Boolean networks and PI-calculus) are widely used to simulate reactive aspects in biological systems (Qian and Dougherty, 2009; Sackmann *et al*., 2006). These techniques often simulate biochemical reactions, gene regulatory and signal transduction (Durzinsky *et al*., 2011; Grafahrend-Belau *et al*., 2008; Qian and Dougherty, 2009). However, the existing simulation platforms are yet to fully support object-oriented programming of multiple autonomous agents (Shoham, 1998) over multiple scales.

The reactive model presented here utilizes the visual language of Statecharts (Harel, 1987) as implemented in Rhapsody, IBM's object-oriented platform (www.ibm.com/software/awdtools/rhapsody/). In Statecharts, objects are associated with 'states' that represent stages in the object's dynamics and 'transitions' that connect one state to another. A collection of states and transitions forms a component. One state in each component is labeled as the initial state, which defines the initial stage of the lifespan of the component. The language allows components to be nested within states and facilitates orthogonality (i.e. the representation of simultaneous mechanisms) in an intuitive way.

Rhapsody compiles Statecharts specifications into executable machine code. At any point in time during execution, each component is associated with an 'active state'. Initially, the active state is the one labeled as the 'initial state'. As the execution progresses, 'events' are generated and trigger the transitions between states. Consequently, the active state moves from one state to another. Events can be sent within a component, across components of the same agents and between agents.

Using Statecharts and Rhapsody, cells in the model are formalized as reactive agents whose components reflect the activity of their molecular mechanisms. For example, a gene whose expression is regulated by an external signal would be defined as a component with two states, active and inactive, and two transitions that indicate the presence and absence of a signal. When an agent senses signal below or above a certain threshold, it generates events that trigger the relevant transition. The active state moves between the active and inactive states accordingly.

All the agents in the simulation act based on the same specification to reflect the identical genetic material of cells. Each cell, however, acts independently and decides on its next step based on its current stage and the external factors it senses. The decision-making in the model is deterministic. Stochastic behavior in the model emerges from subtle differences in the order of events (e.g. the order by which cells proliferate). The model does not contain an internal clock, thus agents update their state asynchronously. Simulations are executed on an Intel Core2 Quad CPU Q9950 PC with 4 GB RAM memory. A typical simulation run with a maximum of 500 agents lasts ~20 s.

2.2 Animation

The activities in the model are represented in a 2D front-end that continuously visualizes the changes in the model. The front-end was designed using the ActionScript 2.0 scripting language of Flash MX2004 (http://www.adobe.com/products/flash/). The front-end is linked up with the reactive model through the generic reactive animation platform of Harel and Setty (2008), which enables bidirectional message passing between the two tools.

Each object in the model has a designated animated figure in the front-end. The front-end visualizes key activities of the cell agents by changing the color, position and size of the animated figures. Specifically, cells are animated as circles; cell proliferation is represented by creating an additional identical figure; cell differentiation is visualized by a change of color and/or size of the figure and cell movement is represented by positioning the animated figure into a neighboring position.

3 ALGORITHM

3.1 Modeling of a synthetic developmental system

The synthetic developmental system presented here retains two key characteristics of real developmental systems in multicellular organisms: (i) cell proliferation whereby cells divide to create new cells and (ii) differentiation whereby precursor cells adopt a specialized cell type. The cell population in the synthetic example occupies a bounded tissue area open at one end and closed at the other (Fig. 1A). A single cell, which secretes a cell-extrinsic ligand, is positioned at the closed end of the bounded area (Fig. 1A, yellow crescent). Cells that sense strong signal of the ligand proliferate at a bounded area at the closed end of the structure (Fig. 1A, yellow circles). As cells move toward the open end of the structure, they enter three sequential differentiation stages before leaving the system: (i) early differentiation stage (Fig. 1A, light orange circles), (ii) fully differentiated stage in which proliferation is blocked and the maturation process is initiated (Fig. 1A, orange circles) and (iii) specialized stage in which the cells have adopted their mature developmental stage (Fig. 1A, double size green circles).

