Appendix S1

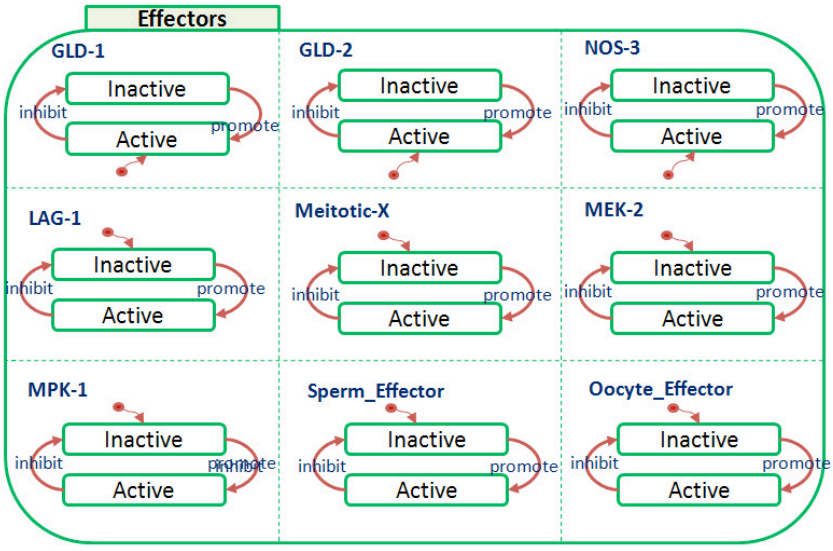
Model Documentation

We provide here a detailed description of the model and its underlying assumptions, including the baseline design and its variants. Source code of the model is available at http://research.microsoft.com/celegans/. In the descriptions below, capitalization of a word indicates its identity as a model feature.

The basic unit in the model is a cell, composed of three elements that specify (1) cell-intrinsic regulation ("Effectors"), (2) cell-extrinsic response mechanisms ("Membrane"), and (3) differentiation and proliferation mechanisms (the "Cell" itself). The Statechart design figures shown here represent the core design, and are thus abstracted and simplified (e.g., text of transition labels is shortened, and certain auxiliary states and transitions are omitted) for presentation purposes.

Designs for cell-intrinsic regulation

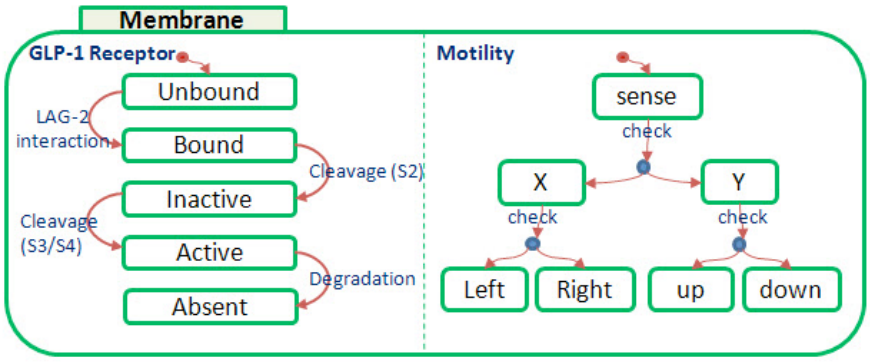
The "Effectors" element specifies cell-intrinsic regulation in a binary fashion. That is, the behavior of each Effector is described by two states, Inactive and Active, with two transitions (Promote and Inhibit) connecting them; all Effector components are orthogonal states and thus run in parallel, so a cell is simultaneously in either the Inactive state or the Active state for each of the Effector components. The initial state (i.e., the state that is chosen as active when the object is initially created) is designated by the default arrow (with the appearance of a stabbed arrow, see Figure, left). The model includes nine effectors: LAG-1, GLD-1, GLD-2, NOS-3, Meiotic-X, MEK-2, MPK-1, Sperm_Effector and Oocyte_Effector. LAG-1 is initially in an Inactive state and its transition (Promote) to the active state occurs in response to GLP-1 activation (in the Membrane element). Similarly, GLP-1 degradation inhibits LAG-1 activity. GLD-1 and GLD-2 components are negatively regulated by LAG-1 activity, that is, are inhibited if LAG-1 becomes active and promoted if LAG-1 becomes Inactive. The Meiotic-X effector is promoted by an intrinsic signal. Here (in the absence of a known



