Cryptic quantitative evolution of the vulva intercellular signaling network in caenorhabditis.

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Cryptic quantitative evolution of the vulva intercellular signaling network in *Caenorhabditis*

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Abstract

Background: The *Caenorhabditis* vulva is formed from a row of Pn.p precursor cells, which adopt a spatial pattern of cell fates - 3°3°2°1°2°3° - centered around the gonadal anchor cell. This pattern is robustly specified by a network of intercellular signaling pathways including EGF/Ras signaling from the anchor cell and Delta/Notch signaling between the precursor cells. It is unclear how the roles and quantitative contributions of these vulva signaling pathways have evolved in closely related *Caenorhabditis* species.
Results: Cryptic evolution in the network is uncovered by quantifying cell fate pattern frequencies obtained after displacing the system out of its normal range, either by anchor cell ablations or through LIN-3/EGF overexpression. Silent evolution in the *Caenorhabditis* genus covers a large neutral space of cell fate patterns. Direct induction of the 1° fate as in *C. elegans*, and possibly lateral signaling, appeared within the genus. *C. briggsae* displays a graded induction of 1° and 2° fates, with 1° fate induction requiring a longer time than in *C. elegans*, and a concomitantly reduced lateral inhibition of adjacent 1° fates. *C. remanei* displays a strong lateral induction of 2° fates relative to vulval fate activation in the central cell (P6.p). This evolution in the space of cell fate patterns can be experimentally reconstituted by mild variations in Ras, Wnt and Notch pathway activities in *C. elegans* and *C. briggsae*.
Conclusions: Quantitative evolution in the roles of graded induction by LIN-3/EGF and lateral signaling through Notch is demonstrated for the *Caenorhabditis* vulva signaling network. This evolutionary system biology approach provides a quantitative view of the variational properties of this biological system.

Introduction

Outputs of many biological systems are robust to various perturbations, including random fluctuations in the system (for example noise in protein concentration or cell position) and variations in its environment [1-4]. This robustness raises two categories of related questions: i) its mechanistic basis and ii) the evolutionary dynamics of robust systems. In recent years, the mechanistic basis of robustness has been studied using a combination of modeling and experimental approaches on various systems [5-10]. The evolutionary context of

robustness has been mostly studied theoretically [2,4,7,11-14], and one likely consequence of robustness to noise and environmental variations is insensitivity to some genetic variation: the system may thus accumulate silent/cryptic variation while its output remains invariant. A key experimental challenge is to uncover the extent and nature of cryptic evolutionary change in biological processes, and ultimately its evolutionary dynamics and significance. Key experiments investigating cryptic intraspecific variation include studies in *Drosophila melanogaster* of the effect of an introduced mutation on the phenotypic variance (lack of robustness) in different wild genetic backgrounds, and of the genetic architecture of this variation [15-17].

The present work utilizes experimental approaches to unravel genus-level cryptic evolution in a well characterized and simple system, vulva cell fate patterning in *Caenorhabditis*. The extensive molecular knowledge of vulval development mechanisms in *C. elegans* and the availibility of phylogenetic information on closely related *Caenorhabditis* species allow cryptic evolution to be studied within a phylogenetic framework using both quantitative system analysis and evolutionary approaches.

The vulva is the egg-laying and copulatory organ of the *Caenorhabditis* hermaphrodite (or female), and is formed from a row of precursor cells, called Pn.p cells, born along the ventral epidermis at the first larval stage (L1). In *C. elegans*, a reproducible spatial pattern of cell fates develops during the L3 stage within the set of six competent cells, P(3-8).p: P6.p adopts an inner vulval fate (1°), P5.p and P7.p an outer vulval fate (2°); P3.p, P4.p and P8.p normally adopt non-vulval fates (3°), yet can replace P(5-7).p and are thus part of the vulval competence group (Fig. 1A). Each Pn.p cell undergoes an invariant cell division pattern that is characteristic of its fate (Fig. 1B). Formation of this '3°3°2°1°2°3°' spatial pattern relies upon an inductive signal (EGF-Ras-MAP kinase pathway) from the uterine anchor cell, which can act as a morphogen, inducing the 1° fate at high doses and the 2° fate at low doses [18,19]. EGF/Ras signaling in P6.p also activates a lateral Delta-Notch signaling pathway, which has two consequences in neighboring cells: induction of the 2° fate and inhibition of Ras pathway activity [20-23].

This developmental system offers the advantage of a small number of cells and a well-characterized molecular network. Like many (but not all) developmental systems, its output – here the cell fate pattern - is robust to noise and a range of environmental variations (C. Braendle and M.-A. F., in preparation). Its network properties that confer robustness of the cell fate pattern include positive feedback loops, pathway redundancy and crosstalk between pathways [24,25]. For example, Ras and Notch pathways display cooperative reactions and positive feedback loops [26-29] that may ensure switch behaviors. A Wnt pathway acts redundantly with the Ras pathway in inducing vulval fates and may contribute to the system's robustness [30,31]. Moroever, the 2° vulval fate can be specified either through LIN-3 action at intermediate doses [18] or through lateral signaling [20-23]. Crosstalk between the Ras and Notch pathways further contributes to robust specification of the three cell fates. In P6.p, a high Ras activity triggers LIN-12/Notch degradation, thus ensuring that it does not adopt a 2° fate [32]. In addition, Ras pathway activity in P6.p triggers lateral signaling, thus inhibiting Ras pathway activity in P(5,7).p [19,23,33]; this interaction helps to robustly specify 2° fates in P6.p neighbors [34]. The molecular network thus displays features that make its cell fate output robust to a range of variations. For example, a two-fold decrease in *lin-3* gene dosage has no phenotypic effect on the fate pattern [35], nor does a 10-fold decrease in Ras pathway activity as revealed by an *egl-17* transcriptional reporter (in *eps-8* mutants [28]). The many 'silent' regulators (such as GAP-1, the Ras GTPase-activating protein [36]) that have no phenotype in single mutants in standard laboratory conditions but

display a synthetic phenotype in double mutant combinations also reveal robustness of the system to variation in Ras pathway activity [27,37,38]. They also suggest that silent evolution is possible in this robust system.

Evolutionary studies on vulval cell fate patterning have been so far confined to other nematode genera. The vulval cell fate pattern undergoes some changes at long evolutionary distances [39,40], and the requirement for the anchor cell in vulval induction varies extensively [40-42]. At relatively long evolutionary distances, comparing *C. elegans* and *Pristionchus pacificus*, gains and losses of cell signaling events and pathway recruitment are detected [43-46], whereas closer to *C. elegans*, the requirement for the Ras pathway in vulval induction appears conserved in *Oscheius tipulae* [47]. The present study analyzes vulval patterning mechanisms at yet a smaller evolutionary scale, within the *Caenorhabditis* genus. Features of evolution (e.g., quantitative or temporal changes in roles of signaling pathways) could be revealed by performing analysis at this level. This genus comprises a diversity of species, eleven of which are available in culture [48,49], and the availability of a phylogeny (Fig. 2A) is in addition a key tool to assess the polarity of changes in vulval development.

Cryptic quantitative divergence is here uncovered in the vulva system among species of the *Caenorhabditis* genus by analyzing cell fate patterns obtained: i) by ablating the anchor cell at successive timepoints, thus uncovering a series of P(5-7).p fate patterns, from '3°3°3°' to the final '2°1°2°' pattern; ii) by overexpressing the EGF/LIN-3 signal at levels below those resulting in all cells adopting a 1° fate (Fig. 1C). Cell fate patterns in either experimental situation reveal relative activities of the different signaling pathways. The molecular architecture of the network is shown to be conserved in *C. briggsae*: Cbr-LIN-3/EGF can activate 2° and 1° fates in a graded manner, and LIN-12/Notch plays a role in lateral inhibition. Observed differences among species are reconstituted by experimentally varying Ras, Wnt and Notch pathway activities in *C. elegans* and *C. briggsae*. Thus, through experimental manipulation of the signaling pathways directing vulva development in *Caenorhabditis* species, this study reveals and characterizes quantitative evolution in the relative roles of these pathways, and suggests that the well-characterized *C. elegans* vulval fate specification mechanism arose within the *Caenorhabditis* genus.

Results

The same Pn.p cell fate and division patterns (Fig. 1B) were found in all *Caenorhabditis* species, except for changes in P3.p competence and division [50] and in the 3° cell division pattern in the species branching most basally, *C.* sp. 1 SB341 (Kiontke et al., in preparation). The wild type fate pattern of P(4-8).p is thus '3°2°1°2°3°' in all species. Cryptic variations in fate specification mechanisms were revealed by displacing the system out of its normal range of anchor cell inducing activity, using i) anchor cell ablation and ii) LIN-3 overexpression.

Cell fate patterns upon anchor cell ablation in different *Caenorhabditis* species

The first experimental paradigm to reveal cryptic change was to eliminate the anchor cell (the source of inductive LIN-3/EGF signaling; [51]) by laser ablation in L3 stage larvae and to follow vulval cell fates thereafter. In all species, early anchor cell ablation resulted in all vulval precursor cells adopting a 3° fate (except *C.* sp. 1 SB341, where inductive signaling originated from several uterine precursors; K. Kiontke et al., in preparation). Between the all-3° pattern and the '3°2°1°2°3°' wild type pattern, several 'intermediate' patterns are possible (Fig. 1C, top), and each pattern was seen in at least one anchor-cell-ablated individual of one species. In each species, several intermediate fate patterns were seen in

different proportions: fate pattern variation is quantitative. Note that these patterns may not represent *temporal* intermediates (see Discussion). Detailed lineages are found in Tables S1-S17, which keep record of ablation time, with landmark divisions of the uterine and vulval precursors as chronological markers.

The different fate patterns (Fig. 1C) being mostly characterized by P6.p fate, a quantitative summary is shown as a ternary plot (Fig. 2C) of the proportion of fates adopted by P6.p in ablations performed before P6.p division, removing 'trivial' all-3° patterns. This quantitative analysis of P6.p intermediate fate patterns is robust to changes in relative timing of developmental events and reveals silent variation. In a few examples, we further distinguished two groups of ablation timepoints, before ('early') versus after ('late') 3° cell divisions (squares in ternary plots).

These experiments revealed ample cryptic change in vulval patterning: the neutral space of cell fate patterns thus defined is fully covered by variation within the *Caenorhabditis* genus (Fig. 2C). When comparing P6.p fate upon anchor cell ablations in reference strains of the three most studied species, *C. elegans* N2, *C. briggsae* AF16 and *C. remanei* PB4641, the first showed a predominance of 1° fates (59%, n=33), the second of 2° fates (72%, n=37) and the third of 3° fates (73%, n=37). Highly significant differences were thus found (N2 versus PB4641, $\chi 2$ test on the proportion of each fate, $p<10^{-7}$; N2 versus AF16, p=0.016). Strains showing a high proportion of 1°, 2° and 3° fates are reviewed successively.

In *C. japonica* DF5079 (highest proportion of 1° fate: 88%, n=29), *C.* sp. 4 PB2801 and CB5161, and predominantly in *C. elegans* N2, the 'transition' from an all-3° to the wild type pattern appeared direct (Fig. 2; Tables S9-S12): only few other patterns were observed. This suggested that P6.p, as soon as it is induced to a 1° fate, activates the 2° fate in P(5,7).p through lateral signaling.

In *C.* sp. 2 DF5070 (93% of 2° fates, n=30), *C. drosophilae* DF5077, C. sp. 5 JU727 and *C. briggsae* AF16, HK104 and JU725, P6.p most often adopted a 'TUUT' cell lineage pattern in intermediate ablations (Fig. 2; Tables 1, S1-S4, S15-S16). This 'TUUT' lineage could correspond to an abnormal primary lineage, or to a mirror-image pattern of two daughters with internal 2° fates (vul'CDDC'; see Pn.p granddaughter nomenclature in Fig. 1B). This lineage was thus further analyzed using Pn.p granddaughter fate markers in *C. briggsae*. The fate markers *Cbr-egl-17::GFP* (expressed in vulC/D in late L4 stage) and *Cbr-zmp-1::GFP* (in vulA and vulE at the L4-to-adult molt) [52,53] were integrated into the *C. briggsae* AF16 genome. After anchor cell ablations, P6.p progeny expressed *Cbr-egl-17::GFP* and not *Cbr-zmp-1::GFP* (Table 1). The same experiment was performed in *C. elegans*: *egl-17* expression was seen in P6.p in only 4/17 animals (ablation time between VU and P6.p divisions); *zmp-1* expression was variable as expected from the late anchor-cell effect on the primary lineage [54]. Thus, the major fate adopted by P6.p upon ablation in *C. briggsae* was an inner 2° fate. For simplicity, we refer to this P(5-7).p fate pattern as '2°2°2°'.

