Supporting Information

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SI Text

A Control Experiment. This section describes a control experiment we performed to test that the emerging structure in the simulation resulted from the specific molecular connection "rules" integrated into the model. We therefore randomized the expression of genes (in the nucleus) in a way that kept the same characteristics as in the original statechart. Accordingly, each state in the randomized statechart has the same number of incoming and outgoing transitions as the corresponding state has in the original statechart. Fig. S1 displays a snapshot of the emerging structure from the randomized model at embryonic day 12. In the randomized model, cells were not specified as pancreatic and eventually died. Thus, the emerging structure failed to initiate the pancreatic bud, as the original model. We repeated the process several times to verify that the emerging structure depicted in the model is the consequence of the biological information specified, rather than a global tendency of the model to develop a cauliflower-shaped structure by itself.

Detailed Description of the Statechart Model for a Cell. This section describes how we formalized a eukaryotic cell as a 3D autonomous entity that senses the environment and acts accordingly. Here, we elaborate on the way we used the language of Statecharts and its implementation in the Rhapsody tool. We show how the statecharts in the model define behavior using a hierarchy of states with transitions, events, and conditions. Among other things, we used the Rhapsody tool to compile the model into executable reactive machine code (for example, in C++).

To explain our model better, we provide here a schematic description of the cell and some of its major statecharts. We also discuss representative pieces of the model and describe their behavior at run-time, using a concrete pathway from the model. The *SI Appendix* lists the elements (i.e., genes, receptors and factors) that appear in the model. However, we deliberately left out the technical implementation and focused only of the conceptual aspects. Some of the technicalities, in particular the related issue of reactive animation, have appeared elsewhere.

Modeling the Eukaryotic Cell. In the model, a eukaryotic cell is considered the basic element. It contains 3 subelements, formalizing the membrane, the nucleus, and the cell itself. The membrane, the surface of the cell, contains many concurrent receptors that are responsible for perceiving external signals. Similarly, the nucleus, the core of a cell that contains the DNA, consists of concurrent genes that generate internal signals in the cell. The Cell itself specifies the behavior of molecular mechanisms (e.g., proliferation) that drive the cell development during its life span. This setup formalizes a cell as an autonomous agent, sensing its environment and acting based on its specified behavior. This is a term taken from artificial intelligence and is used to describe a system that is situated in, and is part of, an environment, and which senses that environment, and acts on it, over time, in pursuit of its own agenda.

The setup of this part of the model is illustrated in Fig. S2, which shows the elements accompanied by schematic versions of their statecharts. Each independent component in the statechart of the Cell specifies the behavior of a concurrent molecular mechanism. Similarly, in the statecharts of the nucleus and the membrane each independent component specifies concurrent behavior of a gene and receptor, respectively. The Cell is visualized in the animated front end, which holds the relevant structural information and as the simulation progresses changes properties (e.g., color) of the animated entities to indicate their development.

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.

Similarly, the nucleus object specifies the behavior of genes that regulate cell development. To model the nucleus, 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 nonactive. The statecharts of these contain two additional states, "Present" and "Active," within the state Expressed.

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

Detailed Descriptions. To elucidate the statecharts in the model, we discuss in more detail representative pieces from the various objects of the model (see Figs. S2–S7). The remaining statecharts are given in Figs. S8–S13 and use similar principles. We do believe that the specification can be understood quite well from the detailed example below.

Two statechart components of the Membrane (Fig. S3 *Left*), represent the FGF2R and ActivinR receptors. Accordingly, the FGF2R receptor is represented by 2 states, Unbound and Bound, and 2 transitions between them. The transition actβ>actβTH goes from state Unbound to state Bound, and the transition actβ<actβTH is drawn in the opposite direction. At run time, the Membrane continuously senses the factors in its vicinity until it senses that the concentration of FGF2 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 ActivinR receptor is implemented similarly as another independent component.

A more advanced case is the motion unit component (Fig. S3 *Right*), which senses the concentration of migration factors (e.g., FGF10) and decides which direction the Cell should move. This motion decision is carried in 2 steps: first the axis is determined (i.e., x, y, or z) and then the left or right direction on the relevant axis.

A similar method was used to specify behavior of genes in the nucleus object. Fig. S4 displays three genes in the nucleus—*SHH*, *Ptc*, and *Pdx1*—that participate in pancreatic development. Each of these is an independent component, which can be either in state Expressed or in state Unexpressed. The transition expSHH is defined from state Unexpressed to state Expressed and represents expression of the *SHH* gene. The gene can be shut down and thus the reverse direction defines the repSHH tran-

sition representing repression of the *SHH* gene. The other two genes, *Ptc* and *Pdx1*, were formalized in a similar way.

Fig. S5 describes in detail the main part of the statecharts of 2 molecular mechanisms in the Cell object, Differentiation and Proliferation. The Differentiation component 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(Pdx1_EXP) 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, the Proliferation component defines a state for each stage of the cell cycle and their corresponding transitions (e.g., the transition evS is drawn from state G1 to state S). Similarly, the transition evM connects 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.

The Model at Run Time. 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. To illustrate the simulation in progress, we describe some of the processes a representative Cell undergoes during its life span.