precise control mechanism to guide the transition from Zygotene to Pachytene stages of prophase of meiosis I in the Proliferation component of the Cell) it is triggered by a cell being in a specific position in the gonad, measured in cell diameters (CD) from the distal tip (>25CD from the distal tip in larval stages and >28CD in the adult). Similarly, MEK-2 and MPK-1 are promoted intrinsically in cells that entered early meiotic stages and are positioned in a specific area of the gonad. These cells, depending on the developmental stage, subsequently promote Sperm_Effector or Oocyte_Effector. Accordingly, at the early stages of the adult stage (up until ~ 55 sec) cells promote Sperm_Effector, while at the later stages Oocyte_Effector is promoted. Thus most of these effectors do not figure prominently (i.e., they are triggered based on position only and do not have a significant link in the regulatory network) in our current modeling but are positioned in the model for future modification.

Designs for cell-extrinsic response mechanisms

The Membrane element consists of two components, the GLP-1/Notch receptor component that interacts with extrinsic ligand and a Motility Unit that determines the movements of the cell. We specified the behavior of the Notch receptor



GLP-1 using five different states: the receptor initiates in the Unbound state, which is connected to the Bound state with a LAG-2 Interaction transition. In reality, after the L3 stage there are actually two ligands (LAG-2 and APX-1) in the distal tip cell (DTC) (Henderson et al. 1994; Nadarajan et al., 2009), however, we modeled only one ligand interaction (LAG-2) for simplicity. When a Cell interacts with LAG-2 in the model, the transition is enabled and the receptor moves from the Unbound to the Bound state. The LAG-2 interaction event is triggered when a cell is positioned within a specified ligand interaction distance (e.g., 3CD in the baseline design) from the distal end. The Bound state of the receptor is connected with a Cleavage (S2) transition to the Inactive state, which is connected to the Active state with a Cleavage (S3/S4) transition. Because this version of the model does not include components of known complexes that regulate these transitions, both Cleavage events are intrinsically triggered. Finally, the Active state is connected to the Absent state with a Degradation transition, which is triggered when the cell crosses a specific threshold (in CD from the distal tip, e.g., 11CD in the larva and 16CD in the adult in the baseline design). This design incorporates a one-to-one relationship between receptor activation and degradation, and its influence on cell

fate. Populations of ligand and receptor molecules can be implemented in future studies.

Another aspect of behavior modeled in this component is the “memory” of a cell with respect to LAG-2 activation. When the GLP-1 Receptor component of the Membrane is in the Active state, upon updating the position of the cell due to movement, it reenters the Unbound state. If memory is not implemented (“memory off”), another interaction with LAG-2 in the new position is required to transition to the Bound state and eventually to the Active state via the Cleavage transitions. If memory is implemented (“memory on”), another interaction with LAG-2 in the new position is not required to reenter the Active state, and a transition to the Bound state and eventually to the Active state will be taken spontaneously. This feature reflects the biological concepts of positive feedback on the undifferentiated state, transmission of mother cell fate to daughter cells upon cell division, and control of receptor pathway activity by active cessation of feedback.