In *Caenorhabditis* species branching basally, i.e. *C.* sp. 1 SB341 (uterine ablation; Kiontke et al., in preparation) and *C. plicata* (Table S17), intermediate ''2°2°2°'' patterns were also observed. Whether P6.p adopted a mirror-image inner 2° fate or a full 2° lineage depended on the species and experimental conditions (anchor cell versus whole-uterus ablation): P6.p usually adopted a full 2° fate in *C.* sp. 1 (uterus ablation), mirror-image outer 2° fates in *C. plicata* and mirror-image inner 2° fates in *C. drosophilae* and *C.* sp. 2, as in *C. briggsae* and *C.* sp. 5 (see Suppl. data). A graded effect of the anchor cell signal (LIN-3 or Wnt family member [55]) may further bias towards inner versus outer 2° lineages.

Finally, in *C. remanei* PB4641 (73% of 3° fates, n=37), PB228 and JU724, anchor cell ablations predominantly resulted in the

somewhat surprising and so far undescribed '2°3°2°' pattern, where P6.p did not adopt a vulval fate while P5.p and P7.p did (Fig. 2B,C; Tables S6-S8). Control ablation of a nearby uterine cell had no effect on vulval lineage (5/5 animals), ruling out that unspecific damage by the laser prevented P6.p from adopting a vulval fate. A plausible hypothesis for this '2°3°2°' pattern was that the anchor cell signal could activate the inductive pathway in P6.p at a level sufficient to upregulate lateral signal transcription (Fig. 1A), yet insufficient for adoption of vulval fates by P6.p. Indeed, when P6.p was ablated with the anchor cell in *C. remanei*, P(5,7).p adopted a vulval fate in only 36% of the cases (n=54), whereas if P6.p was left intact, they adopted a 2° vulval fate in 96% of the cases (n=52; ablation between VU and P6.p divisions; Table S6). These results strongly suggested that the '2°3°2°' pattern was the result of P(5,7).p receiving a signal from a partially induced P6.p cell, even though the latter adopted a 3° fate.

When changes are considered onto the *Caenorhabditis* phylogeny (Fig. 2A), early 1° fate specification seems to have appeared (maybe gradually) between the *C. drosophilae, C.* sp. 3 and *C. japonica* branches. The ancestral '2°2°2°' pattern was previously observed in many other nematode genera (2-step induction). *C.* sp. 3 RGD1 showed a somewhat intermediate state, with a strong temporal component in the data: P6.p first adopted a 2°, then a 1° fate even in ablations performed before its division (*C. elegans* N2 did not display such a temporal component, despite being located at a similar position in ternary plot space; Fig. 2C). The '2°3°2°' pattern, a signature of lateral induction, visible by a deviation from the 1°/2° edge of the plot, appeared around the same time (it was never observed outside the genus). Dramatic evolution occurred in the *Elegans* group (the top five species in Fig. 2A, which are quite similar morphologically), spread over the whole ternary plot space. The position of *C. briggsae* in the 2° corner is a reversal to the ancestral situation.

Cell fate patterns upon LIN-3/EGF overexpression

The second experimental paradigm consisted in scoring deviant cell fate patterns obtained after (mildly) overexpressing the LIN-3 signal from the anchor cell (Fig. 1C, bottom). This experiment evaluates the relative activities of 1° fate induction by the EGF/Ras pathway versus lateral inhibition by the Notch pathway (the latter preventing adjacent 1° fates induced by the former). In *C. elegans*, lateral inhibition is not easily overcome: P(5,7).p still adopt 2° fates upon mild overexpression of *Cel-lin-3* from the anchor cell, at a level that results in P4.p or P8.p sometimes adopting a vulval fate; higher levels of *Cel-lin-3* overexpression caused P(5,7).p to adopt 1° fates that were adjacent to that of P6.p ([18]; J. Milloz and M.-A..F., in preparation).

In contrast, in *C. briggsae*, the first deviant P(4-8).p fate pattern after mild overexpression of *Cbr-lin-3* from its anchor cell promoter was '2°1°1°2°3°' (Table 2A); at a higher dose the predominant fate pattern was '2°1°1°1°2°' (i.e., the same fate transformations occurred on the posterior side; Table 2B). The fate pattern was confirmed by the corresponding loss of *Cbr-egl-17::GFP* L4-stage expression in the adjacent 1° cells (Table 2B). Thus, unlike in *C. elegans*, lateral inhibition was easily overcome in *C. briggsae* by an excess of anchor cell signal, and most easily on the anterior side. When the same *Cbr-lin-3* construct was overexpressed in *C. elegans* (Table 2C), the cell lineage patterns were as observed with *Cel-lin-3*, with P(5,7).p still adopting 2° fates at high LIN-3 levels [18], suggesting that the difference was not due to evolution at the *lin-3* locus.

The anchor cell ablation and LIN-3 overexpression data pointed to a possible quantitative difference among different *Caenorhabditis* species in the three downstream effects of the Ras pathway, namely 1° fate specification, 2° fate

specification and lateral signal (Deltas) transcription (cf. Fig. 5A). Specifically, the *C. remanei* behavior could be explained by a higher threshold for 1°/2° induction than for lateral signaling, and the *C. briggsae* behavior by a higher threshold than in *C. elegans* for 1° induction compared to 2° induction and lateral signaling. These hypotheses were tested by i) examining the roles of Ras and Notch pathways in *C. briggsae* in order to see whether they were overall conserved; ii) experimentally mildly altering Ras and Notch pathway activities in *C. elegans* and *C. briggsae*.

Roles of the EGF/Ras and Notch pathways in *C. briggsae* vulval cell fate patterning

2° and 1° fates are induced in a dose-dependent manner by LIN-3/EGF in C. briggsae

In order to test whether LIN-3 was able to induce 2° and 1° fates at different doses in *C. briggsae*, the LIN-3/EGF domain was expressed under a heat-shock promoter in gonad-ablated animals, as had been performed in *C. elegans* [18]. Gonad ablation ensured that the endogenous LIN-3 signal was removed. Without heat-shock, P(4-8).p adopted a 3° fate (Fig. 3A). In low heat-shock conditions, they started adopting a 2° fate (Fig. 3B), and at a higher dose a 1° fate (Fig. 3C). Moreover, partial inactivation of the *Cbr-lin-3* homolog by RNAi resulted in (few) transformations of 1° to 2° fates (Fig. 4B); poor efficiency of RNAi in some tissues may explain that such transformations were rare and that transformation to 3° fates were not observed. Thus, the LIN-3 signal can induce 1° and 2° fates in a quantitative manner in *C. briggsae*.

The LIN-3/EGF dose-response experiments in *C. briggsae* reveal another kind of cryptic variation in the system, in the relative competence level of different Pn.p cells (as defined by their ability to respond to LIN-3): at a given LIN-3 dose, without spatial information from the gonad, the different Pn.p cells adopted vulval fates in different proportions (Fig. 3). A high competence of a given Pn.p cell is defined by a high proportion of animals where the cell adopts a vulval fate, and a 1° fate rather than a 2° fate. In *C. elegans*, P8.p and to a lesser extent P7.p were shown to be less competent than the more anterior cells, due to *mab-5/Hox* expression [18,56]. In *C. briggsae*, P3.p exhibited a low level of competence to adopt a vulval fate in the LIN-3 overexpression experiments, and was also found to be incompetent to replace ablated P(4-8).p [50]. Moreover, unlike in *C. elegans*, P4.p appeared less competent than P7.p (Fig. 3; Tables S18-S21): after intermediate heat-shock conditions (30-60' at 33°C or 15-30' at 37°C), they adopted a vulval fate in 26% and 51% of gonad-ablated animals, respectively (n=38; $\chi 2$, p=0.03). Note that despite a higher proportion of vulval versus non-vulval fates, the proportion of 1° fates is lower for P7.p compared to P4.p (see lateral inhibition below). Competence is thus a quantitative character, and is overall lower on the anterior side and higher on the posterior side of the competence group in *C. briggsae* compared to *C. elegans*.

In order to visualize Ras pathway activation, the *C. elegans* Ras pathway transcriptional reporter *Cel-egl-17::GFP* [57] was introduced into *C. briggsae*. In *C. elegans*, *egl-17* reporters are expressed at a high level in P6.p in the L3 stage; they can also be detected at a low level in P(5,7).p early on and are then repressed by LIN-12/Notch signaling [19]. In *C. briggsae*, the *Cel-egl-17::GFP* reporter was detected in P6.p from the early L3 stage on, and further activation was dependent on anchor cell signaling (Fig. 4A). Cbr-LIN-3 overexpression resulted in *egl-17::GFP* L3 stage expression in some adjacent Pn.p cells (not shown). Thus, although P(5-7).p all adopted a 2° fate upon anchor cell ablation, P6.p was already different from its neighbors in the early-mid-L3 stage through its higher Ras pathway activity. The absence of *egl-17* reporter detection in P(5,7).p in *C. briggsae* was consistent with a lower Ras pathway activation, but it could not be ruled out that it

was due to overall lower transgene expression in *C. briggsae*.

Lateral inhibition of the 1° fate by LIN-12/Notch in C. briggsae

In *C. briggsae,* mild overexpression of LIN-3 from the anchor cell resulted in adjacent 1° fates (Table 2), raising the question of whether lateral inhibition operated at all in this species. Alternation of 1° and 2° fates could be detected in animals (either gonad-ablated or intact) in which LIN-3/EGF was expressed from a heat-inducible promoter: in many animals, P6.p and P8.p adopted a 1° fate, while P7.p remained 2° (Fig. 3C,D; Tables S18-19 for the phase information). Thus, lateral inhibition, although weak, is present in *C. briggsae* as in *C. elegans*. The difference in fate pattern upon LIN-3 overexpression from the anchor cell thus likely reflected a *quantitative* variation in the strength of lateral inhibition versus direct LIN-3/EGF-induced fate specification.

At the molecular level, the involvement of LIN-12/Notch signaling also appeared conserved: partial inactivation of the *Cbr-lin-12/Notch* homologs by RNAi resulted in adjacent 1° fates in *C. briggsae*, with a bias towards P5.p (Table 3, Fig. 4C). The phenotype resembled that of weak *lin-12* hypomorphs in *C. elegans* [58], with hardly any loss of 2° fates. The presence of two anchor cells (instead of a single one) was also observed in some animals. Adjacent 1° fates of Pn.p cells occurred in animals with either one or two anchor cells. Thus, in *C. briggsae* as in *C. elegans*, the LIN-12/Notch pathway acts both in anchor cell specification and in lateral inhibition of Pn.p cells.

In addition to its role in lateral *inhibition* of the Ras pathway, the Notch pathway has in *C. elegans* a role in *2° fate induction* [21,22]. As described above, the role of lateral signaling in 2° fate induction could be detected in *C. remanei* by co-ablation of the anchor cell and P6.p. Similar experiments did not detect an obvious role in *C. briggsae* (Table S1), nor in *C. elegans* (M. Wang and P.W. Sternberg, pers. comm.), where lateral induction does operate. Again, the difference among species is likely to be quantitative.

In order to visualize Notch pathway activation, the Notch pathway transcriptional reporter *Cel-lip-1::GFP* was introduced into *C. briggsae*. In *C. elegans*, it is activated downstream of LIN-12/Notch signaling and expressed at a higher level in P(5,7).p compared to P6.p [33]. In contrast, in *C. briggsae* animals bearing the integrated transgene *mfIs29[Cel-lip-1::GFP]*, fluorescence levels were in average similar among P(5-7).p (not shown; idem for independent *Cel-lip-1::GFP* transgenes). Evolution in trans-acting factors cannot be ruled out, but this apparent homogeneity in *lip-1* reporter expression is consistent with the weaker lateral inhibition observed in *C. briggsae*.

Silent changes in the molecular network mimic evolutionary variations in the *Caenorhabditis* genus

The previous experiments suggested that the molecular network of Ras and Notch signaling was overall conserved in *C. briggsae* compared to *C. elegans*. In order to test whether quantitative changes in this network could account for the observed differences between species, the signaling pathway activity levels were experimentally manipulated and tested in the anchor cell ablation paradigm (Fig. 5; Tables S22-S29). These experiments made use of mutations or transgenic alterations that were practically silent (at the level of the final cell fate pattern) and thus potentially mimicked the cryptic genetic change among species.

Ras pathway activity was decreased using the *lin-45(n2018)*/Raf hypomorphic mutation [59,60] in the *C. elegans* N2 background. In the anchor cell ablation experiment, this mutation significantly displaced P6.p fate from the 1° fate region to the 2° fate region ($\chi 2$ for the proportion of 1° versus 2° fates compared to N2, $p<0.01$) occupied by wild *C. briggsae* isolates (Fig. 5B). The Wnt pathway appears to act redundantly with the Ras pathway in *C.*

elegans [30,31] and a similar effect was observed when decreasing Wnt pathway activity through the *bar-1(ga80)*/β-catenin null mutation ($p<0.05$). Conversely, hyperactivation of the Ras pathway through *Cbr-lin-3* overexpression in *C. briggsae* mimicked the *C. elegans*/*C.* sp. 4/ *C. japonica* situation. *C. elegans* N2 could be further displaced towards the 1° fate corner through mild Ras pathway hyperactivation (*ark-1* mutant, $p<10^{-2}$ compared to N2) [37] (Fig. 5B). Thus, interspecific variation along the 1°/2° edge could be explained by quantitative differences in Ras (or Wnt) pathway signaling.