Consider the pancreatic specification process, in which endodermal cell is specified as pancreatic (i.e., it expresses the pancreatic marker). Fig. S6 provides an illustration of the process as it appears in one of the related papers (1). In this process, the notochord, a tissue that lies above the endodermal gut, secretes two factors, FGF2 and activin. When a cell comes in contact with these factors, the corresponding receptors bind to the factors and initiate a chain reaction of activities. Eventually, the cell activates the pancreatic marker Pdx1, and thus is specified as a pancreatic cell. At the same time, the cell proliferates and migrates.

This process incorporated the three subelements of the model and is reflected in their statecharts (Figs. S3–S5). When a Cell object senses that the concentration of acitivin goes above a certain threshold, its Membrane enables the transition actβ>actβTH 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 (Fig. S7).

Concurrently, the active state of the Proliferation component moves through the different stages of the cell cycle to carry out cell division. When the active state of the component is G2 and the event evM is generated, the active state becomes M and the Cell duplicates itself by creating an identical Cell instance. Accordingly, at the proper location in the front-end animation, a new identical sphere is created, corresponding to the new Cell instance. However, if at any stage of the process, the cell cannot proceed with the proliferation (e.g., as a result of an environmental signal), the exitCC event is generated, and the active state in the component becomes G0 (i.e., the cell division was blocked). At the same time, the motion unit in the Membrane continuously senses relevant migration factors and directs the Cell accordingly. Thus, when the Membrane senses a gradient of relevant factors toward a certain axis, it initiates a cascade of events that update, if possible, the cell's position. For example, if the Cell senses a gradient of concentration of a relevant factor toward its left on the *x* axis, the Membrane generates events (i.e., CheckAxis and CheckX) that cause a transition of the motion unit to state X and then to state left. At the end of the process, the event move is generated, and the animated cell changes its position to the new location. Finally, the motion unit returns to the initial state, Sense. If at any stage, the Cell cannot move, the motion events (e.g., CheckAxis) cause the Motion Unit to move back to state Sense.

1. Kim SK and MacDonald R (2002) Signaling and transcriptional regulation in the developing pancreas. *Curr Opin Genet Dev* 12:540–547.

Fig. S1. Emerging structure of the randomized model.

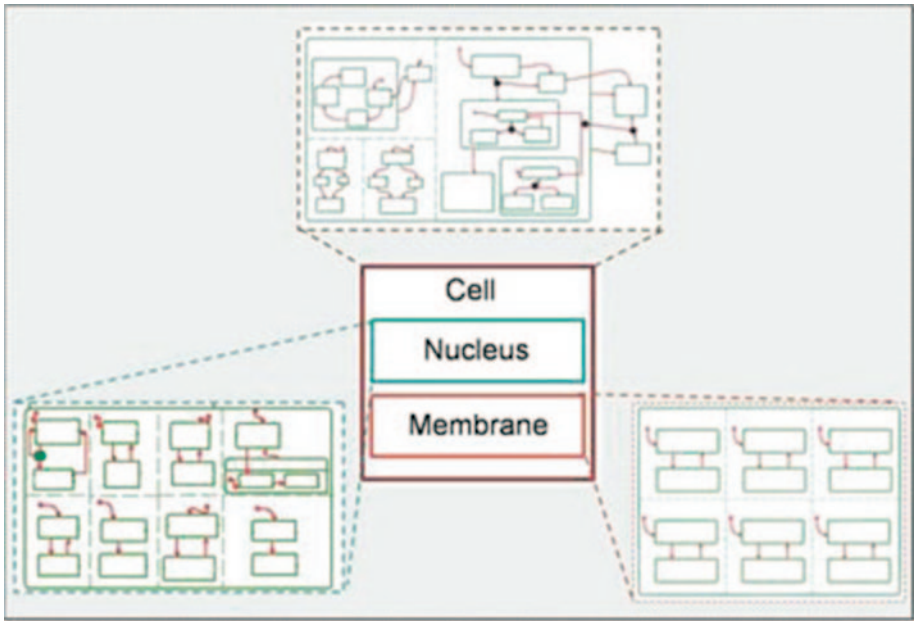


Fig. S2. Schematic description of the Cell object and its statecharts.