For the baseline design and all of the designs for which results are presented in this study, the memory feature is implemented. With memory implemented, after the initial interaction with LAG-2, an individual cell retains the memory of this interaction such that even when the cell moves outside the ligand interaction distance, the receptor will remain active until the cell reaches the critical distance at which the receptor is degraded. This memory will be inherited by the daughter cell upon division. Nevertheless, the current implementation of the model can, in principle, allow a cell to transition to the Early Meiosis state (that is, to differentiate) while reassessing the LAG-2 interaction either upon position update or newly-born daughter cell update. However, due to timing constraints and the fact that this update is a transient state, this is a rare event. If memory were not implemented (“memory off”), the cell would continuously evaluate LAG-2 interaction and would not remain in the Active state once it moved outside the ligand interactions distance, even before reaching the critical distance for receptor degradation. Moreover, if memory is not implemented a daughter cell would need to sense LAG-2 interaction itself in order to prevent differentiation, as the LAG-2 interaction is not inherited from the mother cell. More realistic memory models that incorporate modifications of the “memory on” and “memory off” features can be implemented in future model designs, but are beyond the scope of this study.

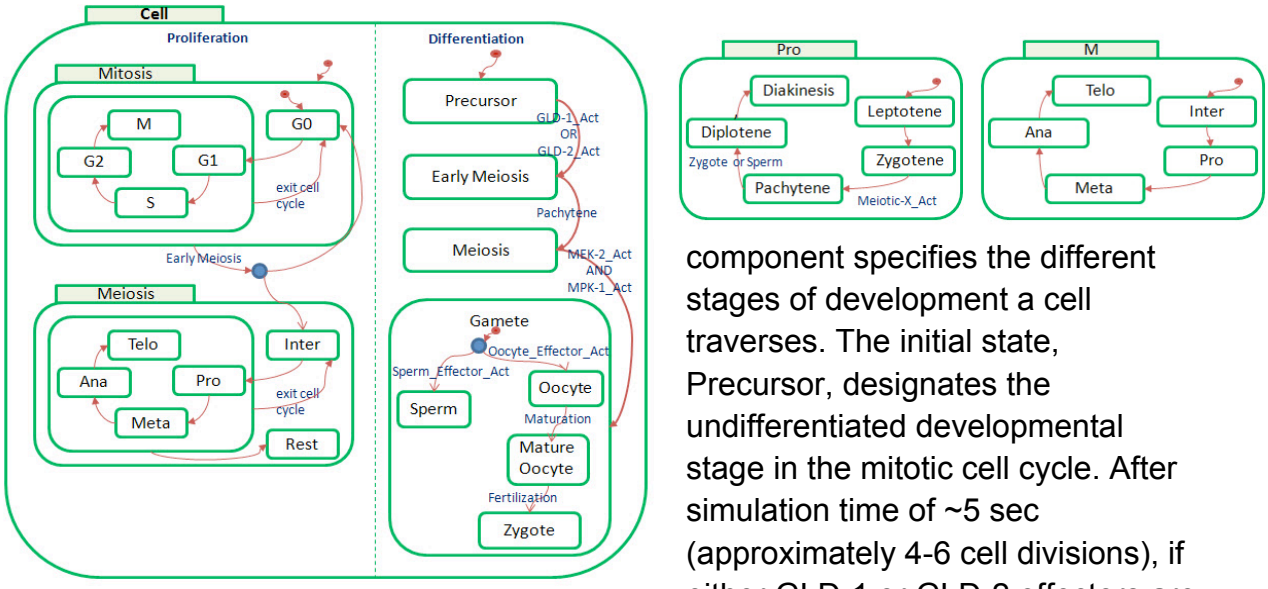
The other component of the membrane, the Motility Unit, continuously seeks possible moves and places the cell in the next possible position based on its developmental stage. The unit checks over the X and Y coordinates and, if applicable, it will decide whether to move Left, Right, Up or Down in the 2D space. In cases where more than one direction of movement is possible, this is a probabilistic decision. The set of rules for the movement decision varies depending on the stage of development: at the early stages of the development the distal gonad migrates centripetally and the germ cells fill the space such that they are associated with the distal-most boundary of the gonad. At the L4-to-adult molt we allowed movement of

cells in a proximal direction whenever possible. That is, while cells are able to move distally, they are instructed to keep the cell population packed and therefore tend to move proximally. This change simulates the forces that push cells proximally by virtue of distal cell proliferation combined with vacancies created by cells maturing proximally.