As mentioned above, the '2°3°2°' pattern observed in *C. remanei* could result from a lower threshold of Notch signaling activation compared to P6.p fate specification (Fig. 5A). The level of Notch pathway activation was thus experimentally altered using silent mutations in *sel-10*, a negative regulator of the Notch pathway [61-63]. *sel-10* mutations resulted in a shift of *C. elegans* towards the 2° and 3° fate regions and in addition, the data showed a strong temporal component, with the '2°3°2°' fate pattern being predominant in early ablations (Fig. 5B). The *C. remanei* situation can thus be partially mimicked by a silent gain-of-function in the Notch pathway in the *C. elegans* background.

Discussion

Cryptic evolution in vulval cell fate patterning mechanisms was uncovered by removing the anchor cell during the induction process and by overexpressing the inductive signal from this cell. Evolution in the *Caenorhabditis* genus covered a large neutral space of possible cell fate patterns obtained in these experimental situations, in the absence of change in the actual final pattern, which forms a robust and invariant trait. Molecular measures and manipulations of the intercellular signaling system in species of the Elegans-group showed that the molecular network was overall conserved yet evolved quantitatively. I first review below the phylogenetic information provided by these (and previous) experiments, and then discuss cryptic quantitative evolution i) in the anchor cell induction pathway and ii) in the network that anchor cell induction forms with lateral signaling.

Evolution of vulval cell fate patterning mechanisms in *Caenorhabditis* and beyond

In most species outside the *Caenorhabditis* genus, such as *Oscheius tipulae* CEW1 and *Pristionchus pacificus* [40,42,64], anchor cell ablations produce the same effect as in the basally branching *Caenorhabditis* species: P(5-7).p adopt an intermediate '2°2°2°' fate pattern. The same holds true for *Prodontorhabditis wirthi* DF5074, a member of the sister group of the *Caenorhabditis* genus (Kiontke et al., in preparation; [65]). The adoption of a 2° fate by P6.p after anchor cell ablation is thus very likely to be the ancestral situation in the *Caenorhabditis* genus, and direct 1° fate induction and concomitant induction of P(5,7).p through lateral signaling as seen in *C. japonica*, *C. elegans* and *C.* sp. 4 is derived. Further evolution occurred in the Elegans species group, with *C. briggsae* apparently 'reverting' to the ancestral pattern and *C. remanei* displaying the peculiar '2°3°2°' fate pattern. Features of vulva development in the model organism, *C. elegans* N2, are thus representive of very few nematode species.

Intra-specific variation can also be detected, at least in *C. japonica* ($\chi 2$, $p<10^{-4}$) and *C.* sp. 5 ($p=0.014$) - note that such male/female species may harbor much larger genetic variation than the predominantly selfing *C. elegans* and *C. briggsae* [66]. More systematic studies among *C. elegans* wild isolates using mutant introgressions and pathway reporters are underway (J. Milloz, I. Nuez and M.-A.F., unpublished).

Whether lateral signaling exists outside the *Caenorhabditis* genus or even in basally-branching *Caenorhabditis* species is unclear. Interestingly, the '2°3°2°' pattern, a distinctive feature of lateral signaling, was

only found within the *Caenorhabditis* genus. Mutant screens outside the genus did not detect lateral signaling either [47]. A two–step mechanism may not require lateral signaling to robustly obtain the fate pattern, and lateral signaling may have appeared within the *Caenorhabditis* genus.

The variety of cell fate patterns obtained in experimental situations could in principle represent an evolutionary potential for innovation. However, the major vulval cell fate pattern changes observed so far at a long evolutionary range concern the number of induced cells (two to four) and the centering of the lineage (between two Pn.p cells rather than between the P6.p daughters) [39,40]. The 1° lineage is split between the central daughters of P6.p and P7.p in the nematode suborder Cephalobina and this is presumably possible because of the late induction of 1° fates after Pn.p division and the putative absence of lateral inhibition [40]. On the other hand, the centering of the vulval pattern on P6.p in the suborder Rhabditina (to which *Caenorhabditis* and *Oscheius* belong) is a prerequisite for the evolution of lateral inhibition as in *C. elegans*. Innovation is here to be found in the developmental mechanism rather than in the final cell fate pattern.

Cryptic quantitative evolution in the induction by the anchor cell

The mechanism at work in *Oscheius tipulae* and other distant species was previously called 'two-step induction', because the anchor cell ablations suggested that P(5-7).p first received a signal specifying 2° versus 3° fates, and that the *daughters* of P6.p were then induced to a 1° vulval fate [42]. The present results show that at least in *C. briggsa*e, the late requirement for the anchor cell after P6.p division is mechanistically the result of a *quantitative* decrease in inductive signaling activity, and provide additional insights into the quantitative state of the system in *C. elegans* N2.

Indeed, LIN-3 is able to act in a graded quantitative fashion to specify 2° and 1° fates in both *C. elegans* and *C. briggsae*. In *C. elegans* N2, P6.p may adopt a full or partial 2° fate after anchor cell ablation (Table S11) and in addition to increasing the frequency of 2° fates for P6.p, the *lin-45(n2018)* mutation delays the induction (Table S22). The same holds true when Wnt signaling is reduced (*bar-1(ga80)* mutant; Table S23), confirming that Ras and Wnt pathways both contribute to vulval fate induction level in *C. elegans* [30,31]. Conversely, the *ark-1(sy247)* mutation results in an apparently earlier induction (Table S28). Therefore, in *C. elegans* as in *C. briggsae*, quantity and duration of inductive signal production appear to positively influence the induction level and the adoption of 1° versus 2° fates.

The observed fate patterns correspond to *final* fate patterns after ablation at intermediate times and not necessarily to *temporal* intermediate cell states that would occur during normal development. Indeed, in *C. elegans* animals bearing the hypomorphic *lin-3(e1417)* allele that reduces LIN-3 expression [67], P6.p, when adopting a vulval fate, adopts a 1° (versus 2°) lineage at higher frequencies than in the anchor cell ablation experiment ([68]; M.-A. F., unpublished). The difference between the ablation and the *lin-3* mutation is the temporal cessation of signal production (which may also include other molecules such as Wnts) in the former case. Using a LIN-3 expression time-course, Wang and Sternberg [69] showed that the Pn.p daughters can still switch from a 2° to a 1° fate, whereas 3°-fated daughters can no longer adopt a vulval fate. Late anchor cell signaling is required for regulation of *Cel-zmp-1::GFP* expression in different P6.p granddaughter sublineages [54]. Overall, these results suggest that P6.p can adopt a 2° fate if it receives low levels of LIN-3, and that in *C. elegans*, 1° fate specification is robust to some variations in concentration and timing of LIN-3 signaling.

The evolution between *C. briggsae* and *C. elegans*, *C.* sp. 4 or *C. japonica* thus likely resides in the *level* of anchor signaling

activity (integrated over time) required to induce the 1° fate, which is compatible with the *longer* requirement for signaling (ablation experiments do not eliminate signaling molecules that are already active at ablation time) and is translated into a heterochronic shift in P6.p induction timing. The molecular variation may reside either in a lower inductive signal production in *C. briggsae* or a lower sensitivity of the downstream signal transduction or transcriptional components (in the Ras or Wnt pathways). The low *egl-17* (Ras reporter) transgene expression in *C. briggsae* is consistent with a low level of Ras pathway activity.

Cryptic quantitative evolution in the vulval intercellular signaling network

In addition to inductive signaling from the anchor cell, robust vulval cell fate patterning requires lateral Notch pathway activation, which, with the Ras pathway, forms an intercellular signaling network (Fig. 5A), at least in the Elegans species group. In *C. briggsae*, lateral inhibition through the Notch pathway appears less active. The overall equal level of *lip-1* (an effector of lateral *inhibition*) expression in P(5-7).p is consistent with low lateral inhibition activity, although in both cases, differences with *C. elegans* may be caused by unrelated alterations in transgene regulation. Interestingly, inhibition of adjacent 1° fates may be little required for robust patterning in this species since 1° fate induction takes longer and is less likely to occur in two precursors. In addition, Notch pathway activity may have increased in *C. remanei*, at least the branch leading from Ras pathway activation to 2° fate *induction.*

As a general conclusion, robustness of vulval patterning mechanisms may allow cryptic genetic change to accumulate, because the system's output is insensitive to a range of variation in molecular activities. In other words, despite conservation in the final phenotype (which is likely maintained by selection via egg-laying and mating efficiencies), the biological processes that construct this final phenotype and mediate the relationship between genotype and final phenotype are sensitive to variation in genotype (and environment) [25]. Such cryptic variation of underlying mechanisms in this robust intercellular signaling network may be one of many examples where the large molecular divergence between *C. elegans* and *C. briggsae* [70] does not result in overt morphological change.

What drives this cryptic evolution in the vulva signaling network? One possibility is that the system's evolution is neutral since a range of variation has no effect on the final vulva fate pattern phenotype - even if it shows an effect at an earlier developmental stage, for example through a signaling pathway reporter [25]. Exploration of the neutral space of intermediate cell fate patterns as defined here may in this case obey neutral evolutionary dynamics. Another possibility is that in natural populations, the system is faced with genetic or ecological contexts that reveal 'cryptic' variation in the form of deviations in the final vulva pattern, which may be deleterious and impose a selective pressure on the system. Finally, the system's evolution may be driven through pleiotropic gene action, for example if levels of activity of the Ras pathway were driven to change through varying selection pressures on one of its many other roles, such as olfaction or pathogen defense [71,72].

Experimental procedures

See Supplemental information.

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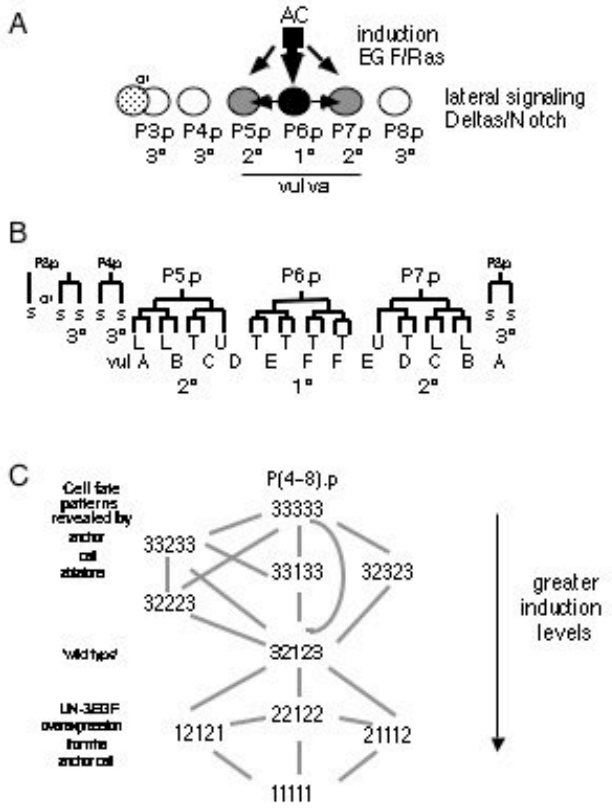

Figure 1

Figure 1. Vulval precursor cell fate patterns.

A. In *C. elegans*, the spatial pattern of three cell fates adopted by P(3-8).p is the result of induction by the anchor cell (AC) and lateral signaling between vulval precursor cells. The anchor cell signal induces the 1° fate at high doses, and may induce 2° fates at low levels. Ras pathway activation in the P6.p activates lateral signaling through Notch, which induces the 2° fate and also inhibits the Ras pathway in the receiving cell (P5.p and P7.p). The '3°3°2°1°2°3°' cell fate pattern is basically invariant within the *Caenorhabditis* genus, except for P3.p competence. Black: 1° fate. Grey: 2° fate. White: 3° fate. Dotted: non-competent.

B. Vulval precursor cell lineages that are characteristic for each fate. L: longitudinal division (antero-posterior) of the Pn.p granddaughter; T: transverse (left-right) division; U: undivided granddaughter; S: non-vulval fate, i.e. fusion to the hyp7 syncytium of Pn.p daughters. The vulA-F granddaughter nomenclature is given below.

C. Cell fate patterns that can be obtained by experimentally disturbing the system out of its buffered range, either by ablating the anchor cell during the induction process (top) or by increasing the LIN-3/EGF dose produced by the anchor cell (bottom). Such cell fate patterns are intermediate in induction levels and are not necessarily temporal intermediates.