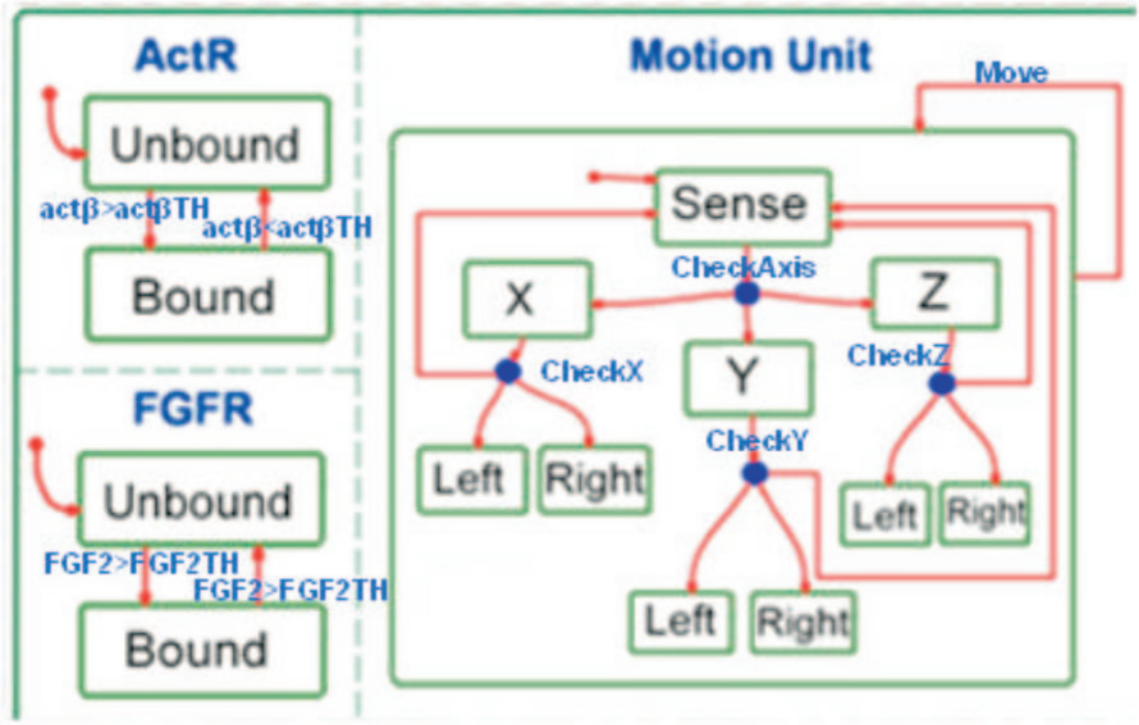


Fig. S3. Detailed description of 3 statechart components in the membrane.

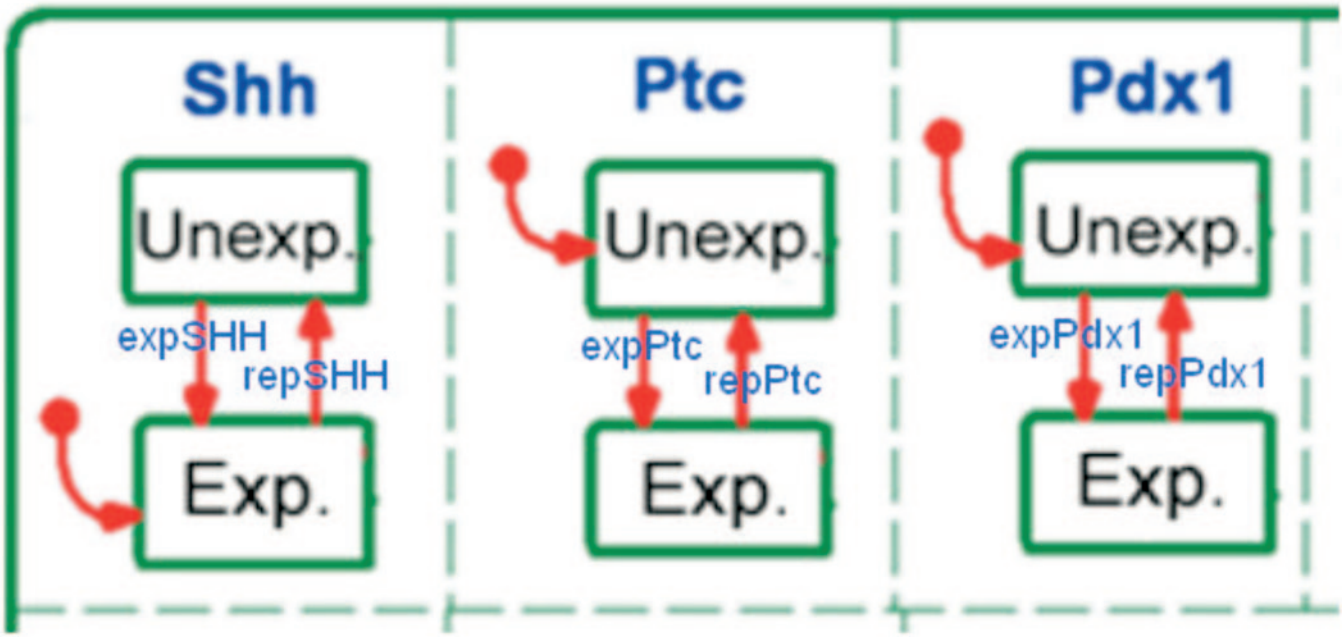


Fig. S4. Detailed description of 3 statechart components in the nucleus.

