SUPPLEMENTARY MATERIAL

TABLE OF CONTENTS:

Figures

Movie Legends

ADDITIONAL SEPARATE SUPPLEMENTARY FILES:

Supplementary User Guide

Supplementary Documentation: universal LSCs

Supplementary Documentation: existential LSCs

Movies

kamsuppmovie1.mov
kamsuppmovie2.mov
kamsuppmovie3.mov
kamsuppmovie4.mov
kamsuppmovie5.mov

SUPPLEMENTARY MATERIAL

Overview of VPC fate specification

The *C. elegans* hermaphrodite vulva forms from a set of six ventral epidermal/hypodermal blast cells (Fig. S1A; Horvitz and Sternberg, 1991). These cells are the posterior daughters (.p) of a larger set of P cells. The entire set of these cells is collectively called Pn.p cells, while the subset that have the potential to form vulval tissue are called P3.p, P4.p, P5.p, P6.p, P7.p and P8.p [P(3-8).p, or vulval precursor cells (VPCs)]. Under normal (wild-type) conditions, only P(5-7).p form vulval tissue, while the remaining three VPCs [P(3,4,8).p] acquire a non-vulval fate [known as the tertiary fate (3º)], in which they divide once and fuse with the surrounding hypodermal syncytium (hyp7). A cell in the overlying gonad, the anchor cell (AC), lies immediately dorsal to P6.p and induces P6.p to acquire the central vulval cell fate, known as the primary (1º) fate. The adjacent VPCs, P5.p and P7.p, acquire a secondary (2º) vulval fate. Many genetic pathways participate in patterning VPC fates, including an inductive pathway mediated by a receptor tyrosine kinase (RTK), a lateral specification pathway mediated by LIN- 12/Notch, and a Wnt pathway involved in polarity. Determining all the components of these pathways and their relative roles and interactions remains at the forefront of this field (Sternberg, 2005).

Biological sources of information for the Core Model

The model presented here is a comprehensive representation of virtually all of the information and experiments reported in three seminal papers that helped establish the field of VPC fate specification in *C. elegans* (Sternberg, 1988; Sternberg and Horvitz, 1986; Sternberg and Horvitz, 1989). This is referred to as the complete, or "Core", model. To represent the information contained in the three core papers in the proper context, the model also incorporates additional selected information from other papers, much of which was knowledge that existed at the time. Specifically, behaviors were incorporated from the description of the normal development of the vulva (Sulston and Horvitz, 1977), the initial data demonstrating regulative behavior between the VPCs (Sulston and White, 1980) and between the AC and the VPCs (Kimble, 1981), and the characterization of *lin-12* (Greenwald et al., 1983). These mechanisms include (a) inductive signal production by the anchor cell and gradient formation, (b) position-dependent response of the VPCs to the inductive signal, (c) VPC production of and response to a lateral signal, and (d) a *lin-15*-dependent hypodermal influence on vulval induction. In addition to these mechanisms that directly affect VPC fate specification, we also needed to represent a number of processes that have indirect effects: (e) the anchor cell/ventral uterine precursor cell ("AC/VU") decision that determines the cellular source of the inductive signal; and (f) a non-deterministic choice of P3.p to participate in the vulva equivalence group (Chen and Han, 2001). How these mechanisms are represented in LSCs is summarized in Table S1 and presented in detail in Supplementary Documentation: universal LSCs.

Since the ultimate goal of our effort is to provide a core model from which to develop a comprehensive, contemporary dynamic model, we represented these critical early results and inferred mechanisms in a way that is largely consistent with our current mechanistic understanding of this system. Occasionally, this required us to make use of the post-1989 literature. Thus, for example, we included a mechanism reported in Ambros (1999) that uses a two-step cell-cycle gated response to *lin-*

12 to implement the influence of the lateral signal on vulval cell fates (Ambros, 1999). One major exception is that we did not model the more recent findings of (Cui et al., 2006) regarding the role of SynMuv genes in preventing inappropriate expression of the inductive signal LIN-3 in the hypodermis. The intended expandability of this model will allow the vast repository of additional relevant data to be added to the model (step-by-step instructions for adding new LSCs are provided as part of the User Guide).

Model structure: Objects

A Play-Engine model is a set of objects and behaviors. The objects have properties, such as names and positions, but no behaviors. (The behaviors of the objects are specified by the set of uLSCs; see below.) Objects can be represented graphically in the graphical user interface (GUI), such as the cells in this VPC fate specification model. Alternatively, certain objects, such as genes and locations, are not represented graphically and are accessed via an "Internal Object" form. There are, in addition, a small number of Play-Engine objects, including the clock and a representation of the external user. These objects are described below.

GUI objects

The anatomy is depicted in the GUI (Fig. S1B), which includes objects representing the VPCs, the gonad, two cells (Z1.ppp and Z4.aaa), one of which will become the anchor cell (AC), and the hypodermal syncytium, the site of action of *lin-15* (Herman and Hedgecock, 1990). Some additional objects have been included for future development, including one of the two sex myoblasts (SM), the complete vulval lineage, and a "thermometer" object that can be used to represent experiments performed at different temperatures.

Internal objects

By contrast to the anatomical objects represented in the GUI, genes are represented as internal objects. Fifteen genes are in our model. Six of these are central to vulval fate specification and the behaviors of the core papers (*lin-3*, *lin-12*, *lin-2*, *lin-7*, *lin-10*, and *lin-15*). Two genes (*let-23* and *dig-1*) are not explicitly referred to in the three papers, but are included in the model for their central importance in structuring and analyzing the model. *let-23* encodes the receptor for the LIN-3 EGF-like (epidermal growth factor) inducing signal (Aroian et al., 1990); the papers refer directly to this receptor, whose existence is inferred by the observed behaviors of the system. It is also the substrate that is localized by the LIN-2, -7, -10 complex (Kaech et al., 1998). Although these components were not known to function as a complex at the time of the core papers, these are key genes in the three papers and are modeled in a way that is consistent with building the core of a current model. Mutations in *dig-1* cause the displacement of the gonad (Thomas et al., 1990), which provides a crucial test for vulval fate specification behaviors during model analysis throughout construction. Two additional genes, *lon-1* and *unc-84*, were used in the three papers for special analytical purposes. *lon-1* is represented in the model in its capacity to change the relationship between the positions of the VPCs and the AC. *unc-84* was used as a genetic means to ablate VPCs. The *unc-84* gene and its potential for mutation are included in the model as an internal object, but its behavior is not specified in the model in the form of any LSC. The User Guide contains instructions on how to add an LSC to specify the *unc-84* mutant behavior. Instead, in the current model, in places where the *unc-84* mutation was used to genetically ablate VPCs, the cognate experiments were represented in the model in the same way as other cell ablation experiments

(that are accomplished experimentally using a laser). Finally, five additional genes (*sem-5, let-60, mek-2, mpk-1* and *lin-45*) are included as internal objects. These genes encode components of the signal transduction pathway that acts downstream of the LET-23 EGFR (EGF receptor) as part of the induction pathway. Although these last five genes cannot be manipulated in the current model, they are included for future expansion.

Allele representation

Genetic mutations can have a wide spectrum of effects on gene function. For many of the genes represented in this model, there are a large number of known alleles, each with their specific allele designation. In *C. elegans*, there are often alleles that are used as representative of particular types of perturbations of specific genes; these are referred to as "canonical" alleles. The canonical alleles referred to in the core papers are available in the model as genetic perturbations, as well as the wild-type (unperturbed) allele. These alleles are referred to by their official names, in order to be able to associate specific phenotypic effects with specific alleles. In certain instances, a large set of alleles behaves similarly (for example, the set of null alleles that eliminate gene function). To reduce the complexity of the model, we have included a procedure to map this set of alleles to their common phenotypic consequences (for example, see LSCs "lin15n309" and "lin15_GS" in Supplementary Model Documentation: universal LSCs, pp. 57-58).