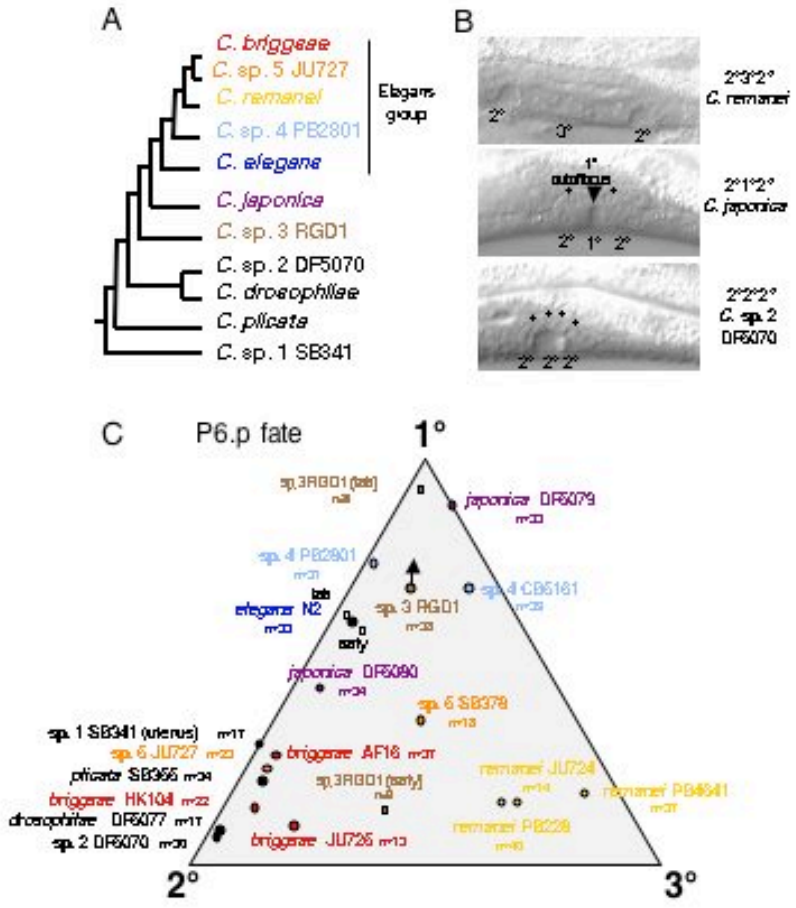

Figure 2

Figure 2. Vulval cell fate adopted by P6.p after anchor cell ablation in different *Caenorhabditis* species.

A. *Caenorhabditis* phylogeny. After [49, 73], K. Kiontke and D. Fitch (pers. comm.).

B. Typical P(5-7).p fate patterns after anchor cell ablations. Nomarski micrographs. The stars indicate the undivided Pn.p granddaughters ('U', vulD) of the 2° lineage. The non-vulval fate (3°) of P6.p in *C. remanei* is clearly visible by the lack of invagination. Lateral view. Dorsal is to the top.

C. Ternary plot showing the proportion of 1°, 2° and 3° fates adopted by P6.p for different wild genotypes of different *Caenorhabditis* species, taking into account all animals ablated before P(5-7).p division, in which at least one of P(5-7).p adopted a full vulval fate. For example, the N2 position corresponds to P6.p adopting the 1° fate in 59% of the animals, the 2° fate in 38% and the 3° fate in 3%. Species are color-coded as in panel A. The data for RGD1 and N2 were separated between times before ('early') and after ('late') 3° cell division (squares): RGD1 shows a strong temporal component (arrow, $\chi 2$, $p<10^{-5}$), whereas N2 does not. See Tables S1-S17 for detailed cell lineages and ablation timing.

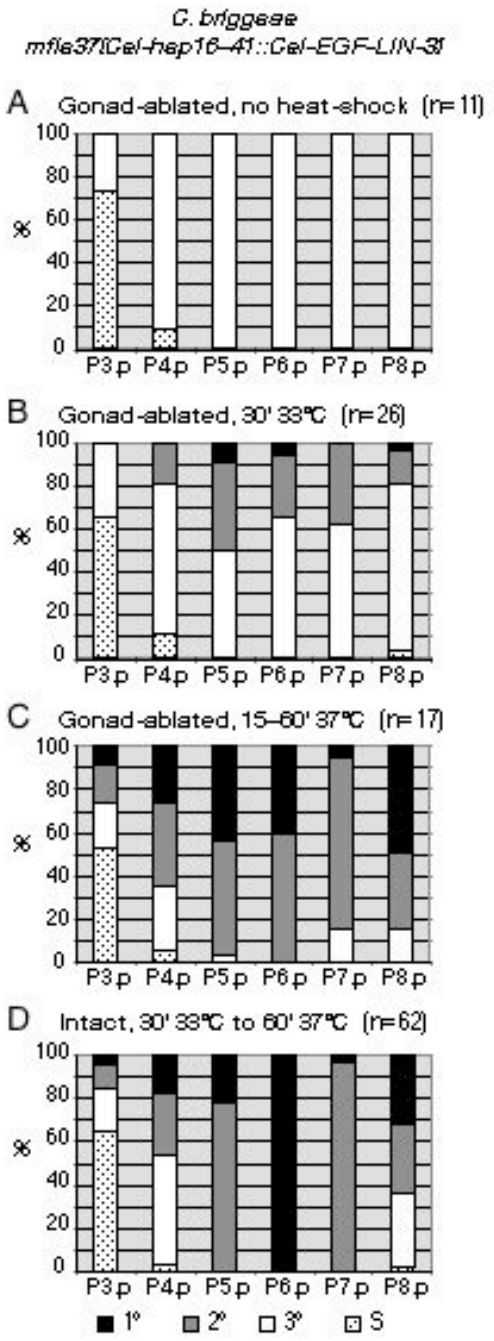

Figure 3

Figure 3. Graded induction of 2° and 1° fates by LIN-3/EGF and lateral inhibition in *C. briggsae*.
The gonad primordium of *mfIs37 C. briggsae* larvae was ablated in the early L1 stage, and the larvae heat-shocked in the late L2 to early L3 stage. Heat-shock conditions are indicated above each panel. Vulval cell divisions were scored at the late L3 to early L4 stage. Each panel represents for each Pn.p cell the proportion of animals showing a given cell fate. Fates are color-coded as in Figure 1. The dotted fate indicates no division and fusion to hyp7 in the L2 stage (non-competent cell; most frequent fate for P3.p in *C. briggsae*). See Tables S18, S19 for detailed lineages.

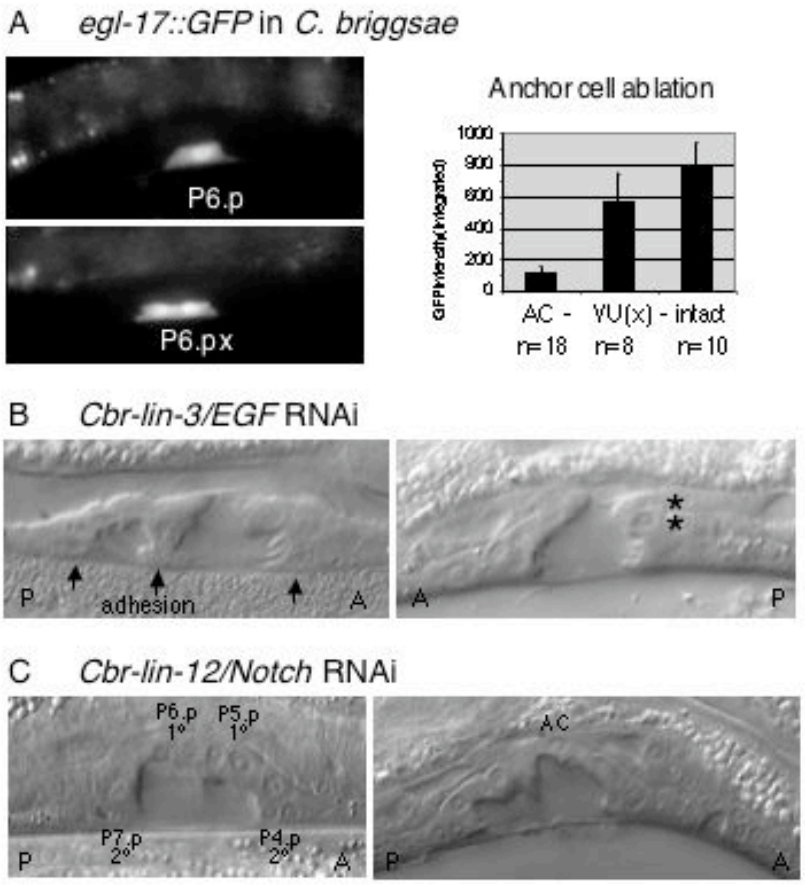

Figure 4

Figure 4. Molecular components of Ras and Notch pathways in *C. briggsae*.

A. The Ras pathway transcriptional reporter *egl-17::GFP* is activated in P6.p and P6.p daughters (fluorescence micrographs, left). Anchor cell ablation (from VU division until P6.p division) reduces *egl-17::GFP* expression (significantly different from VU ablation, $p<0.01$ Mann-Whitney test).

B. Partial inactivation of the *Cbr-lin-3* homolog by RNAi results in aberrant vulval cell fate patterns with transformation of 1° to 2° fates, visible by the attachment to the cuticle characteristic of the outer 2° lineage (left), or by an additional vulD-like cell, characteristic of the inner 2° lineage (stars, right). A: anterior. P: posterior.

C. Partial inactivation of the *Cbr-lin-12/Notch* homologs by RNAi results in aberrant vulval cell fate patterns with adjacent 1° fates and transformation of neighboring 3° cells to a 2° fate.

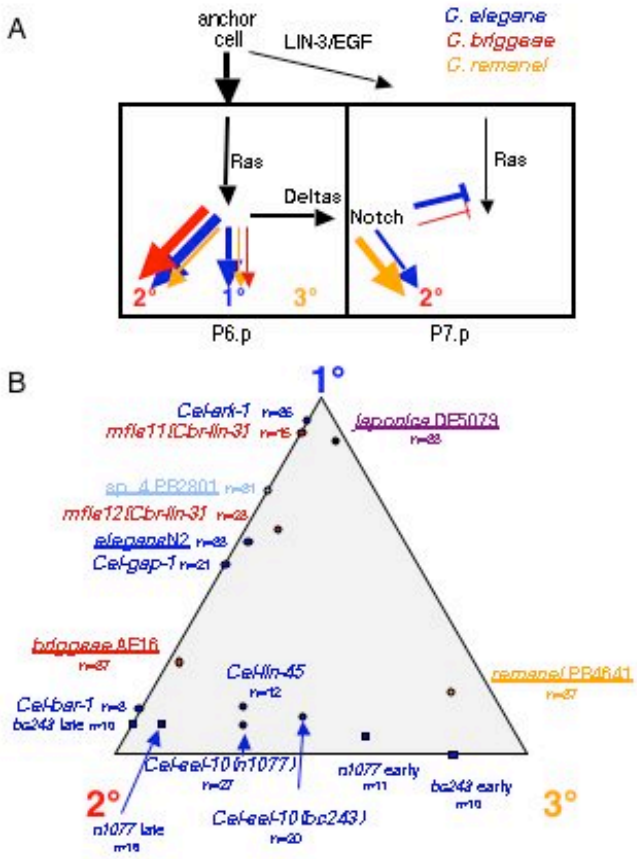

Figure 5

Figure 5. Experimental variations in Ras and Notch pathway activation levels mimic the system's evolution.
A. Hypothesis of quantitative variation in the vulval signaling network. The basic wiring of the EGF/Ras and Delta/Notch network is depicted (P5.p is not shown as it would be similar to P7.p). The behavior of different *Caenorhabditis* species may be explained by evolution in relative strengths of network components, schematized as arrows of different thicknesses. The colors refer to the different species. In *C. elegans* (blue), Ras signaling has several downstream effects: induction of the 2° fate by low levels of Ras signaling, of the 1° fate at higher levels, and of Delta transcription. Notch activation in the neighboring cells has two downstream effects: 2° fate induction and Ras pathway inhibition. '2°2°2°' fate pattern of *C. briggsae* (red) upon anchor cell ablation may be explained by a higher threshold necessary for 1° fate activation; the adjacent 1° fates observed after Cbr-LIN-3 overexpression may correspond to less active lateral inhibition. Finally, the '2°3°2°' fate pattern of *C. remanei* (orange) are explained by a lower relative threshold for Delta transcription compared to P6.p fate specification.
B. Ternary plot of P6.p fate after anchor cell ablations, as in Figure 2. Wild type genotypes are underlined. The *mfIs12* transgene results in almost silent Cbr-LIN-3 overexpression (Table 2). *lin-45(n1018)* is a mild Raf hypomorph and *bar-1(ga80)* is a null mutation in a β-catenin gene; both mutations result in a mild hypoinduction (mean induction index in the 2.5-3 range). *sel-10* mutations result in hyperactivation of the Notch pathway. See Tables S20-S29 for detailed lineages.

Table 1. P6.p adopts a 2° fate upon anchor cell ablation in *C. briggsae* AF16

Cell fate marker	Ablation	Descendants of P5.p	P6.p	P7.p	# of animals
A. *C. briggsae* *mfIs5[Cb-egl-17::GFP]* in AF16 background	intact	LL**T**U	TTTT	U**T**LL	many
	AC -		**T**UU**T** **T**UTT **T**TTT		6/8 1/8 1/8
	VU -		TTTT		4/4
B. *C. briggsae* *mfIs8[Cb-zmp-1::GFP]* in AF16 background	intact	**L**LTU	**T**TT**T** 4 GFP+	UTL**L**	many
	AC -		0 GFP+		9/9
	VU -		4 GFP+ 2 GFP+		12/13 1/13

Upon anchor cell ablation, P6.p mostly adopts a 'TUUT' lineage in *C. briggsae* AF16. Use of granddaughter fate markers shows that this 'TUUT' lineage corresponds to internal 2° lineages. See Fig. 1B for Pn.p lineages. L: longitudinal division (antero-posterior) of Pn.p granddaughter; T: transverse (left-right) division; U: undivided. Cells that adhered to the cuticle are underlined. AC: anchor cell. VU: ventral uterine precursor (control). Bold indicates late L4 stage GFP expression. 'n GFP+' indicates the number of GFP-expressing cells (0-4).