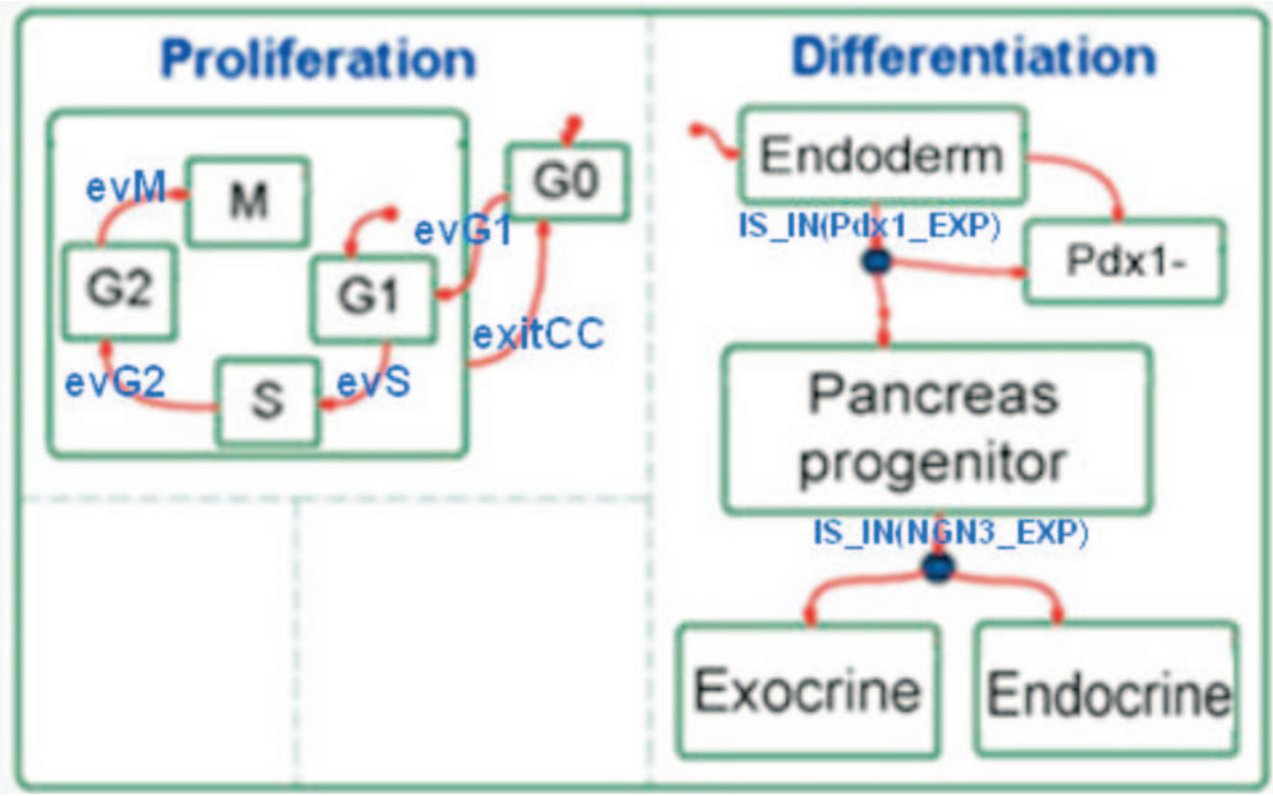


Fig. S5. Detailed description of the statechart components for Proliferation and Differentiation.

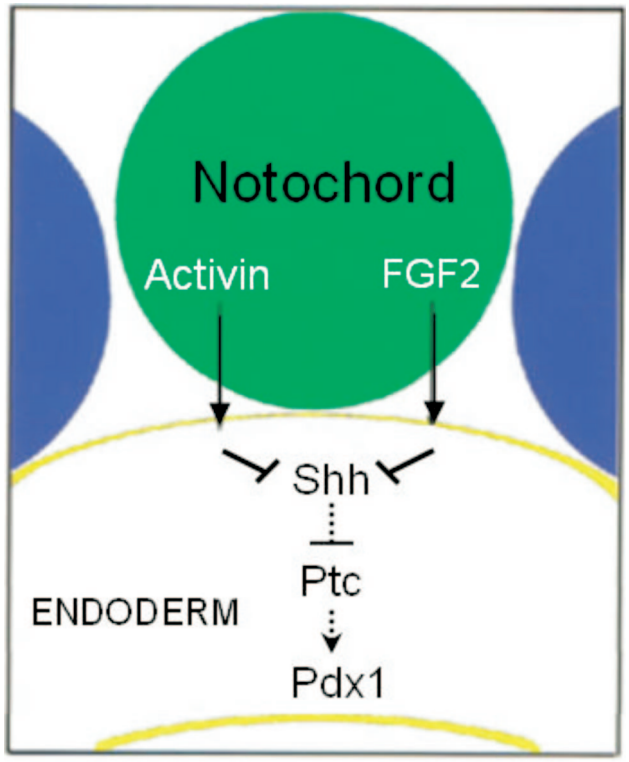


Fig. S6. Illustration of the pancreatic specification process as it appears in Kim *et al.* (2001).

Fig. S7. The pancreatic specification as it appears in the front-end animation view. A red sphere, designating an endodermal cell, changes its color to green, designating a pancreatic progenitor cell.