The development of individual cells is maintained by a regulatory network that generates reactions to extracellular cues (Fig. 1B). Interaction with the cell-extrinsic factor is done through Receptor-1 that regulates cell-intrinsic activity of three intracellular effectors: a transcription factor (TF-1) and two regulators (Regulator-1 and Regulator-2). To illustrate activity over alternative signaling pathways, the regulatory network in the synthetic system consists of two independent pathways for triggering cellular differentiation. When Receptor-1 interacts with the ligand, it enhances the expression of the transcription factor TF-1, which in turn blocks the activity of the two regulators, Regulator-1 and Regulator-2. Consequently, cellular proliferation is

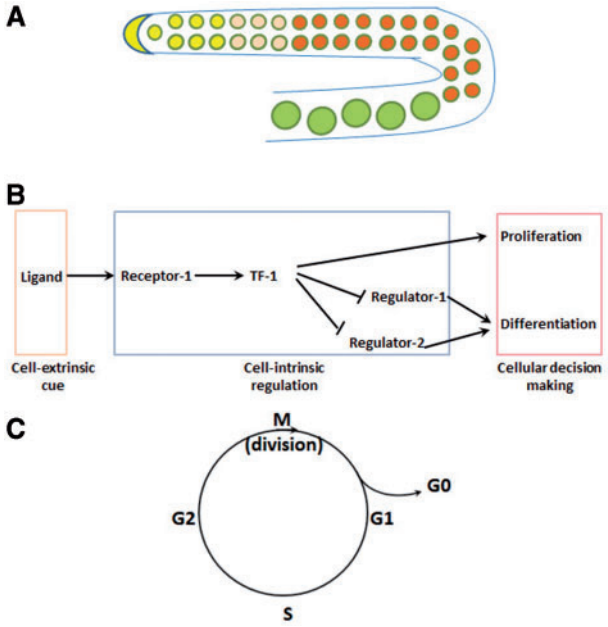


Fig. 1. A synthetic developmental system. (**A**) An illustration of the synthetic cell population, source of the ligand (yellow crescent), precursor cells (yellow circles); early differentiation (light orange); differentiated cells (orange) and specialized cells (green; double size). (**B**) The signaling pathway mediating cell activity in the synthetic developmental system; cell-extrinsic cue (ligand) interacts with the cell intrinsic regulation (Receptor-1, TF-1, Regulator-1 and Regulator-2). (**C**) Schematic description of the four phases of the cell cycle. Proliferative cells go through phases G1, S, G2 and divide in phase M. Cells that cannot proliferate exit the proliferation cycle and enter stage G0

initiated and cellular differentiation is blocked. In contrast, when Receptor-1 is not active (or absent), the genetic regulators are not expressed, thus blocking cellular proliferation and permitting cellular differentiation.

The proliferative cells (i.e. undifferentiated ones) continuously go through the four cell cycle phases, G1, S, G2 and M, and divide to give rise to an identical daughter cell. A differentiated cell exits the cell cycle and remains in the resting G0 phase (Fig. 1C).

3.2 The intracellular activity at the molecular scale

To implement the regulatory network at the molecular scale, the signaling pathways are decomposed into two distinct elements. The first specifies the interactions of the receptors with the cell-extrinsic factors and the second handles the regulation of the intracellular molecules (Fig. 2A and B, respectively).

The activity of Receptor-1 is described by three states: unbound, active or absent. Ligand interaction transition connects the unbound state with the active state, and degradation transition connects the active state to the absent one. The initial state of Receptor-1 is the unbound state. The component triggers the ligand interaction transition when the receptor detects ligand signal above 0.9 (indicating that the cell is positioned in an interaction distance with the ligand source). When the signal amplitude drops below this value, the Degradation transition is triggered and the component moves the active state to absent.