Model structure: Behavior

Developmental time and simulation progression

The progression of developmental time underlies the dynamic behavior of this model (Kam et al., 2004). The time period represented spans from hatching (L1 entry) to the completion of the divisions of the Pn.p cells by L4 entry. One uLSC ["Developmental Time 20c(Core Behaviors)"] describes the progression of time between these larval stages (Fig. S2). All other events are linked to this central uLSC either directly [for example, "PnDivide(Core Behaviors)", Fig. S2] or indirectly. The links between uLSCs are the events that are common between the main chart of one uLSC and the precharts of other uLSCs (for example, the event "L1entry" in Fig. S2). Thus, events compelled to occur in the main chart of one uLSC become the link to events specified in the other uLSC by being part of the triggering conditions. Although vulval fate specification occurs within a narrow window of time from the end of the second larval stage (L2) to the mid-L3 stage, earlier events are crucial for laying the groundwork for this process. Thus, for example, the events that determine which Pn.p cells participate in the vulval equivalence group (the set of VPCs) must be represented as well.

The Play-Engine incorporates the progression of time through the use of a clock function that can be referred to in the LSCs. Clock ticks can be set to advance automatically to mimic the normal progression of time. Within the LSCs, these clock ticks can be correlated to developmental time. In our model, with the exception of the first hour of the L3, clock ticks quantize time progression into 1-hour intervals. The first hour of the L3 is a crucial window of time for vulval fate specification, and fate prioritization requires a more refined quantization of time. Therefore, in the first hour of the L3, each clock tick represents 1 minute. A "Developmental Stage" control has been included in the GUI to aid in the visualization of the progression of developmental time (see Fig. S1B).

Generation of simulations from a set of uLSCs ("scenarios")

How does a series of if-then statements or "scenarios" drive a model simulation (a "run" of the model)? Each simulation begins from a specified set of starting conditions. As a simulation progresses, the Play-Engine monitors the Precharts of all participating LSCs. Once a given Prechart's conditions are fulfilled, the Play-Engine generates the events contained in its main chart, which in turn may activate the conditions in Precharts of additional LSCs. This network of inter-related events captured in the charts forms a cascade of events that drives the simulation.

Overview explanation of a sample simulation "run"

A manual play-out simulation is initiated by the user, thereby allowing the user to pre-set the conditions of a simulated experiment prior to running the simulation. To give an example of what happens during a "run" of the system, we describe what occurs during a sample simulation of the ablation of P5.p and P6.p. The ablation of P5.p and P6.p is set up by menu-driven manipulations of the cells as they are represented in the GUI (see Supplementary User Guide). The GUI reflects the ablation of these cells by eliminating them from view. Genetic perturbations can be similarly performed by manipulating the internal object representations of genes. Once the desired starting conditions are set, the user starts the simulation by clicking the START button on the GUI. Time progresses via automatic clock ticks. All actions that are specified to occur within a specific developmental clock-tick time interval are completed before progressing to the next clock tick; that is, the CLOCK waits for the Play-Engine to complete its tasks before advancing the clock. This feature allows for developmentally "simultaneous" or temporally restricted events to occur properly, even if the model simulation requires additional real time to complete the tasks.

Few events relevant to this model occur during the L1 stage. The major events are the birth of the Pn.p cells, visualized by their change from a dotted outline to white-filled ovals, and the birth of Z1.ppp and Z4.aaa, the AC/VU precursors. A module of uLSCs in the AC/VU Use Case specifies the outcome of the AC/VU decision. Since the starting conditions of the particular simulation we are following (ablation of P5.p and P6.p) does not perturb any aspect of this decision, that process proceeds as in the wild type.

During the second larval stage, the Pn.p cells migrate to fill the positions left available due to the absence of P5.p and P6.p. This movement is governed by a set of uLSCs within the Pn.p Movements Use Case. Both posterior and anterior Pn.p's can move towards the center (the AC position), and moving cells leave spaces that can be filled by adjacent cells. The rules regulating movements have not been experimentally established in a systematic way, so the rules necessary for this model have been inferred from experimental ablation data (see below). Consistent with the available data, the simulated movements are governed by non-deterministic rules (i.e., several different outcomes can result from the same initial conditions). For example, P4.p will not always move to the P6.p position when both P5.p and P6.p are ablated, but instead will often acquire a final position somewhere between the normal positions of P4.p and P6.p, discretized into half Pn.p-Pn.p units).

In the L3, the fates of the VPCs are determined by a set of rules contained in a number of Use Cases including, Hyp7 Inhibitory Signal, Lateral Signaling, and VPC Fate Assumption. Cells that are not determined to acquire either 1º or 2º fates acquire a default 3º fate. Rules governing fate assignments are described in further detail below in section "Mechanisms used to implement VPC fate specification."

Indirect influences on VPC Fate Specification that are included in the model in simplified form

Although this model is focused on the fate specification of the VPCs, earlier influences, both direct and indirect, must be represented in the model to allow the affecting conditions to be accurately represented. Since the indirect influences are only represented so as to provide input into VPC fate specification, they are represented in a simplified manner. Indirect influences include a simplified representation of the AC/VU decision in the gonad (AC/VU) and the fusion potential of P3.p (Pn.p Fusion). The AC plays an important role in *C. elegans* vulval fate specification, making its representation crucial to depict in this model. While much is known about AC development and function, only those aspects of its behavior that are relevant to vulval fate specification are included here (see Sadot et al., 2008, for a model of AC fate acquisition). Thus, the presence or absence of an AC, the secretion of the LIN-3 inducing signal, and the relative positioning of the gonad/AC with respect to the VPCs are all represented in this model as early influences on vulval fate specification. In addition to directly influencing VPC fate specification via lateral signaling, *lin-12* also controls the AC/VU decision, serving to showcase the ability of this methodology to aid in integrating distributed behaviors. The birth of both the VPCs [the division of P(3-8)] and the AC/VU precursors is also represented to aid the accurate representation of behavior via the GUI.

Integration of Events

A severe limitation of using abstract reasoning alone to evaluate an increasingly complex understanding of biology derives from the inability to keep track of a large number of concurrent events. One of the strengths of this modeling methodology is its ability to integrate concurrent events using a rigorous, formal methodology. Computational models can thereby assist the abstract reasoning that biologists use.

Integration of events first must take into account events that depend on previous events or conditions. Some processes in uLSCs are linked by the time-sequence of events, and the Play-Engine naturally integrates these processes. For example, the ability of P3.p to fuse with the hypodermal syncytium occurs *prior to* the subsequent events concerning VPC fate acquisition, thereby influencing the outcome. Even in the wild type, in the L2 stage P3.p displays variability with respect to its decision to fuse with the hypodermal syncytium or remain unfused and be part of the vulval equivalence group (Sulston and Horvitz, 1977); subsequent events will only influence the fate of P3.p if it remains unfused in the L2. Many mutations that affect vulval fate specification also influence the propensity of P3.p fusion (Chen and Han, 2001), and this can be integrated into the model as well, as has been done for *lin-15(n309)* (see the uLSCs in the Use Case entitled “Pn.p Fusion” in Supplementary Documentation: universal LSCs). Nonetheless, because of the temporally distinct nature of these two processes, the Play-Engine naturally integrates these variable behaviors. This model includes a number of similar sub-processes that influence vulval fate specification (e.g., gonad location, AC/VU decision, Pn.p fusion, Pn.p movement). The ability of the Play-Engine to keep track of the ramifications of the events that

occur in each of these sub-processes gave rise to some of the biological insights obtained during the building and analysis of the model.

Other behaviors are controlled by more interdependent events, for example genetic states. To constrain behavior and help integrate the processes affected by various genetic states, the current model uses Forbidden Elements and conditional statements (Harel and Marelly, 2003). Conditional statements can be used to restrict behaviors to the proper set of specific conditions; these are most appropriately used when the restrictions can be assigned to specific events that occur within an LSC. For example, the uLSC "VPCresponse100LIN3 (VPC Fate Assumption)" integrates the response of a VPC to a high level (level = 100) of LIN-3 with the genotype of the genes of the LET-23 signaling complex. Since the response to LIN-3 is dependent upon the activity of the receptor localization complex (LIN-2, LIN-7, LIN-10), the conditional statement allows a full 1º response only in cases where the complex is genetically intact.