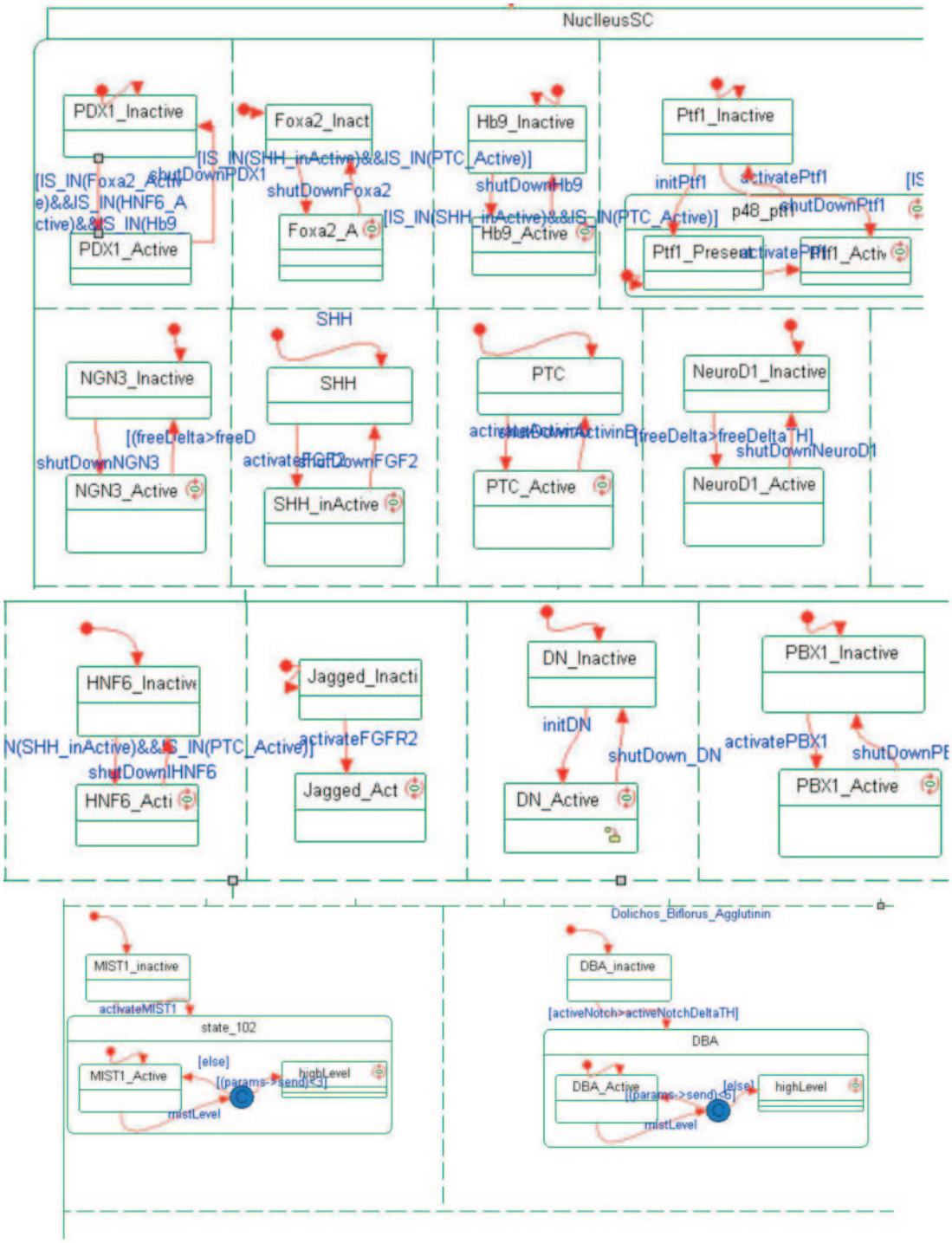


Fig. S8. A rather inclusive view of the nucleus statechart.

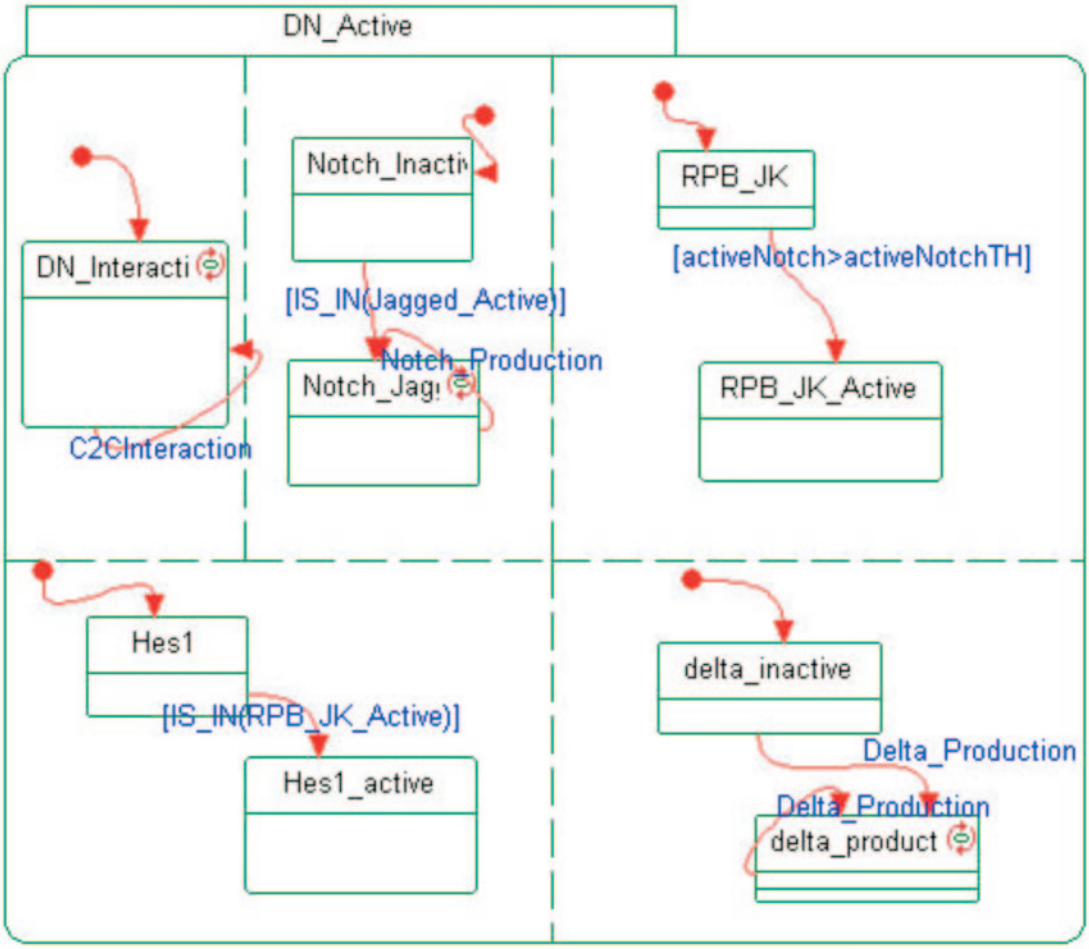


Fig. S9. Statechart for the Delta-Notch genes in the nucleus.