Motility decisions are influenced by the differentiation stage of the cell, its size, and its position. Physical constraints block the cells from leaving the region of the grid that defines the gonad and from moving on to an occupied position. An exception to this rule is the sperm-oocyte interaction where sperm in the spermatheca do not prevent oocytes from entering the spermatheca.

Designs for differentiation and proliferation

The third element of the design specifies behaviors at the cell level and consists of two major components, Differentiation and Proliferation. The Differentiation



component specifies the different stages of development a cell traverses. The initial state, Precursor, designates the undifferentiated developmental stage in the mitotic cell cycle. After simulation time of ~5 sec (approximately 4-6 cell divisions), if either GLD-1 or GLD-2 effectors are active (i.e., their corresponding component is set to the Active state), the cell enters the Early Meiosis state, which corresponds to the early stages of the meiosis division (i.e., leptotene and zygotene). This will occur upon taking the Degradation transition in the Membrane component which causes LAG-1 to become Inactive and GLD-1 and GLD-2 to become Active. Once a cell enters the Pachytene stage of the meiotic division, the Differentiation component enters the Meiosis (as opposed to “Early Meiosis”) state. Further, when MEK-2 and MPK-1 are active, the cell enters the Gamete state, which nests the states for gametogenesis determination. Based on activity of the effectors, the gamete enters the Sperm state (if Sperm_Effector is Active) or Oocyte state (if Oocyte_Effector is Active). The oocyte state specifies an intrinsic increase in volume. Future work can incorporate known genetic controls for these processes. Cells that fail to complete the maturation process due to physical constraints (i.e., could not extend their volume) or temporal constrains (i.e., did not activate the Oocyte_Effector) undergo programmed cell death and are removed from

the simulation. Cells that mature as oocytes move proximally until interacting with sperm in the spermatheca and are fertilized. Zygotes that move into the area designated for the uterus are subsequently removed from the simulation.

The Proliferation component specifies mutually exclusive aspects of the mitotic cell cycle and differentiation. The Mitotic state nests state G0 (as an initial state), that is sequentially connected to stages of G1, S, G2 and M. The M state itself nests the Interphase, Prophase, Metaphase, Anaphase, and Telophase. Once the cell enters the Telophase state, it duplicates by creating an identical instance (copying the entire Statechart configuration from the mother cell) in an adjacent position. Proliferative cells fill the space over time by executing two orthogonal instructions, move and proliferate. The interplay between the movement of existing cells and the creation of new cells by cell division, maintains the proliferative zone. Movement can occur virtually whenever a free place is available (the movement evaluation frequency is approximately every 1-5 msec), whereas proliferation is bounded by the frequency by which cells evaluate their ability to proliferate (for the baseline design this is every 110 msec in larval stages and every 550 msec in the adult). If a cell attempts to divide but no free place is available, it will try again in the next round of evaluation. When a cell moves, it opens space for an adjacent cell. The adjacent cell can either move to the free place or create a new instance (divide). Thus, reducing the frequency by which cells evaluate their ability to divide results in cells that are more likely to move to occupy free places. Similarly, increasing the frequency by which cells evaluate their ability to proliferate results in cells that are more likely to create a new instance (that is, to divide) to occupy an open space. At the population level, the frequency by which cells evaluate their ability to proliferate influences the time between divisions. Thus, changing this frequency provides a proxy for altering the cell cycle time. Cells evaluate their opportunity to divide every 110 msec in the larval stages. This frequency is reduced by a factor of 5 (to once every 550 msec) when the simulation reaches the L4/Adult, to simulate a slowing of the cell cycle in the adult stage relative to larval stages (Maciejowski et al., 2006; Crittenden et al., 2006; Michaelson et al., 2010). This factor of 5 was determined empirically by identifying values that produce the proper developmental pattern; it represents a qualitative change in cell cycle rate rather than an exact ratio.