To represent genetic perturbations that alter behavior, the governing uLSCs must not only trigger the new mutant behavior, but must also disable the affected portion of wild-type behavior. Disabling wild-type behaviors under specific conditions is done effectively via the use of "Forbidden Elements." Forbidden Elements can obviate an entire uLSC and all of its compelled behaviors or prevent an event from occurring (see Harel and Marelly, 2003; for examples, see Supplementary Documentation: uLSCs).

The use of conditional statements and Forbidden Elements is reasonable for this model in which there are a small number of genes, gene activity levels (alleles which are essentially "ON" or "OFF"), and interacting pathways. As the system becomes increasingly complex, however, these elements will become increasingly cumbersome to list and keep track of. We are currently developing alternative methods to integrate genetic interactions for the next phase of this modeling effort.

Mechanisms used to implement VPC fate specification

The core papers represented in this model define two major pathways that influence VPC fate specification: an inductive signaling pathway and a lateral signaling pathway. LIN-3 is the inductive signal, and the lateral signaling pathway is mediated by the Notch-like LIN-12 receptor (Sternberg, 2005). In the wild type, the AC is the major source of LIN-3, which triggers the 1º and 2º vulval fates. Our model includes a *Graded Signaling* mechanism by which high levels of LIN-3 stimulate 1º fate specification while intermediate levels of LIN-3 stimulate 2º fate specification. Our model also includes LIN-12-mediated lateral signaling between VPCs as part of a *Sequential Signaling* mechanism. Lateral signaling provides a mechanism by which VPCs specified as 1º can promote 2º fate specification in their neighbors. Thus, during the normal course of vulval induction, there is a sequential signaling process by which a VPC can acquire a 2º fate specification: the initial LIN-3 signal induces a VPC to be specified as 1º, and the 1º cell subsequently specifies a 2º fate in its neighbors by a lateral signaling mechanism. The combined action of these mechanisms provides two ways for a VPC to acquire a 2º fate: (1) a medium level of LIN-3 inductive signal; and (2) a lateral signal from a 1º neighbor. Further details on the implementation of inductive signaling, the influence of the lateral signal, and on fate acquisition in the *lin-15(0)* background are described below.

VPC lineage representation

Though VPC fate specification can be represented at many different levels of description, for the majority of the experiments reported in the core papers, Pn.p fates are assigned based on the characteristics of the lineage pattern of each Pn.p cell (see Sternberg and Horvitz, 1986 and Katz et al., 1995 for the rules used in our model). The model assigns fates within these categories, but does not associate them with specific lineages (note that the GUI is set up to be able to reflect the actual lineages in the future). Since no rules are known that govern the production of hybrid (mixed vulval/non-vulval) and intermediate (mixed 1º /2º) fates, these categories are not generated in the current version of this model. However, for purposes of completeness in model testing, eLSCs that represent hybrid and intermediate lineages were included (see Testing below).

Implementation of inductive signaling:

The *Graded Signaling* hypothesis postulates that there is a gradient of LIN-3 whose peak is at the source of its production in the AC(s), and that fate acquisition by the VPCs is position-dependent. To implement the effects of the LIN-3 inductive signal on VPC fate, it was necessary to allow VPCs that had altered their position to encounter an amount of the LIN-3 inductive signal that was appropriate for their new position. To implement this, we created a set of discrete locations to "receive" a level of LIN-3 that was appropriate for their position relative to the AC(s). The VPCs would then read the level of LIN-3 from their final location in order to respond appropriately.

Implementation of the influence of lateral signaling:

To represent the interactions between the LIN-12 and the inductive signaling pathways, we included mechanisms in the model inferred from work reported in Ambros (1999). This work suggested that there is a cell-cycle gating of two responses to LIN-12 signaling, both of which influence the acquisition of the 2º fate (Ambros, 1999). Thus, we similarly separated the acquisition of the 2º fate into two steps. In the first step, prior to S-phase, a "non-1º versus 1º" decision occurs. This decision is first influenced by a high level of LIN-3 (which promotes the 1º fate and causes the cell to send a lateral signal to its neighbors that will be received by LIN-12). In our model, any cell that has not adopted a 1º fate and has received a lateral signal is temporarily designated "non-1º." In the second step, after S-phase, each "non-1º" VPC undergoes the "2º versus 3º" decision (adopting the 2º fate in response to persistent reception of the lateral signal). In the absence of these inductive and lateral signals, a cell retains an "undifferentiated" designation until 5 hours after the end of S phase, in which case the 3º fate is triggered.

Implementation of fate acquisition in lin-15(0)

Recent results have demonstrated that the *lin-15*/SynMuv effect reflects ectopic LIN-3 expression in the hypodermis (Cui et al., 2006). Thus, the *lin-15*-mediated "inhibitory signal" is more accurately described as a *lin-15*-mediated inhibition of a plentiful, dispersed activating signal (LIN-3). This more accurate representation will be incorporated into a subsequent version of the model. Nevertheless, the effect on VPC fate specification is equivalent as currently modeled.

In *lin-15(0)* mutants, the VPCs adopt an alternating pattern of 1º and 2º fates due to LIN-12-mediated lateral signaling. When VPCs are isolated in this mutant background, however, essentially all acquire a 1º fate (Sternberg and Horvitz, 1986). Thus, any computational model must address the problem of avoiding a situation in which all VPCs decide independently and simultaneously to become

1°, and allowing the opportunity for 1º VPCs to prevent their neighbors from becoming 1º via LIN-12 signaling. In Fisher et al. (2005), a model that was limited to the data in SH89, the problem was solved under the assumption that fate acquisition is a point event, and a "mutual exclusion" mechanism was employed (Fisher et al., 2005). In the Fisher et al. (2007) model a "scheduler" allows different temporal ordering of interaction between the VPCs, which results in simulations with different outcomes (Fisher et al., 2007).

Here, we provide a somewhat different prioritization mechanism for the 1º vs. 2º fate decision. This prioritization mechanism has two components: (1) an absolute prioritization to AC-dependent inductive signaling; and (2) a stochastic AC-independent prioritization. The combination of these prioritization mechanisms guarantees that some VPCs in a *lin-15(0)* mutant stochastically execute the acquisition of 1º fate specification faster than others, thereby preventing their neighbors from acquiring a 1º fate. The former mechanism is utilized in a *lin-15(0)* background in the presence of an AC. In this case, P6.p "experiences" a high level of LIN-3 and is temporally prioritized to adopt the 1º fate first. A natural consequence of this mechanism is that P5.p and P7.p will always adopt 2º fates (provided that *lin-12* is intact). The latter stochastic mechanism is utilized in all other cases, including P6.p when the AC is not present. To implement the decision process in these cases, in a *lin-15(0)* background each VPC that does not immediately experience a high level of LIN-3 at the start of the L3 chooses a random number between 1-60, representing the time in minutes of the first hour of the L3. When the minute arrives that the cell has chosen, if the VPC has not received a lateral signal from its neighbor, it can adopt a 1º fate. In the relatively unlikely occurrence that two neighboring cells choose the same exact time (that is, they choose the same integer between 1-60), they both can adopt a 1º fate, producing adjacent 1º VPCs. In fact, this mechanism reproduces experimental evidence of rare instances of adjacent 1º VPCs. This "mechanism," which is clearly artificial, is compelled by a lack of understanding regarding the precise mechanism by which the time of fate specification is chosen in a *lin-15(0)* background. However, it nonetheless reflects the likely scenario that stochastic differences among the VPCs influence which ones among them will acquire a prevailing 1º fate. The modeling of this event as a process is further supported by the findings of Yoo *et al.* (2004), in which P5.p and P7.p express a 1º fate marker initially and then respond to the lateral signal from P6.p (Yoo et al., 2004). Our preliminary results also support a similar situation in *lin-15(0)* animals (Fig. S3), as do the results of Fisher *et al.* (2007). These data also indicate that the *egl-17::GFP* marker for the 1º fate is strongly expressed earlier in the *lin-15* mutant than in the wild type (Fig. S3).