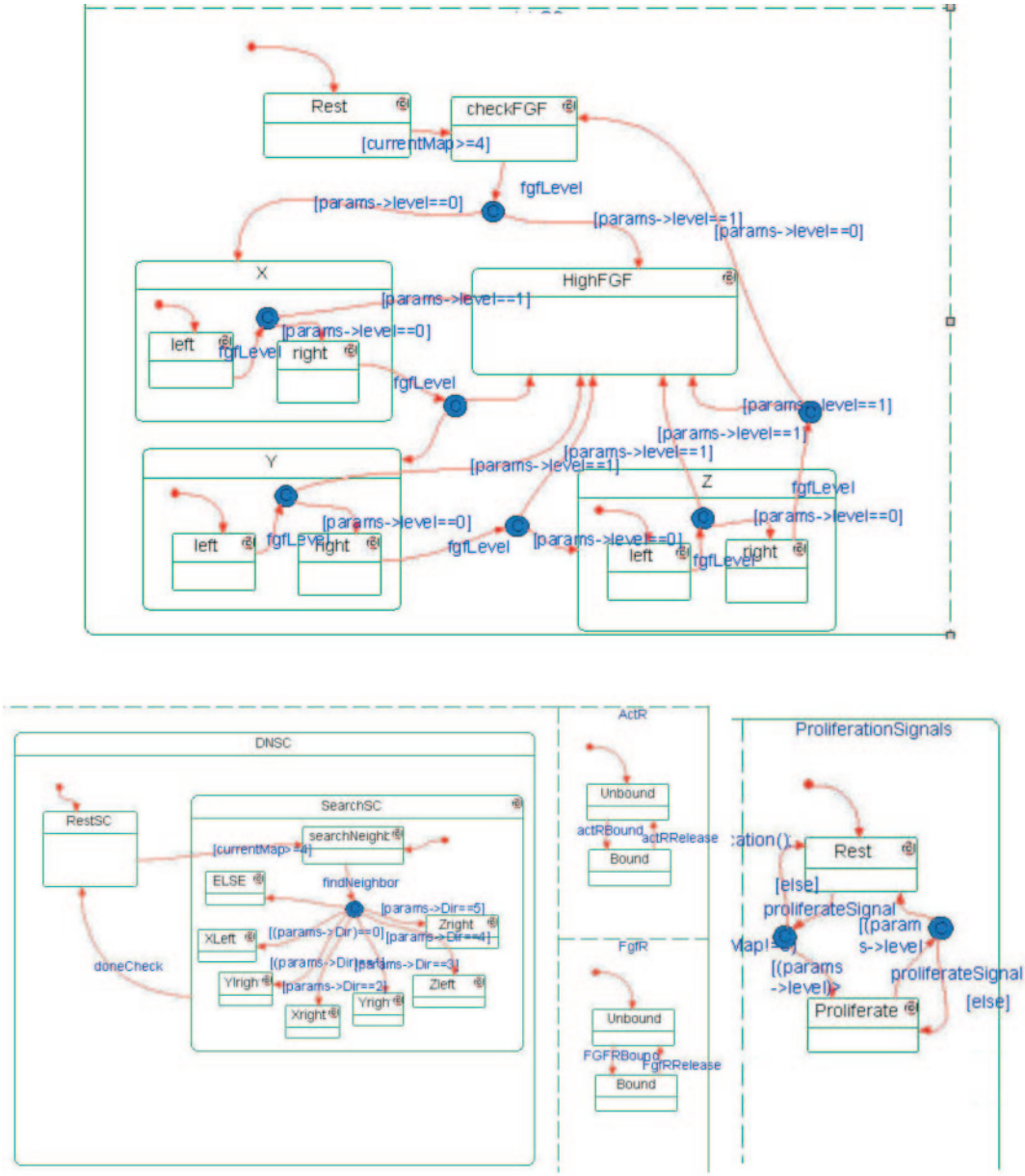


Fig. S10. Statecharts for different receptors in membrane. Level of FGF10 (*Upper*), Delta-Notch (*Lower Left*), pancreatic specification receptors (*Lower Center*), and proliferation signals (*Lower Right*).