At the earliest stages of the simulation, proliferation is signaling-independent (in accordance with findings of Austin and Kimble, 1987), however after the first 4-6 total cell divisions, the ability to proliferate is dependent on GLP-1 receptor activation (i.e., the cell being at the Precursor state in the Differentiation component). Additional GLP-1-independent regulators of the rate of proliferation such as the sheath cell interaction and the insulin signaling pathway (Killian and Hubbard, 2005; Michaelson et al., 2010) are not incorporated into the current model.

Once a cell enters meiotic development, the proliferation compartment switches to the Meiosis state, which nests states for the meiotic stages (Interphase I, Prophase I, Metaphase I, Anaphase I and Telophase I). Prophase I state nests five more states,

Leptotene, Zygotene, Pachytene, Diplotene and Diakinesis. Conditioned by the activity of Meiotic-X effector, a cell moves from Zygotene to Pachytene, which it exits only when it differentiates as Sperm (and undergoes two meiotic divisions), or when it becomes an Oocyte. Subsequent stages such as embryonic development are not implemented in the current version of the model.

Modeling the Extracellular Space

We represent the extracellular space in the simulation using a 2-dimensional grid that overlies the cells positions. The grid contains information regarding the location of the cells as well as cell-extrinsic cues. It defines a bounded area that represents the gonad size at the beginning of its development. The simulation extends the area of the gonad over time in two opposing directions to designate the development of the two arms of the gonad. At the distal end of each gonad arm, we positioned the distal tip cell, which is the source of the LAG-2 ligand. As the simulation advances, the gonad area is gradually extended to form the two symmetric U-shaped arms characteristic of the gonad. As a basis to the dynamic formation of the gonad structure, we extracted 32 snapshots from a previously prepared animation that was based on measurements in live worms [see Stupay and Hubbard at www.wormatlas.org]. The simulation updates the structure every 1.5 seconds. Each grid-pixel is labeled as "invisible", "free" or "occupied". Proliferative and meiotic germ cells and sperm each occupy 1 grid-pixel, early oocytes occupy 2x2 grid pixels, and mature Oocytes and Zygotes each occupy 3x3 pixels. A cell can be positioned (either by proliferation or by movement) only in "free" pixels and occupies the pixels by updating its object ID in the grid. During cell division, when a new cell is born it is given a new ID. During the actual cell division, the mother cell retains its position but is then free to move once the daughter is generated. Similarly, a cell that vacates a grid-pixel erases its object ID from the grid and thus changes the label of the pixel to "free". In the analysis for Figure 4B in the main text, we used this ID system to track mother and daughter cells. Grid pixels that are occupied by sperm are uniquely labeled to designate that oocytes can move to these pixels to trigger fertilization. This implementation restricts the cells into an anatomically bounded area and maintains the relative position of each cell with its neighbors. To reduce the complexity of the calculation of the cell distance from the tip, the value of the distance from the distal tip cell is stored in each grid point. Therefore, cells read their relative position from the grid, rather than using computational resources to calculate this during run time. As the simulation advances and the gonad grows, the positions of the distal tip cells move and the grid updates the relative distance of the pixels.