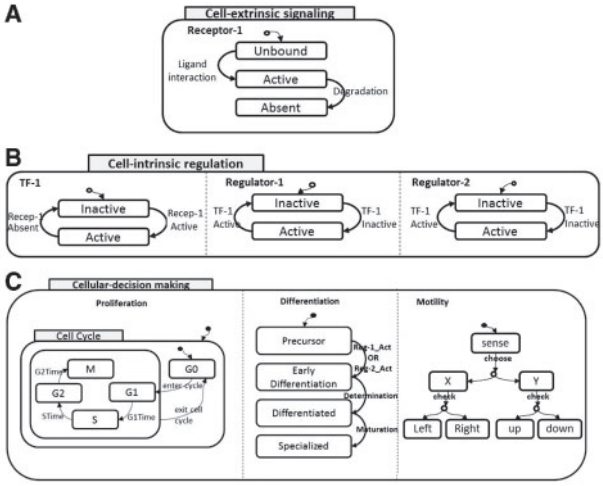


Fig. 2. Reactive molecular regulatory network. (**A**) A representation of the activity of Receptor-1. Three states (unbound, active and absent) that are connected with two transitions. (**B**) A binary representation of the three cell intrinsic elements (TF-1, Regulator-1 and Regulator-2). Each element consists of two states, active and inactive. The states are connected by transitions that represent their regulator activity (TF-1 activity is regulated by the receptor activity; Regulator-1 and Regulator-2 are regulated by TF-1). (**C**) Cellular decision-making—three orthogonal components: proliferation, differentiation and motility

The cell-intrinsic regulation (Fig. 2B) specifies the activity of molecular elements in a binary fashion. Each of the three elements, TF-1, Regulator-1 and Regulator-2, can be either in active or inactive state. The initial state of these elements is set to inactive. Receptor-1 active transition connects the inactive state of TF-1 to its active state and receptor absent connects the opposite direction. TF-1 active transition connects the active state of Regulator-1 and Regulator-2 with their inactive state. TF-1 inactive transition connects their inactive state with the active state.

3.3 The decision-making at the cellular scale

The cellular scale defines a set of rules to simulate the decisions each individual cell agent makes in response to its current developmental stage, its position and the signals it detects. The decision-making consists of three orthogonal components, one for proliferation (Fig. 2C, left), the second for differentiation (Fig. 2C, middle) and the third for motility (Fig. 2C, right). An additional cellular decision, apoptosis, is assigned to cells as a global action that instructs the agent to exit all active processes.

The proliferation component specifies the cell cycle division phases. This component specifies two proliferation states for the cell, G0 whereby the cell rests and a replication stage that defines the four phases of the cell cycle (G1, S, G2 and M). The transitions between the states are connected by transitions that are triggered after a fixed time period (enterCycle 50 ms, G1Time 200 ms, STime 150 ms and G2Time 100 ms; i.e. cells attempt to proliferate approximately every 500 ms by evaluating their ability to divide). An exitCellCycle transition monitors the proliferation and is activated when the cell agent blocks proliferation. A successful proliferation occurs when (i) Receptor-1 is in its active state, (ii) the cell successfully completed the cell cycle phases (i.e. entered the M phase) and (iii)

a free adjacent space is available for the new instance. Once a cell satisfies the three conditions, an identical instance is created in the neighboring position. In cases where any of the conditions cannot be fulfilled, the mother cell blocks proliferation by triggering the exitCellCycle event.

In parallel, the differentiation component determines four sequential differentiation states that designate the developmental stage of the cell agent: precursor, early differentiation, differentiated and specialized (Fig. 2C). The step from the precursor state to early differentiation is guarded by the activity of genes in the molecular network, namely, Regulator-1 and Regulator-2. Similarly, two sequential transitions determination and maturation connect early differentiation to differentiated and differentiated to specialized, respectively. These transitions are triggered by the amplitude of the ligand signal (signal <0.75 and signal <0.5, respectively). The signal amplitude approximates the distance from the tip where cells adopt a specific fate. Once a cell enters the specialized state, it doubles its size. In rare cases where cells do not have available space, they go through programmed cell death and are removed from the simulation.

The third component, the motility, determines the movement direction of the cell agent. This component abstracts the activity of physical forces and possible adhesion molecules that control the motility of a cell from one position to another. This component continuously senses the environment, checking for a free space to reposition the cell (Fig. 2C, bottom right). This component repeatedly checks occupancy in the cell's close vicinity. In each iteration, it first randomly decides on the direction and moves its active state to the relevant X or Y state. In each direction, the cell examines the availability of the neighboring grid entries (left, right, for the X axis and up, down for the Y axis). If an available entry is detected, the cell relocates itself to this position. Otherwise, the active state returns to the initial sense state.