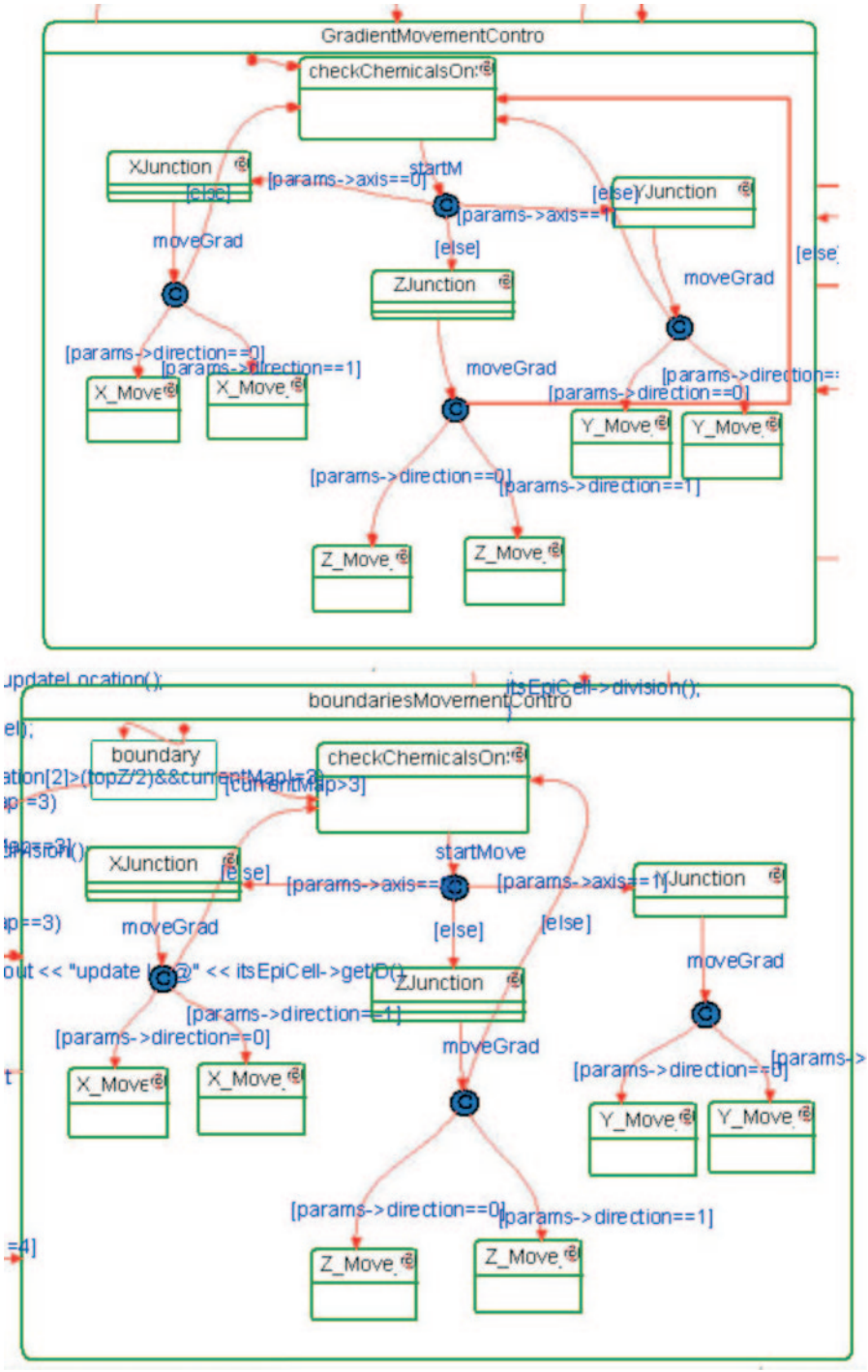


Fig. S11. Motion units in the membrane for endodermal cell (*Upper*) and pancreatic progenitor cell (*Lower*).