Table 2. Vulval cell lineages in *C. briggsae* upon *Cbr-lin-3* overexpression from its own anchor cell promoter

	P3.p	P4.p	P5.p	P6.p	P7.p	P8.p	n
C. briggsae	S	S S	LLTU	TTTT	UTLL	S S	67/82*
AF16	S S	S S	LLTU	TTTT	UTLL	S S	15/82
A.	S	S S	LLTU	TTTT	UTLL	S S	11/27
C. briggsae	S S	S S	LLTU	TTTT	UTLL	S S	9/27
mfIs12[Cbr-lin-3]	S S	S S	LLTT	TTTT	UTLL	S S	1/27
in AF16 background	S S	S S	LLTU	TTTT	TTLL	S S	1/27
	S S	S S	LLTU	TTTT	TTTL	S S	1/27
mean induction index:	S S	LLTU	TUTT	TTTT	UTLL	S S	1/27
3.15 (n=27)	S S	LLTU	TTTT	TTUT	UTLL	S S	1/27
	S	LLTU	TTTT	TTTT	UTLL	S S	1/27
	S S	LLTU	TTTT	TTTT	UTLL	S S	1/27
B.	S	S S	LLTU	TTTT	UOLL	S DL	1/9§
C. briggsae	S	S S	LL**TU**	TTTT	**UT**LL	L**LL**L	1/9
mfIs11[Cbr-lin-3];	S	ss**O**D	DDTT	TTTT	**UTL**L	sssL	1/9
mfIs5[Cbr-egl-17::GFP]	n.d.	LL**T**U	TTLL	TTTT	UTL**L**	S S	1/9
in AF16 background	S S	LLTU	TTTT	TTTO	UTLL	S LL	1/9#
	S S	LL**T**U	TTTD	DLOT	UTTL	U**T**LL	1/9
mean induction index:	S S	LL**T**U	TTTT	TTTT	TTLO	TT**L**L	1/9
4.4 (n=9)	S S	LL**T**U	TTDD	TTTT	TTTT	U**L**LL	1/9
	S S	LL**T**U	TTTT	TTTT	TTLT	U**O**LL	1/9
C.	S	OTTO	LTTT	TTTO	TTLL	TTOL	1/7
C. elegans	LLLL	LOOO	OLTT	TTOL	LLLO	TTTL	1/7
mfIs10[Cbr-lin-3]	LTOL	LLLL	OTTT	TTTT	TTLL	LTTT	1/7
in N2 background	LOOO	OTTT	LLTT	TTTT	TLLL	TOLL	1/7
	LOLL	OLTL	OTTT	TTTT	TLLL	LTTL	1/7
	LLOT	LLLU	TTTT	TLLL	TOLL	LTTL	1/7
	LTTL	LLLL	TTLO	LTTT	TLOL	LLLL	1/7

S/s indicates a 3° non-vulval fate (fusion to the hyp7 syncytium of Pn.p daughters or granddaughters, respectively); L: longitudinal division (antero-posterior); T: transverse (left-right) division; U: undivided; O: oblique division; D: division, orientation not observed. Cells that adhered to the cuticle are underlined (characteristic of 2° fate). In (B), GFP fluorescence was scored in the late L4 stage and corresponds to vulC/D fates (bold). *: data from [50]. §: expression of *egl-17::GFP* in three unidentified progeny of P5.p and two of P7.p. #: expression in one progeny of P7.p.

Table 3. Vulval cell fates upon *Cbr-lin-12* inactivation by RNAi in *C. briggsae*

	P4.p	P5.p	P6.p	P7.p	P8.p	n
wild type	3	2ei	1	2ie	3	
A.	2ei	1	1	2ie	3	4/23
Cbr-lin-12 RNAi	3/v	2e/1	1	1/2e	2i/3	1/23
Adult parents	2e/3	2/1	1	1/2e	3	1/23
	2ei	2e/1	1	2ie	3	1/23
	2e/3	2ei	1	2ie	3	1/23
	3	2ei	1	2ie	2i/3	1/23
	3	2ei	1	1/2e	3	1/23
	3	2ei	1	2ie	3	13/23
B.	2ei	1	1	1/2e	2i/3	1/37
Cbr-lin-12 RNAi	2ei	1	1	2ie	3	1/37
Embryos/L1	2ei	1	1	3	2ie	1/37
	2e/3	2e/1	1	1	2ie	1/37
	2ei	2e/1	1	2ie	3	1/37
	3/v	2e/1	1	2ie	3	2/37
	3	2e/1	1	2i/3	3/v	1/37
	3	2ei	1	2ie	2ie	1/37
	3	2ei	1	1/2e	2i/3	1/37
	3	2e/1	1	1/2e	3	1/37
	3	2e/1	1	2ie	3	2/37
	3	2ei	1	2ie	3	24/37

The stage at which the animals or their parents were placed onto RNAi plates is indicated in the left column. Pn.p fates are indicated by the corresponding number (1/2/3). 2° fates are further separated into the external '2e' fate of the Pn.p daughter (scored using the attachment to the cuticle of 'LL' sublineage) and the internal '2i' fate (scored as a 'TU' sublineage with one undivided daughter). 'v' = undetermined vulval fate.

Supplement

I. Experimental procedures

Strains and culture conditions. Wild isolates of the different *Caenorhabditis* species were obtained from the *Caenorhabditis* Genetics Center, K. Kiontke, D. Fitch and W. Sudhaus, or the wild. *C.* sp. 5 JU727 was isolated from soil near fallen fruits under an unidentified wild tree in Chengyang, Guangxi, China (May 2005). *C. remanei* JU724 was isolated from soil in cultivated fields in Zhouzhuang, Jiangsu, China (May 2005). See [1] for *C. briggsae*, [2] for other *C. remanei* genotypes and [3] for the other species. The animals were cultured at 20°C under standard conditions [4], unless otherwise indicated. *C. elegans* and *C. briggsae* reproduce through self-fertile hermaphrodites and facultative males and laboratory strains are isogenic. All other species reproduce through males and females. PB4641 and PB2801 are inbred derivatives.

<u>*C. elegans* (N2 background)</u>

JU646: *lin-45(n2018) IV* [5] obtained by backcrossing WU48 six times to N2, removing *dpy-20(e1282) IV* (gift of J. Milloz).

PS1461: *ark-1(sy247) IV* [6].

MD1290: *sel-10(bc243) V* (gift of B. Conradt). MT2244: *sel-10(n1077) V*. *n1077* is a dominant negative allele and *bc243* is a putative null allele [7].

AH12: *gap-1(ga133) X* [8].

EW15: *bar-1(ga80) X* [9].

JU703: *ark-1(sy247); gap-1(ga133)* [6] obtained by backcrossing HP13 four times to N2 (gift of J. Milloz).

JU972: *lin-45(n1018); sel-10(n1077).*

JU673: *mfIs4[Cel-egl-17::YFP; Cel-daf-6::CFP; Cel-unc-119(+)]; mfIs10[Cbr-lin-3; Cel-ttx-3::GFP; Cel-unc-119(+)].*

PS3997: *syls77[Cel-zmp-1::pes-10::YFP]; syls59[Cel-egl-17::CFP]* (gift of P. Sternberg).

C. briggsae (AF16 background)

JU610: *mfIs5[Cbr-egl-17::GFP; Cel-myo-2::GFP].*

JU613: *mfIs8[Cbr-zmp-1::GFP; Cel-myo-2::GFP].* GFP expression is variable for vulA in (2° lineage), but consistent for vulE (1° lineage).

JU616: *mfIs11[Cbr-lin-3; Cel-myo-2::GFP].*

JU617: *mfIs12[Cbr-lin-3; Cel-myo-2::GFP].*

JU675: *mfIs5[Cbr-egl-17::GFP; Cel-myo-2::GFP]; mfIs11[Cbr-lin-3; Cel-myo-2::GFP].*

JU690: *mfIs15[Cbr-sma-6(+); Cel-egl-17::GFP (NH#293); Cel-odr-1::DsRed]; sma-6(sy5148).* Unlike the *egl-17* construct in the *mfIs5* transgene, which may only be used as a L4 stage reporter for 2° inner cell fates, the *egl-17* reporter in the *mfIs15* transgene contains the regulatory regions driving L3 stage expression in P6.p and can be used as a Ras pathway reporter [10].

JU944: *mfIs37[Cel-hsp16-41::EGF-LIN-3 (pRH51), Cel-myo-2::GFP, Cel-odr-1::DsRed].*

HC189: *qtIs21[Cel-SID-2::GFP; pEON2(rol-6(su1006))]* (gift of W. Winston and C. Hunter).

Cell lineage and ablation. Cells were ablated with a laser microbeam (Photonics Instruments), using standard methods [4]. All individuals in the L2 lethargus or early-mid L3 stage were selected from a non-synchronized population. The animals were then cultured at 25°C, and cell lineages followed at room temperature (20-25°C) under Nomarski (100x objective) during the late L3 stage to early L4 stage. Some animals were followed continuously, but observations at the L3-to-L4 lethargus (when most divisions occur) and

one-two hours later were usually sufficient to infer the cell lineage. Cell nomenclature was as in [11-13]. The assignment of vulval cell fates from cell lineage and morphology criteria was as in [13]. Briefly, fusion to hyp 7 of a Pn.p daughter ('S') or granddaughter ('s') indicates a 3° non-vulval fate. The 1° lineage ('TTTT') is characterized by division of all four granddaughters and no attachment to the cuticle. The 2° lineage ('LLTU' or 'UTLL') is characterized by attachment to the cuticle (underline) and absence of division ('U') of one granddaughter. An ambiguous cell lineage is 'LLTT', as it may be a modified 2° lineage [13] or a lineage with the anterior daughter adopting a 2° lineage and the posterior daughter a 1° lineage [14]. This lineage pattern was scored as 2° as in [13]. In the experiments in Table 3, the 'TT' progeny clearly aligned with and apparently fused to the adjacent vulE/F rings made by P6.p progeny, and was thus scored as a 2° internal fate (see [15] for the equivalent lineage observed for *lin-12* hypomorphs in *C. elegans*).

Transgenesis. Plasmids were injected into the syncytial germ line of adult hermaphrodites, at a final concentration of 100-150 ng/□l in the needle [16]. Efficiency of extra-chromosomal array formation is lower in *C. briggsae* compared to *C. elegans*, and is even lower in other *Caenorhabditis* species. Transgene expression follows the same trend. The *Cbr-lin-3* gene was PCR-amplified from AF16 genomic DNA using oligonucleotides oMA140 (ctcggatccattccggtggtttcttatgc) and oMA141 (ctcaagcttgatcaggctgcccacctgg) and cloned into pGEM-T/Easy (Promega), yielding plasmid pMA48. The *Cbr-egl-17::GFP* (mk160-161) and *Cbr-zmp-1::GFP* (mk172-173) plasmids were gifts of M. Kirouac and P. Sternberg [17]. *mfIs15* is a spontaneous integrant. Extra-chromosomal arrays were integrated by γ-irradiation (4,000 rad) of 100-150 young adults bearing the transformation marker. Ten irradiated adults were placed on a standard culture plate. Each plate was then chunked twice to a new plate. After 3-4 generations, integrants were identified by isolating 15-20 (marker-positive) animals

per plate and scoring for 100% segregation of the marker in their progeny. The integrants were backcrossed 5-10 times to the corresponding wild background.

RNAi. Bacterial-mediated RNAi was performed as in *C. elegans* [18] using the sensitive HC189 *C. briggsae* strain (W. Winston and C. Hunter, pers. comm.). A *Cbr-lin-3* gene fragment was amplified using oMA154 (aggcggccgCAGTCTTCATCCTCGGCC) and oMA155 (tgccatggTTAAAGATGCATATGAGGA) oligonucleotides. A fragment of the *Cbr-lin-12* gene fragment was amplified using oMA158 (agccatggTACTTGTTCTTC) and oMA159 (tggcggccgcTTCTCACTGAACATT); the amplified fragment is 99% identical to the duplicated *Cbr-lin-12* paralog [19] (www.wormbase.org) and therefore likely to inactivate both copies. PCR products were cloned into pPD129.36 using NotI/NcoI restriction sites and plasmids grown in *E. coli* HT115. HC189 animals (five adults or about 50 embryos/L1s) were placed on feeding RNAi plates and incubated at 25°C. In some experiments, the *Cel-rol-6* clone from the Ahringer library was added to suppress the Roller phenotype of HC189 (this however decreased RNAi efficiency for the gene of interest).