While isolated VPCs in *lin-15(n309)* animals essentially all acquire a 1º fate (Sternberg and Horvitz, 1986), some VPCs have been observed to acquire a 2º fate via a non-standard mechanism, that is, in a way that can not be easily explained by the cell-cell interactions described above (lateral signaling from a neighboring 1º cell or intermediate distance from AC). For example, in Table 1E, line 3 of S88, P3.p is neither adjacent to a 1º cell (and therefore is not responding to a 1º-dependent lateral signal) nor close to the AC (and therefore responding to an intermediate level of the inductive signal; in fact, in this case, the AC is absent). To enable our model to represent these results, we included a "third" unspecified mechanism by which a VPC can acquire a 2º fate: we tempered the intrinsic VPC response to a *lin-15(n309)* background from always acquiring a 1º default fate to one in which they will acquire a 2º fate by default at a frequency of 10% (described in the uLSC "Lin15_GS" (Hyp7 Inhibitory Signal)). These results suggest that there is an as-yet unaccounted-for mechanism that affects VPC cell fate acquisition in *lin-15* mutants. Based on the results of Cui et al. (2006) demonstrating that *lin-3* is ectopically expressed in the hypodermis of similar *lin-15* mutants, one possibility is that the LIN-3

signal from the hypodermis is not efficiently received by VPCs such that some VPCs experience an intermediate level of the LIN-3 signal. Alternatively the physical distribution of LIN-3 from the hypodermis may be patchy. Additional experiments will be necessary to distinguish between these (and other) possibilities.

We also found that given a very large number of simulations, this "10%" mechanism, together with the "graded" mechanism but without the lateral signaling mechanism, can, in fact, reproduce all of the patterns of fates reported in the core papers for *lin-15* animals. However, our testing shows that although all the experimentally observed VPC fate patterns can occur, they do not occur with the correct (experimentally observed) probabilities (see below). This observation suggests that rigorous testing of models of biological phenomena can help distinguish between plausible and implausible mechanisms by comparing the probability distribution of experimental results with those of multiple simulations.

Testing

Testing a model's mechanisms against the experimental results

Manual play-out allows a user to test whether individual simulations match the expected behavior of the system. While this is important for model development, the model must also be tested more thoroughly to ensure that the model's mechanisms will produce outcomes that match the experimental results that were used originally to infer those mechanisms. This requires a large number of runs. Ideally, the entire set of experimental starting conditions must be simulated multiple times, and the simulated results then checked to see if they reproduce the observed biological results. The Play Engine makes this feasible if the experimental conditions and their paired results are represented as existential LSCs (eLSCs).

As one of its normal functions, the Play-Engine monitors the progress and state-of-completion of all active LSCs during a run. By monitoring the state-of-completion of the eLSC representations of experimental results, the Play Engine automatically checks whether a simulation matches a specific biological result. We used this function to test the *Core* model on all of the experimental results reported in the three papers, SH86, S88 and SH89.

Even for a model that represents a small number of papers, this is a large task that involves running simulations describing each independent experimental result that was reported (over 250 total results). Furthermore, the non-determinism of experimental biology and its representation in the model requires multiple iterations for each independent experimental set-up (over 450 total runs). To help this testing procedure, a number of features of the Play-Engine were developed to facilitate automated simulations and their analyses. Jump starts provide the automated setting of the initial simulation conditions. Batch-Run play-out automates the running of multiple simulations. Various aspects of the results of these simulations were automatically recorded in a number of different formats. These automated features (described in detail below) allowed systematic test runs conducted according to a comprehensive test plan, to test the match between the outcome of runs and the results of each of the reported experiments in the core papers. Initially, these test runs were performed to identify bugs in the model; later they were used to systematically test the model as indicated below. Based on these runs, we have shown that our model can reproduce essentially all of the results observed for each experiment that was conducted in the core papers.

In general, model testing can detect three broad categories of inconsistencies between a model and the data: bugs (the model does not do what we wanted it to do), "acceptable failures" (model does not completely satisfy observations due to some approximations we made, or any other limitation we are willing to accept as a reasonable level of abstraction), and "interesting failures", which may lead to new insights. Our iterative model development and testing aims to filter out the first category, assure that we are aware of the second, and draw our attention to the third. The inconsistencies in the third category are of particular interest, since they point out areas where mechanistic hypotheses are not consistent with the experimental data.

Our tests showed that most but not all experimental results were reproduced by the *Core* model. Results that were not reproduced included experiments in which Pn.p daughter cells were ablated (our model does not yet extend to the divisions of the Pn.p cells and so these data were not tested), and the twenty-three independent results listed in Supplementary Table 2A (below). These results are largely from experiments in which partial induction patterns were observed in some of the *vul* mutants (*lin-2, -3, -7, -10*). The inability of the model to reproduce these results derives from several sources. First, the current model does not include the rules to generate hybrid or intermediate fates, since these are still not understood. Second, in the model, the inductive pathway mutants block the pathway completely, whereas in reality they block partially, a caveat that was appreciated by the field at the time. Third, experimental results are sometimes summarized in ways that are ambiguous or that do not strictly agree with observations (though they are consistent with the preponderance of the data they summarize). And, fourth, very rare results, such as a 2º fate in a *lin-12(0)* mutant, remain unexplained (Supplementary Table 2). The ability to detect inconsistencies between a model and the data it is based on is crucial to the model's usefulness, and is a key feature of our modeling methodology.

Testing experimental results against competing mechanistic hypotheses

Biologists are often faced with more than one mechanistic hypothesis that appears compatible with the available experimental data. The next task is to identify which specific results are compatible with one or more hypotheses but not others. The structure of the Play-Engine easily accommodates multiple mechanistic hypotheses without the need to create completely separate models. This is accomplished within the settings of the Execution Configuration within the Play-Engine (Tools/Execution Configuration). A complete set of uLSCs that encompasses all of the postulated mechanisms for the various hypotheses can be created and stored within a Play-Engine project. Our complete "Core" model contains both "Graded" and "Sequential" signaling mechanisms. Using the Execution Configuration form, the user can choose which uLSCs will participate in driving simulated model behavior and which are left out (see the User Guide). Thus, each alternative mechanistic hypothesis can be represented by a subset of uLSCs within the model project, stored as a specific execution configuration. Simulated runs can be conducted using the specific set of hypothesis-specific uLSCs specified by the execution configuration, thereby testing that specific hypothesis. The behaviors of different mechanisms can easily be compared by simply changing to a different execution configuration and conducting similar tests. Batch-Run allows for choosing an execution configuration for each of the twelve simulation types specified in the Batch-Run form.

We used the same large set of experimental results to test a famous example of two competing mechanistic hypotheses that concern VPC fate specification: the *"Graded"* versus the *"Sequential"*

signaling hypotheses (Sternberg and Horvitz, 1986). These hypotheses account for 2º fate specification by different mechanisms. The *Graded* signaling hypothesis postulates that a VPC that directly receives an intermediate level of LIN-3 signaling will adopt a 2º fate, while the *Sequential* hypothesis postulates that 2º fates are induced by lateral signaling from an adjacent VPC that receives a high level of LIN-3 and adopts a 1º fate (Fig. S1B). These hypotheses have been a subject of controversy ever since the publication of the papers covered in our model (see Katz et al., 1995; Kenyon, 1995; Simske and Kim, 1995; Sundaram, 2004). Both mechanisms are operational in our *Core* model, each being represented by a small number of uLSCs.

The *Sequential Signaling* model was created from the *Core Model* by removing the two uLSCs, "VPCresponse50LIN3" and "VPCresponse50LIN3B" (found in the Use Case "VPC Fate Assumption") from the set of participating uLSCs. This alteration eliminates the mechanism by which intermediate levels of LIN-3 (Location.LIN-3exp = 50) can specify a 2º fate. All other participating uLSCs from the *Core* model remain as such. This change is illustrated in Fig. S4.