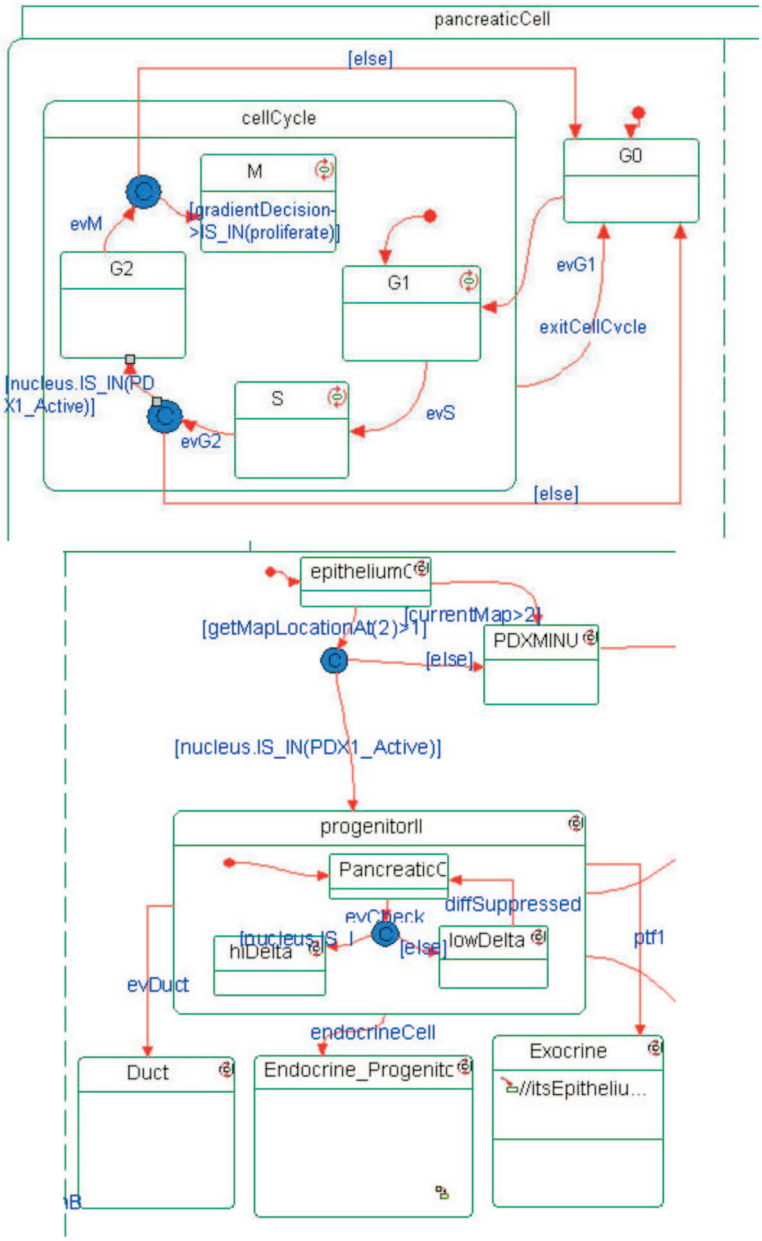


Fig. S12. Statechart components of the Cell object, specifying Proliferation (*Upper*) and Differentiation (*Lower*) in the Cell object.

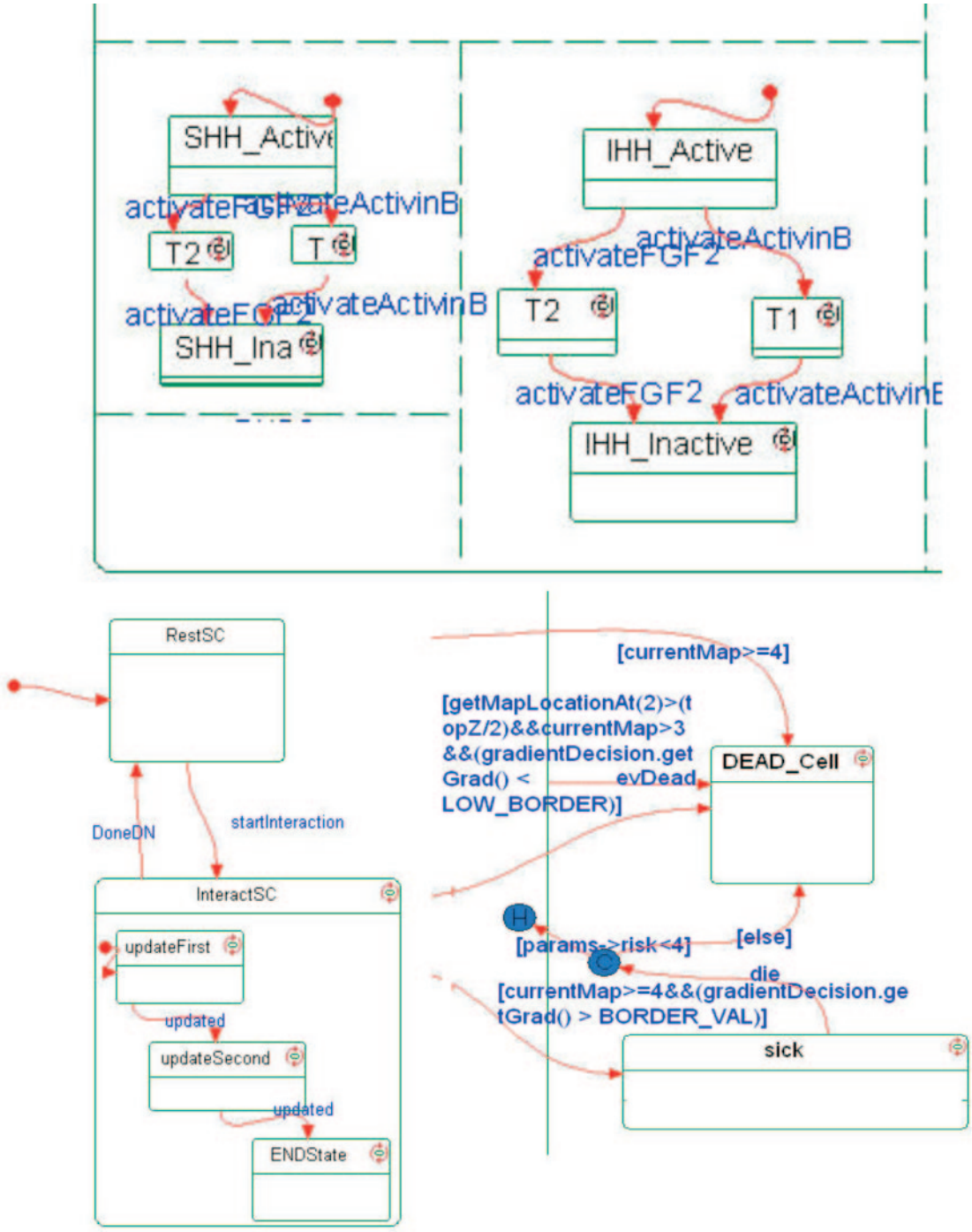
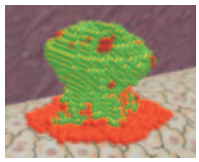
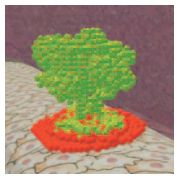


Fig. S13. Statechart components of the Cell object that specify pancreatic specification (*Upper*), Delta-Notch interaction (*Lower Left*), and apoptosis (*Lower Right*).



Movie S1. Recorded simulation of the early stages of pancreatic organogenesis.

Movie S1 (MOV)



Movie S2. The emerging lobulated structure of the pancreas.

Movie S2 (MOV)

Other Supporting Information Files

SI Appendix