Fluorescence. The animals were observed under GFP epifluorescence (100x) with an AxioImager M1 (Zeiss) microscope equipped with a CoolsnapES (Roper Scientific) camera. For quantification, animals were immobilized using 10 mM azide in the mounting M9 solution. A picture was taken at the same exposure time of 100 ms for all animals, without prior illumination to avoid photobleaching. Fluorescence intensity was measured after background substraction and integrated using the contour feature of Metaview.

II. Tables of vulval cell lineages

- Anchor cell (and P6.p) ablations at successive time points in different strains and species of the *Caenorhabditis* genus (in the order of Fig. 1A, from top to bottom):

Table S1	*C. briggsae* AF16 (anchor cell and P6.p ablations)
Table S2	*C. briggsae* HK104
Table S3	*C. briggsae* JU725
Table S4	*C.* sp. 5 JU727
Table S5	*C.* sp. 5 SB378
Table S6	*C. remanei* PB4641 (anchor cell and P6.p ablations)
Table S7	*C. remanei* PB228
Table S8	*C. remanei* JU724
Table S9	*C.* sp. 4 PB2801
Table S10	*C.* sp. 4 CB5161
Table S11	*C. elegans* N2
Table S12	*C. japonica* DF5079
Table S13	*C. japonica* DF5080
Table S14	*C.* sp. 3 RGD1
Table S15	*C.* sp. 2 DF5070
Table S16	*C. drosophilae* DF5077
Table S17	*C. plicata* SB355

- Heat-shock induction of the LIN-3 EGF domain in *C. briggsae*:

Table S18	Gonad-ablated *mfIs37* animals
Table S19	Intact *mfIs37* animals

- Anchor cell ablations in *C. briggsae* strains overexpressing *Cbr-lin-3* from its own anchor cell promoter:

Table S20	*mfIs11* (high overexpression)
Table S21	*mfIs12* (mild overexpression)

- Anchor cell ablations in different *C. elegans* mutants:

Table S22	*lin-45(n2018)*
Table S23	*bar-1(ga80)*
Table S24	*sel-10(n1077)*
Table S25	*sel-10(bc243)*
Table S26	*lin-45(n2018); sel-10(n1077)*
Table S27	*gap-1(ga133)*
Table S28	*ark-1(sy247)*
Table S29	*ark-1(sy247); gap-1(ga133)*

The cell lineages are coded in the following manner:

S: fusion of the Pn.p daughter (or the Pn.p cell itself) to hyp7.

s: fusion of a Pn.p granddaughter.

U: undivided Pn.p granddaughter.

L: longitudinal division of a Pn.p granddaughter. Not every division was observed during mitosis: direction of division was sometimes inferred from the daughter's position in the early L4 stage.

T: transverse division.

O: oblique division.

V: dorso-ventral division.

D: division, orientation not observed.

X: ablated cell.

Italics indicate that the daughter cells divided once more.

Underline indicates attachment to the cuticle in the early L4 stage.

Ind.: induced cell, undetermined cell lineage (the progeny number is sometimes indicated).

AC: anchor cell.

DU: dorsal uterine precursor.

VU: ventral uterine precursor.

The ablation timepoints follow several cell lineage landmarks: molt and Pn.p or uterine precursor divisions. Instead of dividing before the 3° Pn.p cells, VUs - and even the DUs in *C. japonica* - divide in some animals at the same time as the 3° Pn.p cells: in this case, the Pn.p cells are used as reference.

Table S1. Anchor cell and P6.p ablations in *Caenorhabditis briggsae* AF16

Cell(s) Ablated	Time of ablation	Descendants of			# of animals
		P5.p	P6.p	P7.p	
-	-	LLTU	TTTT	UTLL	
AC	early L3	S S	S S	S S	3/10
		LL S	S S	S ss	1/10
		S S	OT S	S S	1/10
		DO S	S TT	S S	1/10
		LOTU	S S	S Os	1/10
		LLss	S UU	S LL	1/10
		sLTU	TU S	S LO	1/10
		LLTU	TTUT	UTLL	1/10
AC	DU dividing	ssss	DU ss	LU S	1/6
		LL S	OUUT	S S	1/6
		LOss	*O*TUO	ssLL	1/6
		LLOU	LUTO	UTLL	1/6
		LLTU	TTTT	UDUs	1/6
		LLTU	OOOO	UTLL	1/6
AC	DU divided VU 1-cell or dividing	LLTU	TUUT	UTLL	4/7
		LLTU	TTTT	UTLL	2/7
		LLTU	TTUT	UTLL	1/7
AC	3° dividing or divided	LLTU	TUUT	UTLL	17/23
		LLTU	TUTT	UTLL	3/23
		LLOU	TUUT	UTLL	1/23
		LLTU	OOOT	UTLL	1/23
		LLTU	TDDT	UTLL	1/23
AC	P(5-7).p dividing	LLTU	TTUT	UTLL	2/9
		LLTU	TTOT	UTLL	2/9
		LLTU	TTTT	UTLL	2/9
		LLTU	TUUT	UTLL	1/9
		LLTU	TUTT	UTLL	1/9
		LLTU	TOTT	ULLL	1/9
AC	Pn.p 2-cell stage	LLTU	TTTT	UTLL	6/7
		LLOU	TTTT	UTLL	1/7
AC + P6.p	DU dividing	LLTU	X	UTLL	2/2
AC + P6.p	DU divided	LLTU	X	UTLL	5/5
AC + P6.p	3° dividing	LLTU	X	UTLL	13/13
AC + P6.p	3° divided	LLTU	X	UTLL	6/6

Table S2. Anchor cell ablations in *Caenorhabditis briggsae* HK104

Cell(s) Ablated	Time of ablation	Descendants of P5.p	P6.p	P7.p	# of animals
-	-	LLTU	TTTT	UTLL	
gonad	early L1	S S	S S	S S	4/5
		total =	13	progeny	1/5
AC	L2 lethargus	ss S	S S	S S	2/8
		S S	S ss	S ss	1/8
		S ss	ss S	S ss	1/8
		S S	LL S	S S	1/8
		S S	LOUT	S S	1/8
		ss S	ind. S	all S	1/8
		LLsU	DUDD	UT*sL*	1/8
AC	early L3	LL S	S S	S S	1/4
		LL S	UUss	sssL	1/4
		S UL	ssss	UUUU	1/4
		S ind.	ind.	S S	1/4
AC	DU dividing or divided	S S	S LL	S LL	1/2
		LDDs	*L*OUO	UTLL	1/2
AC	3° dividing	LLss	LOUT	UTLL*	1/3
		LLTU	DUDD	UTLL	1/3
		LLTU	TTTT	UTLL	1/3
AC	3° divided	LLTU	TUUT	UTLL	10/15
		LLTU	TUTT	UTLL	1/15
		LLTU	TTUT	UTLL	1/15
		LLTU	TDTT	UTLL	1/15
		LLOO	DUUT	UTLL	1/15
		LLOU	DDDL	UOLL	1/15
AC	P(5-7).p dividing	LLTU	TUUT	UTLL	4/4
AC	P(5-7).p early 2-cell stage	LLTU	TUUT	UTLL	1/3
		LLTU	TUTT	UTLL	1/3
		LLTU	TTTT	UTLL	1/3
AC	P(5-7).p late 2-cell	LLTU	TTTT	UTLL	3/3

Table S3. Anchor cell ablations in *Caenorhabditis briggsae* JU725

Cell(s) Ablated	Time of ablation	Descendants of P5.p	P6.p	P7.p	# of animals
-	-	LLTU	TTTT	UTLL	
AC	L2 lethargus	S S	S S	S S	6/6
AC	early L3	S S	ss S	S S	1/6
		ss S	S S	S S	1/6
		S S	ss S	S ss	1/6
		ss S	ss S	S S	1/6
		S UU	S S	S LL	1/6
		LLL*L*	TUUT	UTL*L*	1/6
AC	DU dividing	LUUL	UUUs	S DD	1/2
		LLTU	TDTT	UTLL	1/2
AC	DU divided	LL S	S S	UOLL	1/1
AC	3° dividing	S S	ssss	S LL	1/5
		LLTU	TUUT	UTLL	4/5
AC	3° divided	LUTL	S S	UTLL	1/5
		LLTU	TUUT	UTLL	4/5
AC	P(5-7).p dividing	LLTU	TUUD	UTLL	1/2
		LLTU	TTUT	UTLL	1/2
AC	Pn.p 2-cell stage	LLTU	TTTT	UTLL	2/2

Table S4. Anchor cell ablations in *Caenorhabditis* sp. 5 JU727

Cell(s) Ablated	Time of ablation	Descendants of P5.p	P6.p	P7.p	# of animals
-	-	LLTU	TTTT	UTLL	
AC + 3 VUs	early L2	S S	S S	S S	6/6
AC	L2 lethargus	LL S	S S	S S	1/1*
AC	early L3	LLTU	TUUT	UTLL	6/12
		LLTU	LLUT	UTLL	1/12
		LLsU	UTUU	UULL	1/12
		LLUU	UUUU	UO S	1/12
		S UU	TDDD	UU S	1/12
		LLUT	S TL	S	1/12
		LLTU	TTTT	UTLL	1/12
AC	DU dividing or divided	LLTU	TUUT	UTLL	2/3
		LLTU	TTTT	UTLL	1/3
AC	P4.p dividing	LLTU	TTUT	UTLL	1/1
AC	P8.p divided	LLTU	TUUT	UTLL	4/7
		LLTU	TTTT	UTLL	2/7
		LLTU	DDUT	UTLL	1/7
AC	P(5-7).p dividing	LLTU	TUTT	UTLL	1/2
		LLTU	TTTT	UTLL	1/2
AC	Pn.p 2-cell stage	LLTU	TTTT	UTLL	4/4

* : other animals showed regulation in AC specification and are not shown.

Table S5. Anchor cell ablations in *Caenorhabditis* sp. 5 SB378

Cell(s) Ablated	Time of ablation	Descendants of			# of animals
		P5.p	P6.p	P7.p	
-	-	LLTU	TTTT	UTLL	
AC	early L3	LLss	ss S	S S	1/6
		ssss	sUTL	S ss	1/6
		s???	ssss	ssss	1/6
		LL S	ssss	ssLL	1/6
		LDDU	ssss	UTLL	1/6
		LLsU	LLTU	UTLL	1/6
AC	DU dividing	DDss	ssss	UULL	1/3
		LLTU	TUUT	UTLL	1/3
		LLTU	TTTT	UTLL	1/3
AC	DU divided	LLTU	ssss	UTss	1/5
		LLTU	S S	UOLL	1/5
		LLTU	TU S	UTLL	1/5
		LLTU	TTUT	UTLL	1/5
		LLTU	TTTT	UTLL	1/5
AC	VU dividing	LLTU	sDDs	UTLL	1/2
		LLTU	sUTT	UTLL	1/2
AC	3° dividing	LLTU	TUUT	UTLL	1/3
		LLTU	TTTT	UTLL	2/3
AC	3° divided	LLTU	TUUT	UTLL	2/3
		LLTU	TTTT	UTLL	1/3
AC	P(5-7).p dividing	LLTU	TUUT	UTLL	1/4
		LLTU	TUTT	UTLL	2/4
		LLTU	TTTT	UTLL	1/4
AC	Pn.p 2-cell stage	LLTU	TTTT	UTLL	2/2

Table S6. Anchor cell and P6.p ablations in *Caenorhabditis remanei* PB4641

Cell(s) Ablated	Time of ablation	Descendants of P5.p	P6.p	P7.p	# of animals
-	-	LLTU	TTTT	UTLL	
AC	L2 lethargus	S S	S S	S S	1/1
AC	early L3	S S	S S	S S	23/30
		S DU	S S	S S	1/30
		S DU	S S	S S	1/30
		LLTU	S S	S S	1/30
		LLTU	S S	UTLL	1/30
		LLTU	S S	ULLL	1/30
		LLTU	S ss	UTLL	1/30
		S S	DDDD	S S	1/30
AC	DU dividing or divided	S S	S S	S S	4/11
		LLss	S S	S S	1/11
		UUUU	S S	sLss	1/11
		LLTU	S S	UTLL	3/11
		LLDD	S S	UTLL	1/11
		sLTU	S LT	LOUU	1/11
AC	VU dividing or divided	UUUU	S S	S S	1/9
		LLTU	S S	ULLs	1/9
		LLTU	S S	UTLL	3/9
		LLTU	S DD	UTLL	1/9
		LLTU	DD S	UDDD	2/9
		LL S	LDDD	DTLL	1/9
AC	3° dividing or divided	DDDU	S S	UDDD	4/17
		LLTU	S S	UTLL	1/17
		LLTU	S S	UTTL	1/17
		LLUU	S S	UTLL	1/17
		LOLU	S S	OLLL	1/17
		LLTU	S UD	UTLL	1/17
		LLTU	S DD	UTLL	1/17
		LLTU	S OO	LTLL	1/17
		LLTU	DU S	UTLL	1/17
		LLTU	TU S	S S	1/17
		LLTD	LT S	UTLL	1/17
		LLTU	TTUT	UTLL	1/17
		LLTT	TTTT	OTLL	1/17
		LLTU	TTTT	UTLL	1/17
AC	P(5-7).p dividing	LLTU	TTTT	UTLL	8/11
		LLTU	TTUT	UTLL	1/11
		LLTU	TUTO	UTLL	1/11
		LLTU	OO S	ULLL	1/11
AC	Pn.p 2-cell stage	LLTU	TTTT	UTLL	6/6
AC + P6.p	DU dividing	S S	X	S S	1/1
AC +	DU divided	S ss	X	S S	1/2