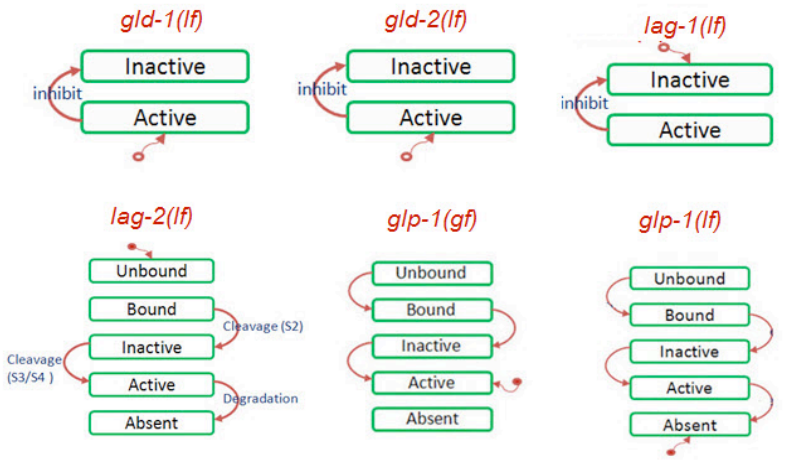
The simulation at run time

When the simulation initiates, four germ cells are packed at the designated area. The cells are in a precursor state and are visualized in the Flash-based animated front-end as yellow circles (Movie S1); at this stage the cell components are set to their initial state. As the gonad grows, the cells proliferate and fill the available space. Proliferation is driven by the Proliferation component such that cells go through the

different stages of the Mitotic part and duplicate as the cell enters Telo state in M phase. Once a new cell is created, a corresponding animated cell appears at the proper position in the interface. Concurrently (after the LAG-2 independent cell divisions are completed), the cells at the distal part of each arm respond to the LAG-2, and the GLP-1 receptor component moves from the Unbound to Bound state. The intrinsic Cleavage (S2) event changes the receptor state to Inactive and a sequential intrinsic Cleavage (S3/S4) event moves the receptor state to Active. As a result, the LAG-1 component (in the Effectors component) sets its state to Active. Consequently, the GLD-1 and GLD-2 components set their state to Inactive. The GLP-1 receptor maintains the Active state (with transient moves back to the Unbound state upon movement, see description of the Membrane component) until degradation occurs (i.e., when the cell crosses the degradation threshold) and then sets the receptor state to Absent. Consequently, LAG1 enters the Inactive state and GLD-1 and GLD-2 move to Active. The Differentiation component of the cell is then set to Early Meiosis and initiates meiosis in the cell. With the entry into meiosis, the animated cell changes color, from yellow to light orange. At the same time, the Proliferation component of the cell switches from Mitosis to Meiosis by way of the Early Meiosis transition. As the run proceeds, a given cell that has entered meiosis will be directed to a more proximal position, whereupon it continues meiotic progression. In this version of the model the underlying biology was simplified such that control of subsequent meiotic progression and gametogenesis events is dependent upon position of the cell relative to the distal tip. Thus, once a cell crosses a pre-defined distance from the distal tip, an intrinsic event promotes the activity of the Meiotic-X effector, which enters the Active state. In turn, The Proliferation component moves from Zygotene to Pachytene (in the PRO I state which is nested in the Meiosis division state of the component) and consequently the Differentiation component moves from the Early Meiosis to the Meiosis state. Consequently, the color of the corresponding animated cell is set to orange. As the run proceeds further, when the cell reaches a specific position more proximally, an intrinsic event will be generated to promote gametogenesis. If the cell reaches this point before the adult stage, the cell triggers a "Promote Sperm_Effector" event, that sets the state of the Sperm_Effector component to Active and the Differentiation component (of the Cell element) sets its state to Sperm. Accordingly, the corresponding animated cell changes color to blue. The Proliferation component then exits the Pachytene state, progresses through two meiotic division cycles, producing four identical sperm. Alternatively, if the cell reaches this point after the adult stage, it triggers the "Promote Oocyte_Effector" event, and the Oocyte_Effector component enters the Active state advancing the Differentiation component to Oocyte state. Once in this state, the corresponding animated cell doubles its size. As the simulation advances, the corresponding figure grows and changes its color to green. Once an oocyte enters the spermatheca, the Differentiation component sets its state to Fertilized and the animated cell changes its shape and color to a grey oval zygote. A zygote that enters the area designated for the uterus is removed from the simulation, and disappears from the animation.