The *Graded* signaling model was created from the *Core* model by removing two different uLSCs, "VPCEarlyReceiveLIN12" and "Sphase_receiveLIS" (also found in the Use Case "VPC Fate Assumption") from the set of participating uLSCs. This eliminates the LIN-12-mediated lateral signal influence on VPC fate specification. All other active uLSCs from the *Core* model were left active. Thus, mutations in *lin-12* still affect VPC fate specification (for example, via direct genetic effects through condition statements and forbidden elements), but not via a lateral signaling mechanism triggered from a 1º VPC.

We tested whether our modeling approach could "find" the experiments that were inconsistent with each hypothesis by altering the settings in a feature of the Play-Engine called the *Execution Configuration* and then repeating the testing (over 900 additional runs). The *Execution Configuration* allows one to define the set of uLSCs that will be active in defining the behavior of the model. Leaving all other uLSCs from the *Core* model active, the *Sequential* model was created by inactivating the two uLSCs that enable the mechanism by which intermediate levels of LIN-3 can specify a 2º fate. The *Graded* model was similarly created from the *Core* model by removing two different uLSCs that enable the LIN-12-mediated lateral influence on VPC fate specification. Systematic testing of each model independently revealed a set of experimental results that were not reproduced according to the testing procedure that had been successful for the *Core* model. The un-reproduced results under the *Sequential* model correspond to cases in which VPCs adopt 2º fates in the absence of adjacent 1º VPCs (7 independent results; Supplementary Table 2B; see also Supplementary Movies 3 and 4). Testing under the *Graded* model showed that non-wild-type alternating 1º/2º fate patterns are difficult to reproduce (21 independent results; Supplementary Table 2C). One example is the result described in Table 2 C, line 1 of SH89. The pattern of fates for this *lin-15(n309)* mutant is 1º/2º/2º/1º/2º/1º from P3.p to P8.p, respectively. The graded signaling mechanism alone cannot generate a 2º fate for P4.p, since it is too far from the anchor cell, and 2º cell fates in *lin-15(n309)* animals derive predominantly from lateral signaling (Sternberg, 1988). Data collected since the publication of these papers suggest that both mechanisms contribute to the pattern, as modeled in the *Core*. Nonetheless, these systematic tests demonstrate the potential of this methodology for testing competing hypotheses against an established set of experimental results, and for supplying lists of results that are/are not reproduced under similar testing protocols running under the alternative hypotheses.

<u>*Unsuccessful runs*</u>

A chart is "completed" once the events of its main chart are successfully executed. If one chart logically conflicts with another chart elsewhere in the set of participating LSCs, the user is alerted (either directly during a simulation or by virtue of the fact that the chart remains uncompleted at the end of a run). Many charts can be active at one time, in various states of completion, conceptually paralleling the concurrent dynamics of developmental processes in biology.

Jump Starts

Each Jump Start sets the initial parameters of the model to match specific experimental conditions. Thus, the Jump Start "Init" initializes a wild-type starting condition, while the "lin-15 Gonad ablated" Jump Start initializes a simulation in a *lin-15(n309)* genetic background in which the gonad has been ablated. Jump Starts can also be used with manual play-out to set up new simulation conditions.

Batch-run mode

Batch-Run Play-out mode allows up to twelve simulation types to run automatically for any specified number of iterations. Thus, the user can specify Batch-Run to run two iterations of the wild type starting condition, ten iterations of simulations of an experiment in which the gonad has been ablated in *lin-15(n309)* animals, and similarly for a total of twelve different starting conditions. Since VPC fate specification is complete by the end of L3, the automated simulations are terminated upon execution of the L4entry method (actually, the "Gather Data(Reporter)" uLSC, whose sole event in the prechart is L4 entry). A more detailed description of Batch-Run and its advanced features are described in (Kugler et al., 2007).

Batch-Run data collection

Various records are kept to document Batch-Run simulations. First, a run log is created for each batch-run. This run log documents the list of uLSCs used to drive behavior in the particular simulation run, violations of any LSCs, and the successful eLSC traces. Second, the values of key object properties are recorded in an Excel spreadsheet (a feature of the "GatherData (Reporter)" uLSC). These properties include the final fates and positions of Z1.ppp, Z4.aaa, and all of the VPCs. Finally, a full set of the events for each batch-run is recorded. This batch-run record can be re-run using the Play-Engine, allowing the run to be reviewed. For each run, these analysis documents provide a record of the run conditions, a summary of the end results, a list of all mechanistic rules that were not followed properly during the run, a list of the experiments that were matched by the run, and a recording to allow a detailed dynamic follow-up analysis.

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Table S1. List of uLSCs organized by Use Case.

Use Case LSC Name	Abbreviated Statement of Purpose	Page # (in Supplementary Documentation: uLSCs)
Core Behaviors		
DevelopmentalTime20c	Timing of the larval stages in the context of model time progression or ticks	2
PnDivide	Ordering and timing of the division of the Pn cells	3
VPCborn	Birth of P3.p - P8.p	3
PnpAssumeFate	Time constraints for VPC fate assumption, especially relative to S-phase	4
ACVU		
ACVUborn	Z1.ppp/Z4.aaa birth	5
ACAdoptFate	Setting of the AC/VU fate	6
WT_AC/VU	Timing and triggering of the AC/VU decision in WT	7
AC/VUdecision	Nondeterministic AC/VU fate decision in WT	8
lin12(d)_AC/VU	Timing and outcome of the AC/VU decision in *lin-12(d)* homozygotes	9
lin12(d/0)_AC/VU	Timing and outcome of the AC/VU decision in *lin-12(d/0)* worms	10
lin-12_AC	Timing and outcome of the AC/VU decision in *lin-12(0)* homozygotes, including AC positioning	11
AC_WTposition	AC anterio-posterior position in WT	12
Gonad2AC	AC/VU anterio-posterior position resulting from Gonad movement	13
GonadAblation	AC/VU ablation upon Gonad ablation	13
GonadAblation2	Triggering of AC/VU ablation upon interactive Gonad ablation	14
dig-1	Effect of *dig-1* mutation on Gonad position	15
lon-1	Effect of *lon-1* mutation on Gonad position	16
Xloc2boxes	AC/VUs' anterio-posterior positions relative to the Locations	17
ACFormedlocUpdate	Triggering of adjustment of VPC's record of its distance from AC upon AC movement	18
UpdateAC_location	Adjustment of VPC's record of its distance from AC upon AC movement	19
LIN-3		
LIN-3expression	Timing of AC-derived LIN-3 expression, given WT *lin-3*	20
LIN-3InBoxes	AC depositing of LIN-3 into proximal Locations	21
Lin-3 50 Diffusion Anterior	Anterior diffusion from a Location with 100 units (a high level) of LIN-3	22
Lin-3 50 Diffusion Posterior	Posterior diffusion from a Location with 100 units (a high level) of LIN-3	22
Lin-3 20 Diffusion Anterior	Anterior diffusion from a Location with 50 units (a medium level) of LIN-3	23
Lin-3 20 Diffusion Posterior	Posterior diffusion from a Location with 50 units (a medium level) of LIN-3	23
Medium2100	Summing of two doses of 50 units (a medium level) of LIN-3 in a Location, resulting in 100 units (a high level) of LIN-3	24
ClearLIN-3	Immediate clearing of LIN-3 levels in all Locations, upon ablation of both Z1.ppp and Z4.aaa	24
ClearLIN-3(b)	Immediate clearing of LIN-3 levels in all Locations, upon ablation of the one and only AC	25
Boxes2VPCs(@StartSpec)	Transfer of LIN-3 in Locations to the resident VPCs	25
Pn.p Movements		
ClearAblatedLocation	If a VPC is interactively ablated, its former Location updates its record of its resident cell, and the cell clears its own record of its neighbors	27
BatchClearAblatedLocation	If a VPC is ablated, its former Location updates its record of its resident cell, and the cell clears its own record of its neighbors	28
Box2MovingVPC	Transfer of LIN-3 from a Location to its new resident VPC	28
ClearNeighbors	If a cell is ablated, its neighbors update their own records of their neighbors	29
FreedUpdatesHalfs	Triggering of the updating of the Locations previously containing fragments of a now ablated or moving cell	30