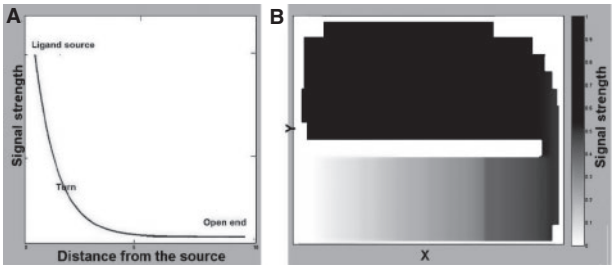
3.4 Cell-extrinsic cues and anatomy at the tissue scale

The highest scale the model tackles is the tissue scale that facilitates a 'playground' for cells in the simulation. A 2D grid defines the structure of the tissue by overlaying the U-shaped anatomy of the synthetic example. Each entry in the grid contains a zero if it is outside of the anatomical structure, a one if it is inside the structure and is free and the cell ID if it is inside the structure and is occupied. Cells cannot move (or proliferate) out of the structure boundaries or into a position that is already occupied (i.e. each grid box hosts at most one cell). The cell–ligand interaction is simulated as a signal that gradually decreases as the distance from the ligand source grows. The signal strength is calculated by a simple diffusion equation as a function of two variables—time, t, and distance, x:

$$\frac{df}{dt} = D\frac{d^2f}{dx^2} - \gamma f(x) + \delta(x - X_{\mathrm{LS}}).$$

Parameter X_{LS} determines the distance of the ligand source from the open end and δ, D and γ are the diffusion coefficient, the production rate and the degradation rate, respectively. X_{LS} is set to 10.

To make the equation easily solvable, the values of the equation rates were normalized to 1. Solution of the equation on steady-state conditions (i.e. $d(x)/d(t) = 0$) approximates the signal amplitude over the space as a function of the distance from the source (Fig. 3A).



C

Transition	Signal amplitude	Developmental stage
Receptor-ligand Interaction	>0.9	High signal amplitude indicating that the receptor retains activation by the ligand.
Degradation	<0.9; >0.75	Cells moved away from the ligand source and the receptor loses activation and degrades.
Determination	<0.75; >0.5	Cells are at a distance from the closed end that promotes cellular determination.
Maturation	<0.5	Cells crossed the turn in the structure and initiated the specialization process.

Fig. 3. Extrinsic signaling and structure anatomy. (**A**) Solution of the diffusion equation for ligand signal as a function of distance from the source of the ligand. The amplitude is maximal close to the source of the signal and gradually decreases approaching the open end of the structure. (**B**) Heat map of the ligand signal as a function of the position in the structure. Maximal strength at the closed end of the structure (black) gradually decreases as the position moves toward the open end where the signal amplitude equals zero (white). (**C**) Transitions: the value ranges that trigger them and the developmental progression they represent

In order to fit the 1D solution to the 2D anatomy, the ligand diffusion is assumed to occur only over the proximal axis (i.e. the ligand is constant on the perpendicular axis). To minimize the complexity required for run-time calculations, the solution values are stored in the grid as shown in Figure 3B. The various transitions, their cause and the developmental progression are summarized in the table in Figure 3C.

This implementation is a highly simplified version of the ligand–cell interaction in reality. However, it could be easily extended to cover anatomical spatial changes over time possibly extracted from observations in live organisms. Similarly, a more realistic implementation of the receptor–ligand interaction could account for physical constraints that exist in reality.

4 IMPLEMENTATION AND TESTING

4.1 Development over time from cross-scale activity

Executions of the model begin with four precursor cells that occupy the area that is assumed to physically interact with the source ligand (i.e. within distance zero from the closed end). As the simulation progresses, these cells proliferate and move toward the open end, filling the empty space of the structure (Fig. 4A and Supplementary Movie S1). At the molecular scale, precursor cells (Fig. 4A, yellow) interact with the ligand (i.e. the ligand interaction transition is enabled). In turn, the active state of Receptor-1 moves from unbound to active and TF-1 is active and the two regulators are inactive. Consequently, at the cellular scale, the proliferation component moves through the four cell cycle states creating a new instance at the