P6.p		ssTU		UTLL	1/2
AC +	VU	S S	X	S S	1/6
P6.p	dividing	S S		ss S	1/6
	or divided	S S		UU S	1/6
		LL S		TTLL	1/6
		LLTU		DD S	1/6
		LLTU		ss S	1/6
AC +	3° dividing	S S	X	S S	3/21
P6.p	or divided	ss S		S S	1/21
		S ss		S S	1/21
		S DU		S S	1/21
		LL S		S S	1/21
		ss DD		S S	1/21
		LLDD		S LL	4/21
		LLDD		DDLL	2/21
		LLDU		DDLL	1/21
		LLDD		UDLL	1/21
		LLTU		UTLL	3/21
		LLDD		DDDD	1/21
		DDDs		DDDD	1/21
AC +	P(5-7).p	LLTU	X	UTLL	3/6
P6.p(x)	dividing	LLTU		UTLs	1/6
	or 2-cell stage	LLTU		TTLL	1/6
		LLDD		DTLL	1/6

Table S7. Anchor cell ablations in *Caenorhabditis remanei* PB228

Cell(s) Ablated	Time of ablation	Descendants of P5.p	P6.p	P7.p	# of animals
-	-	LLTU	TTTT	UTLL	
AC	L2 lethargus	S S	S S	S S	1/1
AC	early L3	S S	S S	S S	2/9
		LLTU	S S	S LL	1/9
		LL S	S S	UOLL	1/9
		LLTU	ss S	UOLL	1/9
		LOOU	S S	UOLL	1/9
		LLTU	UU S	UTLL	1/9
		LLTU	TU S	UTLL	1/9
		LLTU	TUTT	UTLL	1/9
AC	DU dividing or divided	S S	S S	S S	10/20
		S S	S S	ssss	1/20
		LLTU	S S	UTLL	2/20
		LLOU	S S	UTLL	1/20
		LLOU	S S	UOLL	1/20
		LLLU	S S	ULUU	1/20
		LLLU	S ss	UTLL	1/20
		LLLU	S TT	UTLL	1/20
		LLTU	TUUT	UTLL	1/20
		LLTU	TTUT	UTLL	1/20
AC	VU dividing	LLTU	S S	UOLL	1/7
		LLTU	S S	UODD	1/7
		LLTU	S ss	UTLL	1/7
		LsTU	S S	UTsL	1/7
		LLTU	S S	UTsL	1/7
		LLTU	S UT	UTLL	1/7
		LLTU	QUUT	UTLL	1/7
AC	3° dividing or divided	LLTU	S S	UTLL	1/17
		LOOU	S S	UTLD	1/17
		LLTU	S ss	UTLL	1/17
		LLTU	S TT	UTLL	1/17
		LLTU	S UT	UTLL	1/17
		LLTU	S UO	UTLL	1/17
		LLTU	TU S	UTLL	1/17
		LLLU	DD S	UTLL	1/17
		LLTU	TUss	UTLL	1/17
		LLTU	TUUT	UTLL	3/17
		LLTU	TUTT	UTLL	3/17
		LLTU	TUOT	UTLL	1/17
		LLTU	TTTT	UTLL	1/17
AC	P(5-7).p dividing	LLTU	TUUT	UTLL	3/7
		LLTU	TTTT	UTLL	3/7
		LLTU	TUTT	UTLL	1/7
AC	Pn.p 2-cell stage	LLTU	TTTT	UTLL	4/4

Table S8. Anchor cell ablations in *Caenorhabditis remanei* JU724

Cell(s) Ablated	Time of ablation	Descendants of P5.p	P6.p	P7.p	# of animals
-	-	LLTU	TTTT	UTLL	
AC	L2 lethargus	S S	S S	S S	1/2
		LLTU	S TT	UTLL	1/2
AC	early L3	S S	S S	S S	3/8
		LLss	S S	UU S	1/8
		LDLD	S S	S S	1/8
		LLTU	S S	ssLL	1/8
		LOOU	S S	LL S	1/8*
		LLTU	TUTT	UTLL	1/8
AC	DU dividing	S UU	S S	S S	1/4
		S S	S S	UULL	1/4
		LLOU	ss S	sULL	1/4
		LLTU	S S	UTLL	1/4
AC	DU divided	LL S	ssss	ss S	1/1
AC	VU dividing	LLTU	ss S	UTLL	1/2
		LLTU	S UT	UOLL	1/2
AC	3° dividing	LLTU	S UU	UTLL	1/3
		LLTU	TUUT	UTLL	1/3
		LLTU	TTUT	UTLL	1/3
AC	3° divided	LLTU	TTUT	UTLL	1/1
AC	P(5-7).p	LLTU	TUUT	UTLL	1/2
	dividing	LLTU	TTTT	UTLL	1/2
AC	Pn.p	LLTU	TTTT	UTLL	3/4
	2-cell stage	LLTU	TTUT	UTLL	1/4#

*: centered on P7.p: lineage shown for P(6-8).p.
#: centered on P5.p: lineage shown for P(4-6).p.

Table S9. Anchor cell ablations in *Caenorhabditis* sp. 4 PB2801

Cell(s) Ablated	Time of ablation	Descendants of P5.p	P6.p	P7.p	# of animals
-	-	LLTU	TTTT	UTLL	
AC	early L3	S S	S S	S S	12/18
		S ss	S S	S S	1/18
		S S	S S	LL S	1/18
		S LD	DL S	DD S	1/18
		S DD	S DD	DL S	1/18
		S DD	DD S	DD S	1/18
AC	DU undivided	S S	S S	S S	1/8
		S UU	S S	S S	1/8
		S S	S S	UL S	1/8
		S OU	LLTT	ULLL	1/8*
		sDDD	DDDD	DD S	1/8
		LLTU	TTTT	UTLL	1/8
		LLOU	UTTT	UDLL	1/8
		LLOU	TTTT	UTLL	1/8
AC	DU dividing	S S	S S	LT S	1/10
		S LL	S S	LL S	1/10
		LL S	ss UU	S LL	1/10
		S sU	LLTT	UT S	1/10
		S LL	OOUT	UO S	1/10
		S OU	UUUU	OTLL	1/10
		LLTU	LTTT	TL S	1/10
		LLTU	UTUT	UT S	1/10
		LLTU	TTTT	UL S	1/10
		LLTU	TTTT	UTLL	1/10
AC	DU divided	LDDT	LLTT	UTLL	1/4
		LLLL	TTTT	UTLL	1/4
		LLLL	TTOT	LOLL	1/4
		LLTU	TTTT	UTLL	1/4
AC	VU dividing	LLTU	TTTT	UTLL	3/3
AC	3° dividing or divided	LLTU	TTTT	UTLL	8/12
		LLTO	TTTT	UTLL	1/12
		LLOU	TTTT	UTLL	1/12
		LDDU	TTTT	UTLL	1/12
		LLTU	TUTT	UTLL	1/12
AC	P(5-7).p dividing	LLTU	TTTT	UTLL	5/6#
		LLTU	TTUT	UTLL	1/6
AC	Pn.p 2-cell stage	LLTU	TTTT	UTLL	8/8

*: centered on P5.p
#: 1 with P8.p also induced (LLLL)

Table S10. Anchor cell ablations in *Caenorhabditis* sp. 4 CB5161

Cell(s) Ablated	Time of ablation	Descendants of P5.p	P6.p	P7.p	# of animals
-	-	LLTU	TTTT	UTLL	
AC	early L3	LL S	S S	S S	1/17
		ssTU	S S	S S	1/17
		LL S	ss S	S S	1/17
		ss S	S S	S LL	1/17
		LLTU	S S	S LU	1/17
		LLTU	S S	S LL	1/17
		LLTU	S OT	S S	1/17
		LL S	LL S	UTLL	1/17*
		LL S	S TT	UTLL	1/17
		LLTU	S S	UTLL	1/17
		LLTU	OLLT	S UL	1/17
		ssTU	TTTT	UTLL	1/17
		LLTU	LLTU	UTLL	1/17*
		LLOU	LLTT	UTLL	1/17
		LLTU	TTTT	UTOL	1/17
		LLTU	TTTT	UTLL	2/17
AC	DU dividing	LLTU	TTTT	UTLL	3/9
		LLLU	DUDD	UOLL	1/9
		LLsU	TTTT	UTLL	1/9
		LLTU	UU S	UTLL	1/9
		LLTU	S S	UTLL	2/9
		LLTU	S ?0	UOLL	1/9
AC	VU dividing	LLTU	TTTT	UTLL	2/4
		LLTU	TTUT	UTLL	1/4
		LLTU	S S	UTLL	1/4
AC	3°dividing or divided	LLTU	TTTT	UTLL	13/13
AC	P(5-7).p dividing	LLTU	TTTT	UTLL	2/4
		LLTU	DDDD	UTLL	1/4
		S DD	S ss	UUUU	1/4
AC	Pn.p 2-cell stage	LLTU	TTTT	UTLL	5/5

*: centered on P5.p

Table S11. Anchor cell ablations in *Caenorhabditis elegans* N2

Cell(s) Ablated	Time of ablation	Descendants of			# of animals
		P5.p	P6.p	P7.p	
-	-	LLTU	TTTT	UTLL	
AC	L2 lethargus	S S	S S	S S	3/3
AC	early L3	S S	S S	S S	4/7
		ssOL	ss S	S S	1/7
		S S	LTTT	S S	1/7
		LLTU	TUTT	UTLL	1/7
AC	DU dividing	S S	S S	S S	2/2
AC	DU divided	S S	S S	S S	1/12
		S S	ss S	S S	1/12
		S UU	sssU	S S	1/12
		S S	S ss	UO S	1/12
		S S	S OU	S S	1/12
		S TU	S S	UOss	1/12
		S ss	VTLL	S S	1/12
		S S	LLUT	UT S	1/12
		LsTU	TUUT	S S	1/12
		LL S	TTTT	UT S	1/12
		LLOU	S S	UOLL	1/12
		LLTU	OUUO	UTLL	1/12
AC	VU dividing	LLTU	TTTT	UTLL	1/8
		LLTU	TTTT	OOLL	1/8
		LLTU	TDOT	UTLL	1/8
		LLOU	TOTT	UTLL	1/8
		LLTU	TUTT	UTLL	1/8
		LLTU	OOUT	UTLL	1/8
		LLTU	TULL	UTLL	1/8
		LLTU	TUUT	UTLL	1/8
AC	VU divided	LLTU	TTTT	UTLL	2/4
		LLTU	TTUT	UTLL	1/4
		LLTU	TUUT	UTLL	1/4
AC	3° dividing	LLTU	TUUT	UTLL	3/8
		LLOU	TUTT	UTLL	2/8
		LLTU	TTUO	UTLL	1/8
		LLTU	TTTT	UTLL	2/8
AC	3° divided	LLTU	TTTT	UTLL	4/5
		LLTU	TUTT	UOLL	1/5
AC	P(5-7).p dividing	LLTU	TUUT	UTLL	3/5
		LLTU	TUTT	UTLL	1/5
		LLTU	LTTT	UTLL	1/5
AC	Pn.p 2-cell stage	LLTU	TTTT	UTLL	8/11
		LLOU	TTTT	UTLL	2/11
		LLTU	TUTT	UOLL	1/11

Table S12. Anchor cell ablations in *Caenorhabditis japonica* DF5079

Cell(s) Ablated	Time of ablation	Descendants of P5.p	P6.p	P7.p	# of animals
-	-	LLTU	TTTT	UTLL	
AC	early L3	S S	S S	S S	8/18
		S S	S ss	S S	1/18
		S S	ss S	S S	1/18
		S S	ssss	S S	1/18
		S S	S S	S ss	1/18
		S S	S S	ss S	1/18
		ss S	S S	ss S	1/18
		ss sD	S S	S S	1/18
		S LU	S S	S S	1/18
		S S	S S	UT S	1/18
		LLTU	TTTT	UTLL	1/18
AC	DU dividing	S UO	S S	UT S	1/3
		LLTT	TD S	LTLL	1/3
		LLOL	LDTD	UTLL	1/3
AC	DU divided	LLTU	TOTT	OL ss	1/1
AC	VU dividing	LLTU	S ss	LT ss	1/3
		LOTU	TTDT	UTLL	1/3
		LLTU	TTTT	UTLL	1/3
AC	VU divided	LLTU	DDDD	OTLL	1/1
AC	3° dividing	LLTU	TTTT	UTLL	18/25*
	or divided	LLTU	TTTT	TTLL	1/25
	(DU/VU may	LLOT	TTTT	UTLL	1/25
	or may not	LLLU	TTDT	UDLL	1/25#
	have divided)	S S	TUTT	UTLL	1/25
		ss S	ODUT	DO S	1/25
		LLTU	S S	UTss	1/25
		LLTT	ssTT	TTLL	1/25
AC	P(5-7).p	LLTU	TTTT	UTLL	1/2
	dividing	LLTU	TOTT	UTLL	1/2
AC	Pn.p 2-cell stage	LLTU	TTTT	UTLL	3/3