Table S2A. Results not reproduced under the *Core* model

			VPC fate pattern[a]							
Paper	Table	Line(s)	P3.p	P4.p	P5.p	P6.p	P7.p	P8.p	Gonad	Genotype
SH89	1C	3	N	H	1º	1º	2º	3º	+ +	*lin-12(0)*
SH89	2A a	3,4	3º	3º	3º	1º	3º	3º	+	*lin-3*
SH89	2A a	5	3º	3º	H	1º	3º	3º	+	*lin-3*
SH89	2A a	6	3º	3º	3º	1º	H	3º	+	*lin-3*
SH89	2A b	2	3º	3º	3º	3º	1º	3º	+	*lin-7*
SH89	2A b	3	3º	3º	H	1º	3º	3º	+	*lin-7*
SH89	2A b	4	3º	3º	2º	3º	H	3º	+	*lin-7*
SH89	2A b	5	3º	3º	H	1º	H	3º	+	*lin-7*
SH89	2A b	6	3º	3º	2º	1º	H	3º	+	*lin-7*
SH89	2A c	6	N	3º	3º	H	2º	3º	+	*lin-2*
SH89	2A c	7	3º	3º	2º	1º	2º	3º	+	*lin-2*
SH89	3E a	1	N	3º	H	1º	3º	3º	+ + +	*lin-12(0); lin-3*
SH89	3E a	2	N	3º	3º	1º	3º	3º	+ + +	*lin-12(0); lin-3*
SH89	3E b	4,5	3º	3º	3º	1º	3º	3º	+ +	*lin-7; lin-12(0)*
SH89	3E c	1	N	H	1º	3º	3º	3º	+ +	*lin-10; lin-12(0)*
SH89	3E c	4	N	3º	3º	1º	3º	3º	+ +	*lin-10; lin-12(0)*
SH89	3E d	1	N	H	1º	1º	2º	3º	+ +	*lin-12(0); lin-2*
SH89	3E d	3	N	1º	1º	1º	H	3º	+ +	*lin-12(0); lin-2*
SH89	3E d	4	N	H	1º	1º	3º	3º	+ +	*lin-12(0); lin-2*
SH89	3E d	5	N	3º	H	1º	H	3º	+ +	*lin-12(0); lin-2*
SH89	3E d	6	3º	3º	1º	1º	3º	3º	+ +	*lin-12(0); lin-2*
S86	3C	5 (inferred)	2º	2º	X	X	X	1º	+	wild type
S86	3C	7 (inferred)	2º	2º	X	X	X	2º	+	wild type

[a]Pattern legend: N, non-VPC; X, ablated cell; H, Hybrid fate. SH86 T1, lines 15-23 are not within the scope of this version of the model, since they include ablations of daughters of Pn.pcells. The current version of the model does not generate Hybrid (vulval/non-vulval combination) or Intermediate (1º/2º combination) fates. “Inferred” refers to specific outcomes predicted by summary information but not represented in the paper by actual experimental results. We modeled all combinations of inferred results in these cases.

Table S2B. Results not reproduced under *Sequential* signaling model

			VPC fate pattern[a]					
Paper	Table	Line(s)	P3.p	P4.p	P5.p	P6.p	P7.p	P8.p
SH86	1	5,6,8	3º	3º	2º	X	2º	3º
SH86	1	7	N	3º	2º	X	2º	3º
SH86	1	14	N	3º	X	X	2º	H
SH86	4	3	2º	X	X	X	X	X
SH86	4	5	X	2º	X	X	X	X
SH86	4	15	X	X	X	X	X	2º
SH86	3C	3(inferred)	3º	2º	X	X	X	2º

All experiments were performed in a wild-type background. [a]Pattern legend: N, non-VPC; X, ablated cell; H, Hybrid fate.

Table S2C. Results not reproduced under *Graded* signaling model

			VPC fate pattern[a]							
Paper	Table	Line(s)	P3.p	P4.p	P5.p	P6.p	P7.p	P8.p	Gonad	Genotype
SH89	2C	1	1º	2º	2º	1º	2º	1º	+	*lin-15*
S88	1D	1,2	2º	1º	2º	1º	2º	1º	+	*lin-15*
S88	1D	3,4	1º	2º	2º	1º	2º	1º	+	*lin-15*
S88	1J	1-10[b]	X	X	X	X	1º	2º	+	*lin-15*
SH89	3H	1	2º	1º	2º	1º	2º	1º	+	*lin-12(d/0); lin-15*
SH89	3H	2	2º	2º	2º	1º	2º	1º	+	*lin-12(d/0); lin-15*
S86	3C	6 (inferred)	2º	1º	X	X	X	1º	+	wild type
S88	1E	3	2º	2º	1º	2º	2º	1º	-	*lin-15*
S88	1E	4,5	2º	1º	2º	1º	2º	1º	-	*lin-15*
S88	1E	6	1º	1º	2º	1º	2º	1º	-	*lin-15*
S88	1E	7	1º	2º	2º	1º	2º	1º	-	*lin-15*
S88	1E	8	1º	2º	1º	2º	1º	2º	-	*lin-15*
SH89	2D	3	1º	2º	2º	1º	2º	1º	-	*lin-15*
SH89	2D	4	2º	1º	2º	1º	2º	1º	-	*lin-15*
S88	1I	7	2º	X	X	X	1º	X	-	*lin-15*
SH89	2D	1	1º	2º	2º	1º	2º	X	-	*lin-15*
SH89	3F	3	2º	2º	2º	1º	2º	1º	-	*lin-12(d); lin-15*
SH89	3F	4	2º	2º	2º	I	1º	2º	-	*lin-12(d); lin-15*
SH89	3F	5	1º	2º	2º	1º	2º	1º	-	*lin-12(d); lin-15*
SH89	3G	1	2º	1º	2º	1º	2º	1º	-	*lin-12(d/0); lin-15*
SH89	3G	2	1º	2º	2º	1º	2º	1º	-	*lin-12(d/0); lin-15*

[a]Pattern legend: X, ablated cell; I, Intermediate

[b]The model could theoretically produce these results – albeit at a very low frequency – under the following conditions: (1) P7.p and P8.p moved to a position in which P8.p would receive an intermediate level of LIN-3 signal and (2) if a mechanism were activated at a low probability such that a 2º fate could be adopted spontaneously in the *lin-15*mutant background independent of the fate of the neighbors. This latter mechanism is, in fact, included and active at a 10% frequency in both the *Core* and *Graded* signaling models and thus could theoretically produce these results. In our testing protocol, however, these results were successfully reproduced in the *Core* model, but not in the *Graded* model. Theoretically, additional experimental results in Table 1C could similarly be reproduced at a very low frequency.