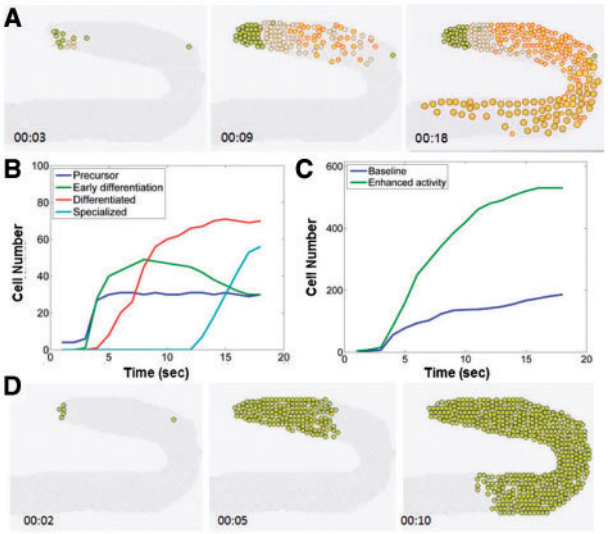


Fig. 4. (**A**) Emergence of cell population dynamics from the collective activity of individual cells. Three stages of the emergent pattern in the simulation at run time (also see Supplementary Movie S1). A small population of precursor cells (yellow) proliferates and enters over time early differentiation stage (light orange), followed by differentiated stage (orange) and eventually adopts a specialized fate (double size green circle). (**B**) Distribution of the different cell types in the synthetic developmental system over time (blue, green, red and cyan designate number of precursor, early differentiation, differentiated and specialized cells, respectively). (**C**) Total number of cells in the simulation under baseline (blue) and mutated network (green). (**D**) The simulation under the mutated network. Precursor cells continuously proliferate and fill the entire structure

M state. In parallel, cells move away from ligand source creating free space into which neighboring cells divide. When a cell moves away from the external ligand source, its receptor loses the interaction with the ligand and degrades. Consequently, at the molecular scale, the degradation transition is triggered and the receptor enters the absent state. In turn, the Receptor-1 absent transition is triggered in the TF-1 component. Once TF-1 enters the inactive state, the TF-1 active transition of Regulator-1 and Regulator-2 is triggered, moving the regulators' active state from inactive to active. As a result, at the cellular scale, the differentiation component moves from the precursor state to early differentiation (Fig. 4A, light orange). As cells move toward the open end, intrinsic signals trigger the determination transition, which causes the cells to enter the differentiated state (Fig. 4A, orange). Cells that crossed the turn of the structure trigger the maturation event and enter the specialized state (Fig. 4A double size green circles).

The tissue-scale pattern that emerged from the simulation consists of three distinct domains. Each domain is primarily occupied by cells of a specific type. At the initial stages, the domain closest to the closed tip is filled with precursor cells. As the simulation progresses domains for the different cell types are formed (Fig. 4A). Proliferative cells occupy the closest domain to the closed end (Fig. 4A, yellow and light orange), followed by a domain filled by differentiated cells (Fig. 4A, orange) and a domain of specialized cells (Fig. 4A, double size green circle). The emergent distribution of the different types over time is given in Figure 4B. Initially, the precursor-cell population increases until it reaches it full capacity (~30 cells; Fig. 4B, blue). The early differentiation population grows in a delay and reaches a maximum of ~40 cells (Fig. 4B, green). Since these cells go through a specialization process, the early differentiated population gradually decreases, while the differentiated population increases over time. Equilibrium between the two populations is reached when the early differentiation population size is ~30 cells and the differentiated population size is ~70 cells (Fig. 4B, green and red respectively). In parallel, the specialized cell population increases its size to ~50 cells (Fig. 4B, cyan). Overall, the population size achieves a maximum size of ~200 cells (Fig. 4C, blue). Notably, neither the population size nor the growth timing was explicitly programmed or restricted in the model. Rather, they emerged from the molecular interactions and the collective decision-making of the cells in simulation.

4.2 *In silico* experimentation: a molecular modification leads to a distinct tissue pattern

An important aspect of the multi-scale modeling approach is the ability to perform empirically grounded *in silico* experimentation. This allows analyzing the effects of changes in the molecular regulatory network on cellular and tissue development. These experiments simulate possible *in vivo* experiments.

An example of *in silico* experimentation is simulating genetic manipulations that enhance receptor activity by preventing its degradation. This is a modification at the molecular scale, that is implemented in the model by removing the degradation transition that connects the active state to the absent state in the Receptor-1 component (Fig. 2A). When the simulation is executed, Receptor-1 is continuously active and thus TF-1 is continuously active. Consequently, both Regulator-1 and Regulator-2 remain inactive. In turn, at the differentiation component of the decision-making, the transition between the precursor state and the early differentiation state is never triggered (i.e. cells block differentiation). Consequently, at the cellular scale, the decision-making was restricted to proliferation and motility (i.e. mutated cells can either proliferate or move).