Baseline design modifications

First we tested changes that mimic mutations that elevate or reduce GLP-1/Notch signaling or its downstream components. For example, to simulate elevated activity of the GLP-1/Notch receptor, we set its initial state to be Active (rather than Unbound), and removed the step that permits receptor degradation. Under these conditions, all germ cells in the simulation remain in the proliferative state and fill the entire gonad. The results correspond with known *glp-1(gf)* mutations (e.g., *oz112(gf)*) that develop a tumorous gonad (Berry et al., 1997). To simulate loss of activity of the GLP-1/Notch receptor, we set the initial state of the receptor to Absent. This design resulted in a simulation that produced few germ cells (by virtue of early GLP-1-independent cell divisions; Austin and Kimble, 1987), all of which immediately enter meiotic development upon loss of receptor activity. Similarly, the design to mimic loss of *lag-2* activity prevents the step between the Unbound state and the Bound state of the receptor. We simulated *lag-1(lf)* by eliminating the connection between receptor activity to activation of LAG-1 (that is, the transition from the Inactive state of the LAG1 component to the Active one). Under all these conditions, the simulation produces a population of cells that enter the meiotic stage rapidly, in general agreement with the in-vivo phenotypes observed in *glp-1(lf)*, *lag-2(lf)*, *lag-1(lf)* mutants.



The model incorporated components that are negatively regulated by GLP-1 activity. We tested simulations mimicking loss of these components alone and in compound in-silico mutations. First we ran conditions mimicking the absence of both *gld-1* and *gld-2* under the simplifying assumption that all of the activity of GLP-1 is accounted for by inactivation of these two effectors. We altered the design to mimic the *gld-2 gld-1* double mutant condition by removing the Promote step that moves these factors from the Inactive to the Active state, a step that would normally be taken in response to reduced GLP-1 activity. The simulation produced a tumorous germ line with an extended proliferative zone over the entire gonad, as expected under our initial simplifying assumptions. In this version of the model we did not incorporate meiosis entry via a possible pathway of a "Factor X" parallel to *gld-2* and *gld-1*. Thus the simulations do not entirely recapitulate the phenotype of *gld-2(lf)* and

gld-1(lf) double mutant (Hansen et al., 2004). Nevertheless the simulation displays a tumorous pattern as expected.

To test the model's ability to recapitulate known epistasis relationships between *glp-1*, *lag-1* and *gld-1* and *gld-2*, we altered the designs for each mutation separately. Consistent with known mutations, in-silico epistasis experiments testing conditions that mimic *glp-1(gf)*;*lag-1(lf)* double mutants result in the meiotic all cells entering meiosis (the *lag-1(lf)* phenotype), while design conditions mimicking the combined loss of *glp-1*, *gld-1* and *gld-2* resulted in a tumorous gonad in which the proliferative zone was extended over the entire gonad (the *gld-2 gld-1* phenotype).

Hardware

Model simulations were performed on a 2-core machine with 2.83 GHz Intel Xeon CPU with 8GB of RAM, the generic reactive animation server (Harel and Setty, 2008) was running on a 4-core machine with 3.39 GHz Intel Pentium CPU with 2 GB of RAM.

Software

We linked the Rhapsody model with a real-time front-end that was designed using the ActionScript 3 programming language in Adobe Flash Professional CS3 environment.

Code

A zipped file including the full Rhapsody model is available in file StemCellModel.zip

To run the model, open it in Rhapsody version 7.3 or higher and then select in the Code menu Generate/Make/Run. The main C++ source files generated from this model via Rhapsody are also available for reference in the file code.zip (includes body.h, body.cpp, cell.h, cell.cpp, synchronizer.h, synchronizer.cpp, worm.h, worm.cpp, def.h).

We used MATLAB (version R2008b) to simulate the theoretical scenarios in Fig. 4A and to calculate the average distance of cells from the distal tip and for analyzing data from the Statechart based model. The Matlab code is available in file theoreticalscenarios.m