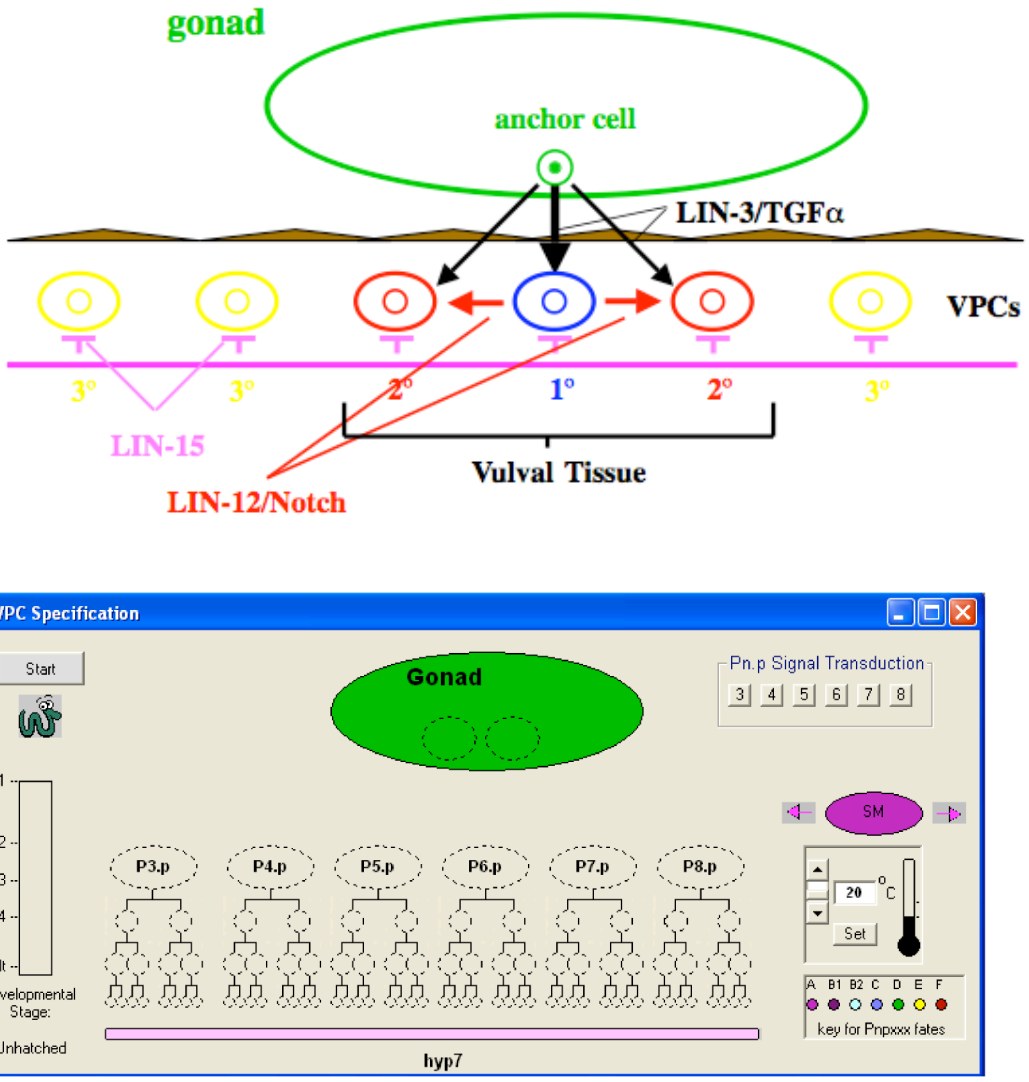

Figure S1. Graphical representations of VPCs. (A) The VPC "static pictorial diagram". (B) The graphical user interface (GUI). The structure of the GUI at the beginning of a simulation.

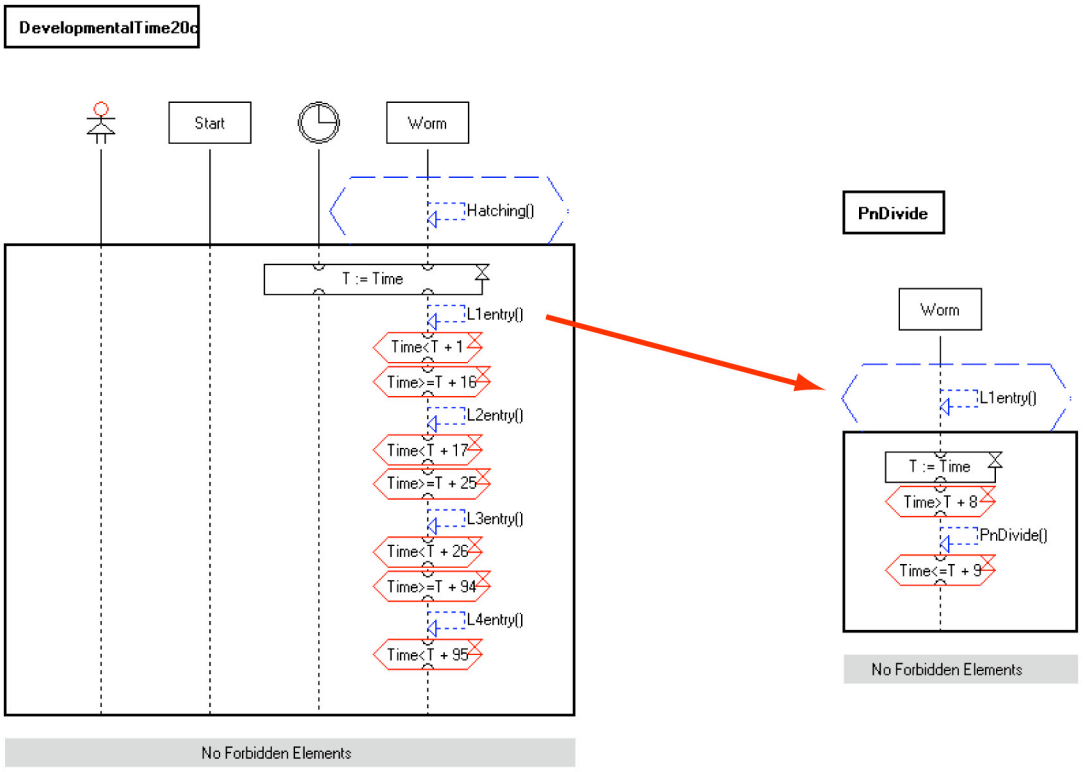

Figure S2. The events in the main chart of one LSC triggers an event in the prechart of another LSC. (A) The "Developmental Time 20C(Core Behaviors)" LSC. The Developmental Time uLSC that lies at the heart of the VPC model project. The arrow indicates the direct link between the Developmental Time uLSC and the PnDivide uLSC. Note the time constraints within the red hexagon conditions. The numbers refer to hours post-hatching, except for after L3 entry (which include 60 minutes for the first hour of L3). (B) "PnDivide(Core Behaviors)."

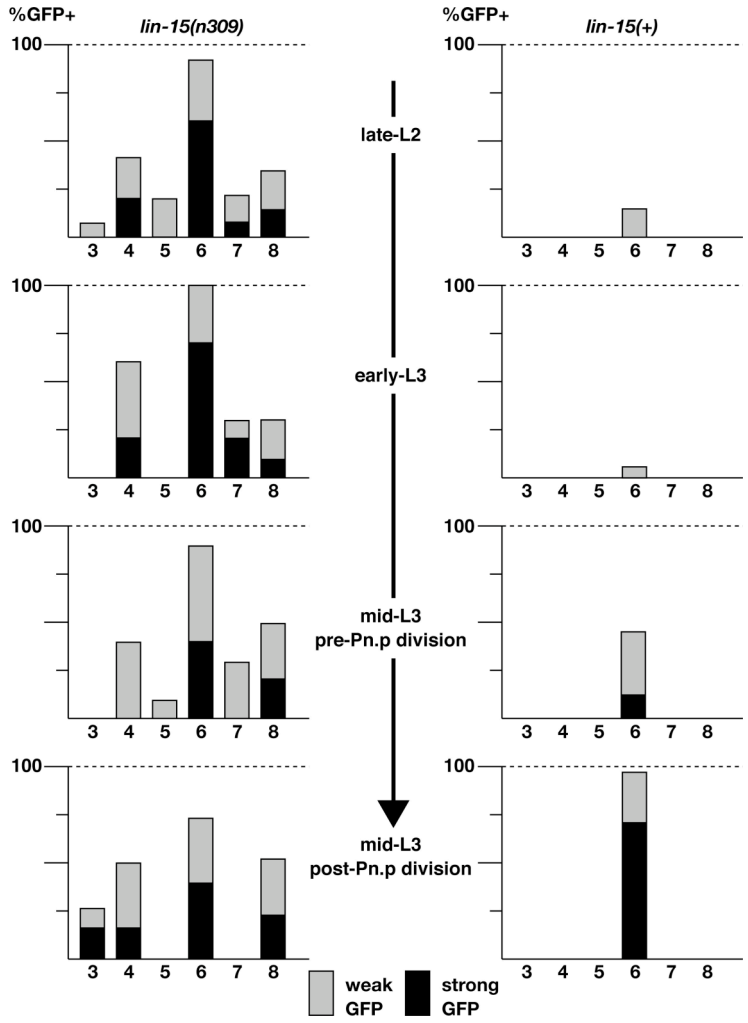

Figure S3. 1º fate specification in *lin-15(n309)* occurs very early. The *ayIs4[P*$_{egl-17}$*::GFP]* reporter (Burdine et al., 1998) was used to score 1º fate specification (GFP+) in each of the Pn.p cells (numbered along the *x*-axis) a *lin-15(n309)* (A) and a *lin-15(+)* (B) background at various timepoints. P6.p expresses the marker of 1º fate specification more frequently and more strongly than other Pn.p cells in *lin-15(n309).* 1º fate specification is also shifted dramatically earlier in *lin-15(n309)* than in *lin-15(+).* These results are consistent with an alternative, hypodermal source of the LIN-3 inductive signal rather than an AC source in *lin-15(n309)* animals (Burdine et al., 1998).