This molecular-scale modification leads to a distinct tissue-scale emergent behavior. In contrast with the three domains observed in the wild-type simulations, the simulation develops packed precursor cell population that fills the structure entirely (Fig. 4D and Supplementary Movie S2). As a result, the emergent distribution of cells is considerably altered. The size of the precursor population increases to ~500, ~2.5 times the total number of cells (Fig. 4C) and 15 times more than this population (Fig. 4B) in simulations under wild-type conditions.

4.3 Comparison with a real developmental system

The germline development in the *C. elegans* nematode particularly illustrates the reality of the synthetic example (Hubbard, 2007). This developmental system consists of a self-renewing cell population that occupies the distal end of a blind-ended two U-shaped arms tube (Fig. 5A). The population maintains interactions with a tip cell that is positioned at the distal end of structure (Fig. 5A, yellow crescent). Precursor germ cells (Fig. 5A, green circle) that interact with the tip cell proliferate to create new instances. Cells are 'pushed' by the proliferating neighboring cells toward the uterus (Fig. 5A, light green). When these cells initiate differentiation, they

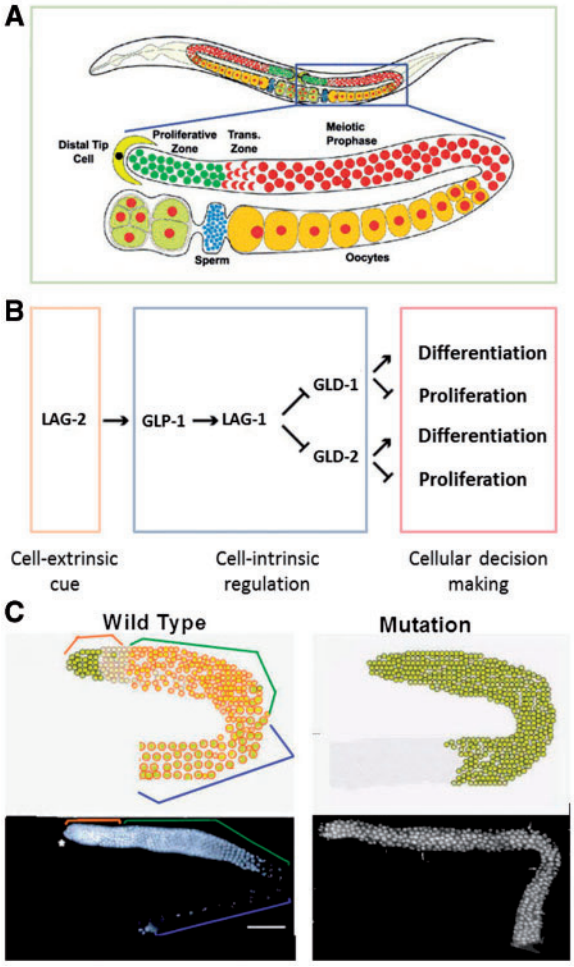


Fig. 5. Germline development [reproduced with permission from Hansen *et al.* (2004)]. (**A**) Illustration of one arm of the adult germline in the gonad that displays a distal-to-proximal axis of germline development. (**B**) The genetic pathway mediating germ cell activity; cell-extrinsic cue (LAG-2) interacts with the GLP-1 receptor that regulates the activity of the cell-intrinsic transcription factor LAG-1. LAG-1 negatively regulates GLD-1 and GLD-2, two regulators (differentiation markers in this context) that promote differentiation and inhibit proliferation. (**C**) Comparison between the synthetic example and germline development. Left: three distinct areas in wild-type animal: proliferative (orange), meiotic (green) and gamete (blue). Right: tumorous development in the glp-1(oz112gf) mutation. Reproduced with permission from Hubbard and Greenstein (2000) and Pepper *et al.* (2003)

go through three phases: early meiosis stage (Fig. 5A, red crescent), from which they adopt a pachytene fate (Fig. 5A, red circle) and, finally, mature gametes (Fig. 5A, orange for oocyte and blue for sperm).

In vivo experimentations characterized the genetic network that controls the cell-intrinsic regulation (Fig. 5B). The cell-extrinsic ligand, LAG-2, i.e. expressed by the tip cells, regulates the signaling pathway by binding to the receptor GLP-1. The active receptor triggers the expression of the transcription factor LAG-1, which in turn promotes the expression of two regulators GLD-1 and GLD-2 that determine the cellular decisions. Cells that express either one of these regulators initiate differentiation and block proliferation.

Although simplified, the simulations of the synthetic example are comparable with experimental observations in the gonad of the *C. elegans* nematode. The emergent patterns in the model share similar characteristics with images from live wild-type and mutant nematodes (Fig. 5C). The pattern of the three distinct domains in the simulation is similar to patterns observed in the gonad of wild-type nematodes: a proliferative zone positioned close to the distal tip (orange) followed by a zone consists of differentiated cells zone (green) and specialized cells (i.e. matured gamete) (blue).

Similarly, the emergent pattern in enhanced receptor activity simulations is comparable with observations in mutations whose GLP-1 receptor is engineered to gain function. In this mutant, similar to the enhanced receptor activity design of the simulation, the receptor remains active through the cell's entire lifespan. Similar to the pattern emerged in the altered simulations; this mutant develops a tumorous tissue that consists only of precursor cells that are packed in the structure (Fig. 5C, right).

5 DISCUSSION

5.1 Applications of the approach

The synthetic example presented here serves to highlight the underlying principles of the approach. Nevertheless, the approach supports complex real systems that consist of multiple signaling pathways and multiple cell types. Indeed, this approach inspired to date three models that simulate development of real organs and stem-cell population in distinct, evolutionarily diverse, biological systems: pancreatic organogenesis in mice (Setty *et al.*, 2008), germline development in *C. elegans* (Setty *et al.*, 2012) and neuronal migration in rodents (Setty *et al.*, 2011). The models served to analyze the biological systems through extensive analysis of *in silico* genetic manipulations. Notably, these studies investigated dynamic aspects that could not be drawn from the isolated elements and, instead, had to be analyzed at the multicellular tissue level. The models recapitulated key features of the development and predicted altered formation under modified conditions. Several of the predictions were validated in *in vivo* experiments, further affirming the potential of the approach.

The approach can be utilized to model a broad variety of biological systems in various evolutionary diverse organisms. This approach particularly suits modeling of systems where the pattern and structure is dominantly regulated by an agent population that responds to external cues. Among these are stem-cell systems such as the *Drosophila* ovariole and testis (Fuller and Spradling, 2007). Similarly, this approach could serve in modeling the dynamic emergence of uncontrolled malignant cancer cell population in healthy tissues. A support for this direction can be obtained by the tumorigenesis-like phenomenon that emerged in the synthetic example (although only partially adheres to the 'hallmark of cancer' (Hanahan and Weinberg, 2000).

5.2 Future extensions

The approach as discussed here tackles three essential scales; however, it can be extended in a modular fashion nonetheless for creating models with additional scales. One possibility is to extend the current discrete representation of the molecular activity

with a more realistic version, through a specification of activity at scales below the molecular one. The extended scales should cover interactions between molecules incorporating parameters for protein concentrations, binding rates, physical forces and so forth. This could be undertaken using the more traditional reaction equations (Edelstein-Keshet, 2005) or by computational techniques (Priami and Quaglia, 2004; Regev *et al*., 2001).

A complementary extension could assemble models of different tissues and the relevant interactions between them to yield a comprehensive model at an organism scale. Such effort may serve as a platform to study organism-level development and function as well as detailed analyses of diseases and abnormalities.

Another possible direction is adding support for triggering decision-making by a combination of stimuli, thus extending the present implementation of a single stimulus (i.e. signal amplitude) that triggers the multiple decision-making in the simulated agents. A possible additional stimulus could be derived from the density of entities in the agent's vicinity. This property could be of particular interest in applying the presented technique in modeling quorum systems (e.g. bacteria population and ant colony) whose response is correlated to population density (Goryachev *et al*., 2006).

The principles underlying this approach could serve in a tool for *in silico* modeling and analysis, facilitating integration of experimental knowledge in computational visual models for a wide range of developmental systems.

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