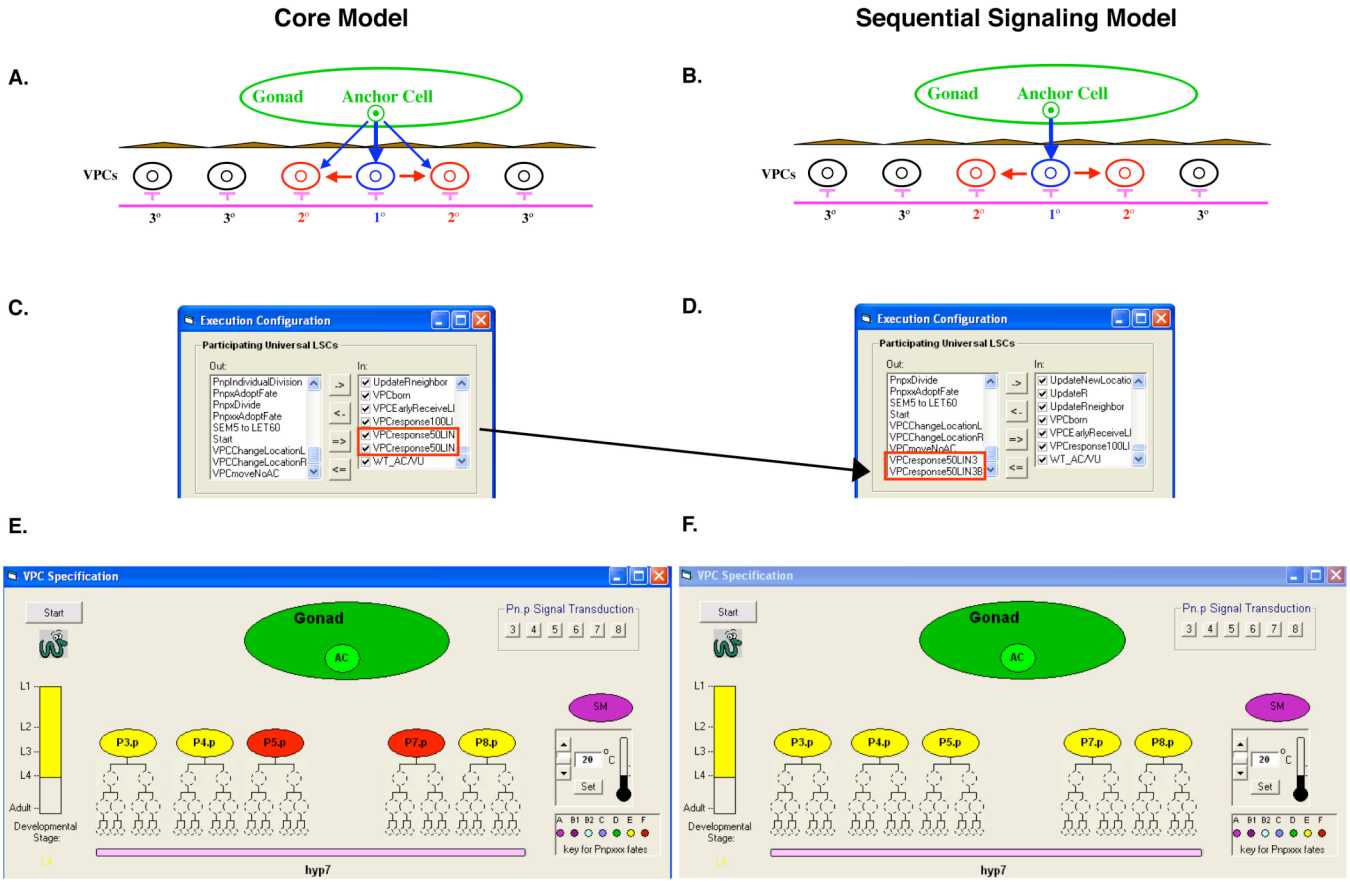

Figure S4. Hypothesis testing using different *Execution Configurations*. (A) All mechanisms are operational in the *Core* model. (B) Medium levels of inductive signaling that induce secondary VPC fates are omitted in the *Sequential* signaling model. (C,D) The relevant portion of the *Execution Configuration* showing the set of participating *uLSCs* for the *Core* model (C) and *Sequential* signaling model (D). The *Sequential* signaling model was generated from the *Core* model by moving the two *LSCs* (boxed in red) "out" from the set of participating *uLSCs*. Moving *uLSCs* "in" and "out" of these sets is accomplished by clicking on the arrows between the lists of sets. These two *uLSCs* describe the behavioral response of VPCs to a medium level of inductive signal (the thin blue arrows in (A)). When these *uLSCs* are "out" of the set of participating *uLSCs*, VPCs do not acquire a secondary fate in response to medium levels of inductive signal during simulations. Only a small portion of the sets of *uLSCs* is visible. (E,F) The final fates of the VPCs depicted in the GUI for a run which matches the result of an experiment in which P6.p was ablated in a wild-type background and neither of the adjacent VPCs moved to replace it (Table 1, lines 5,6,8 of SH86). Simulations using the *Execution Configuration* for the *Core* model (E) or the *Sequential* signaling model (F). Only the *Core* model reproduces the result obtained in this experiment.

Legends for Supplementary Movies:

In each movie, the GUI contains color codes. The developmental stages are coded as follows: no fill, unhatched; aqua, L1 and L2; green, L3; yellow, L4. The VPCs are coded as follows: no fill, VPCs are not yet at the ventral cord; white, VPCs present but unspecified; blue, 1º; orange, non-1º; red, 2º; yellow, 3º.

Movies 1 and 2:
(1) kamsuppmovie1.mov
(2) kamsuppmovie2.mov

These are recordings of a run of the *Core* model under wild-type conditions. In both (1) and (2) the progress and outcome of the simulation as driven by the participating *LSCs* is displayed in the GUI. In (1), a subset of the active *LSCs* also appear on the screen as the simulation proceeds. As the conditions in the Prechart of each *LSC* are met, the *LSC* is activated, and its progress can be followed in the background behind the GUI. Once the behaviors in a given Main Chart occur satisfactorily, the *LSC* is closed.

Movie 3:
(3) kamsuppmovie3.mov

This recording shows a run of the *Sequential* model (see text for details) under conditions in which all VPCs are absent except P4.p. In this particular run, P4.p moves to the position normally occupied by P5.p and nevertheless adopts a 3º fate since there is no neighboring 1º VPC to induce it to adopt a 2º fate.

Movie 4:
(4) kamsuppmovie4.mov

This recording shows a run of the *Graded* model (see text for details) under the same starting conditions as (3) above, in which all VPCs are absent except P4.p. Here, however, P4.p moves to the position normally occupied by P5.p and adopts a 2º fate in response to the intermediate level of LIN-3 that it experiences.

Movie 5:
(5) kamsuppmovie5.mov

This recording shows a run of the *Core* model in a *lin-15(n309)* mutant condition. The temporal prioritization of 1º fate specification of P6.p can be seen. In this particular run, the specification pattern that results for P3.p-P8.p is 2º/1º/2º/1º/2º/1º, respectively.