*: 1 animal with a normal cell lineage but centered on P7.p
#: centered on P5.p

Table S13. Anchor cell ablations in *Caenorhabditis japonica* DF5080

Cell(s) Ablated	Time of ablation	Descendants of P5.p	P6.p	P7.p	# of animals
-	-	LLTU	TTTT	UTLL	
AC	late L2	S S	S S	S S	2/2
AC	early L3	S S	TUTT	S S	1/9
		LLTU	UTLL	S S	1/9
		DDLU	DDUT	UTLL	1/9
		LLTU	TUUT	UTLL	3/9
		LLTU	TTUT	UTLL	2/9
		LLTU	TTTT	UTLL	1/9
AC	DU dividing	sLTU	S S	UTLL	1/3
		S TU	S UT	UTLL	1/3*
		LLTU	TUTT	UTLL	1/3
AC	DU divided	LLTU	TUUT	UTLL	1/1
AC	VU dividing	LLTU	TUUT	UTLL	1/1
AC	3° dividing	LLTU	TUUT	UTLL	1/7
	DU divided	LLTU	TUUT	UOLL	1/7
		LLTU	TUUT	UTLL	1/7
		LLTU	TOUT	UTLL	1/7
		LLTU	TTUT	ULLL	1/7*
		LLTU	TUTT	UTLL	1/7
		LLTU	UUUU	UTDL	1/7
AC	3° divided	LLTU	TTTT	UTLL	4/8
	DU divided	LLTU	TTUT	UTLL	2/8
		LLTU	TUUT	UTLL	1/8
		LLOU	OTDO	UTLL	1/8
AC	3° dividing	LLOU	TTTT	UTLL	1/2
	VU dividing	LLTU	TTTT	UTLL	1/2
AC	3° divided	LLTU	TTTT	UTLL	2/3
	VU dividing	LLTU	TTUT	UTLL	1/3
AC	P(5-7).p dividing	LLTU	TTTT	UTLL	4/4
AC	Pn.p 2-cell stage	LLTU	TTTT	UTLL	3/3

*: centered on P5.p.

Table S14. Anchor cell ablations in *Caenorhabditis* sp. 3 RGD1

Cell(s) Ablated	Time of ablation	Descendants of			# of animals
		P5.p	P6.p	P7.p	
-	-	LLTU	TTTT	UTLL	
AC (1 or 2)	L2 lethargus	S S	S S	S S	8/10
		S ss	S S	S S	1/10
		S S	LL S	S S	1/10
AC	early L3	S S	S S	S S	10/22
		S ss	S S	S S	1/22
		S S	LL S	S S	3/22
		S S	LU S	S S	1/22
		S S	S LL	S S	1/22
		LLUU	S S	S S	1/22
		LUUL	S S	S S	1/22
		S S	DDUD	S S	1/22
		LL S	S LL	S S	1/22
		LL S	LLTU	S S	1/22
		LL S	TODD	S S	1/22
AC	DU dividing	S S	S S	S S	1/7
		LLUU	S S	S S	1/7
		LLUU	TT S	S S	1/7
		LLTU	UTLL	S S	1/7
		LLTU	OTTU	S S	1/7
		ind.7	DD S	S S	1/7
		LLss	LLTU	S LL	1/7
AC	DU divided	LL S	TUDD	ssLL	1/1
AC	VU dividing	LL S	LLDU	S S	1/9
		ind.	ind.	S S	1/9
		LLTU	DDDD	S LL	1/9
		LLTU	TTDD	ssLL	1/9
		LLTU	DDDD	TTLL	1/9
		LLTU	OTTO	UTLL	1/9
		LLOU	TTTT	UTLL	1/9
		LLTU	DUDD	UTLL	1/9
		LLTU	TTTT	UTLL	1/9
AC	VU divided	LLTU	TTTT	UTLL	14/18
		LLTU	OTTT	UTLL	1/18
		LLTT	TTTT	TTLL	1/18
		LLTU	TDUT	UTLL	1/18
		LLTU	TTTT	sULL	1/18
AC	P(3-8).p dividing	LLTU	TTTT	UTLL	13/13
AC	Pn.p 2-cell stage	LLTU	TTTT	UTLL	8/9
		LLTU	TTUT	UTLL	1/9

Table S15. Anchor cell ablations in *Caenorhabditis* sp. 2 DF5070

Cell(s) Ablated	Time of ablation	Descendants of P5.p	P6.p	P7.p	# of animals
-	-	LLTU	TTTT	UTLL	
AC	L2 lethargus	S S	S S	S S	1/1
AC	early L3	S S	S S	S S	1/8
		S S	ss S	S S	1/8
		S ss	S S	S S	1/8
		S S	S UL	S S	1/8
		S S	S S	UU S	1/8
		S S	TUUT	S S	1/8
		S S	TUUT	UU S	1/8
		S UU	OUUT	S S	1/8
AC	DU dividing	S UU	TUUU	S S	1/3
		LLUU	TUUT	UULL	1/3
		LLTU	DLDD	UTLL	1/3
AC	DU divided	LLTU	TUUT	UTLL	1/1
AC	VU dividing	S TU	TUUL	UU S	1/5
		S TU	TUUT	UULL	1/5
		LLUU	TUUU	UTLL	1/5
		LLTU	LUUU	UTLL	1/5
		LLTU	UUUU	UTLL	1/5
AC	VU divided	LLTU	TUUT	UTLL	5/13
		LLLU	TUUT	UTLL	1/13
		LLTU	TUUT	ULLL	1/13
		LLTU	TUUT	UULL	1/13
		LLTU	TUTT	UTLL	1/13
		LLTU	TTUT	UTLL	1/13
		LLDU	UUUU	UULL	1/13
		S UU	TUUT	UULL	1/13
		S DU	LUUT	UU S	1/13
AC	3° dividing or divided	LLTU	TUUT	UTLL	3/5
		LLTU	TUUU	UTLL	1/5
		LLTU	UUUT	UTLL	1/5
AC	P(5-7).p dividing	LLTU	TUUT	UTLL	3/4
		LLTU	UUUT	UTLL	1/4
AC	Pn.p 2-cell stage	LLTU	TTTT	UTLL	7/7

Table S16. Anchor cell ablations in *Caenorhabditis drosophilae* DF5077

Cell(s) Ablated	Time of ablation	Descendants of P5.p	P6.p	P7.p	# of animals
-	-	LLTU	TTTT	UTLL	
AC	L2 lethargus	S S	S S	S S	1/1
AC	early L3	S S	S S	S S	1/4
		S S	LLUL	S S	1/4
		S S	LUUO	S S	1/4
		S S	LUUL	S S	1/4
AC	DU divided	S S	LUTO	ss S	1/3
		S S	DDDD	S S	1/3
		LLTU	TUUT	UTLL	1/3
AC	VU dividing	LLUU	ULLL	UULL	1/2
		LLUU	TUUT	UTLL	1/2
AC	VU divided	LLTU	TUUT	UTLL	2/7
		LLTU	TUOT	UTLL	1/7
		LLTU	TUUU	UTLL	1/7
		LLOU	TUUU	ULLL	1/7
		LLTU	UUUU	UTLL	1/7
		LLUU	TUUT	S S	1/7*
AC	3° dividing or divided	LLTU	TUUT	UTLL	1/2
		LLTU	TUUT	ULLL	1/2
AC	P(5-7).p dividing	LLTU	TTUT	UTLL	2/5
		LLTU	TTTT	UTLL	2/5
		LLTU	OLTT	UTLL	1/5
AC	Pn.p 2-cell stage	LLTU	TTTT	UTLL	3/3

*: this animal had already undergone several VU/DU divisions and had a uterine lumen at the time of ablation (heterochrony compared to standard development).

Table S17. Anchor cell ablations in *Caenorhabditis plicata* SB355

Cell(s) Ablated	Time of ablation	Descendants of P5.p	P6.p	P7.p	# of animals
-	-	LLTU	TTTT	UTLL	
AC	L2 lethargus	S S	S S	S S	6/9
		S S	DDDL	S S	1/9
		S DL	LOLL	S S	1/9
		LLOO	OOOO	S S	1/9
AC	early L3	S S	S S	S S	5/22
		S S	ssss	S S	1/22
		S S	S LL	S S	1/22
		S S	S LL	S S	1/22
		S LL	S S	S S	1/22
		S LL	LL S	S S	1/22
		S S	UUUU	S S	1/22
		S S	LLLL	S S	1/22
		S S	LLDD	S S	1/22
		S DD	S S	DD S	1/22
		S DD	LTLL	S S	1/22
		LDLL	S LL	S S	1/22
		DDDD	S LL	S S	1/22
		DD S	DDDD	S S	1/22
		S S	DDOD	DLLL	1/22
		S LL	LDLL	LL S	1/22
		LLLL	DDDD	LL S	1/22
		LLTU	DDDD	UT S	1/22
AC	DU dividing or divided	S S	S S	S S	3/8
		S S	LLTL	S S	1/8
		S S	DOLL	LL S	1/8
		S S	LLLD	S S	1/8
		S S	LLLT	LLUO	1/8
		LUUD	UULL	S S	1/8
AC	VU dividing	S S	LLLL	DD S	1/8
		S S	LDDD	DD S	1/8
		S S	LLDD	UTLL	1/8
		ind. 7	LLOT	S S	1/8
		S LL	LLDD	UTLL	1/8
		LLTU	QTTO	UTLL	1/8
		LLOU	LOOL	UTLL	1/8
		DDDU	DDDD	UDDD	1/8
AC	VU divided	LLTU	TTTT	UTLL	3/7
		LLTU	TTT*T*	UTLL	1/7
		LLTU	TOQO	UTLL	1/7
		LLTU	QOLT	UTLL	1/7
		LDDD	DDDD	UTL*s*	1/7
AC	P(5-8).p dividing	LLTU	TTTT	UTLL	3/5
		LLTU	TTTT	TTLL	2/5
AC	Pn.p	LLTU	TTTT	UTLL	2/2

2-cell stage

Table S18. Gonad-ablated *C. briggsae* bearing *mfIs37[Cel-hsp16-41::Cel-EGF-LIN-3]*

Heat-shock	P3.p	P4.p	P5.p	P6.p	P7.p	P8.p	n
none	S	S S	S S	S S	S S	S S	7/11
	S S	S S	S S	S S	S S	S S	2/11
	S	S	S S	S S	S S	S S	1/11
	S S	S S	S S	S S	S S	ss S	1/11
33°C 15 min	S	S S	S S	S S	S S	S S	6/13
	S S	S S	S S	S S	S S	S S	7/13
33°C 30 min	S	S S	S S	S S	S S	S S	3/31
	S S	S S	S S	S S	S S	S S	5/31
	S	S	S S	S S	S S	S S	2/31
	S	S S	ss S	S S	S S	S S	1/31
	S	S S	S S	S DU	S S	S S	1/31
	S S	S S	**DD** S	S S	S S	S S	1/31
	S	S	UULL	S S	S S	S S	1/31
	S	S S	LDDU	ssL?	S S	UL S	1/31
	S S	S S	LLUU	UUUU	S Os	S S	1/31
	S	S S	LLTU	LL S	UTLL	S S	1/31
	S	LLLU	LUUL	S S	LLOU	S S	1/31
	S S	S LL	LLTU	LLTU	UDLL	S S	1/31
	S	LLTU	**TTTT**	S **TT**	ULLL	S	1/31
	S	LLTU	**TTTT**	DDDD	S UU	S S	1/31
	S	DD S	LLTU	UO S	UDLL	LL S	1/31
	S	S S	LLTD	UL S	LLLU	**TLTL**	1/31
	S S	S S	S LL	UOLL	TUUT	UUUU	1/31
	S	S S	LLTU	LLDD	DDDD	DDDD	1/31
	S	LDDU	LLTU	**TTTT**	UTLL	ULLO	1/31
	S	S S	S ind.	ind.	UD S	S S	1/31
	S	S S	DDDD	DDDD	ind.	ind.	1/31
	S	S S	ind.	ind.	ind.	ind.	1/31
	S S	S S	ind.	ind.	ind.	ind.	1/31
	S	ind.	ind.	ind.	ind.	ind.	1/31
33°C 60 min	S	S S	LLUU	**TT**UT	ULLD	**TTTT**	1/16
	S	S ind.	ind.	ind.	ind.	ind.	1/16
	S	ind.	ind.	ind.	ind.	S UU	1/16
	S	ind.	ind.	ind.	ind.	ind.	8/16
	S S	ind.	ind.	ind.	ind.	ind.	3/16
	S ss	ind.	ind.	ind.	ind.	ind.	1/16
	UU S	ind.	ind.	ind.	ind.	ind.	1/16
37°C 15 min	S	S LU	**TTTT**	DDDU	S S	UO S	1/11
	S S	S S	LLTU	**TTTT**	UT U	ind.7	1/11
	S	**TTTT**	S **OT**	TOTL	OODL	LOUU	1/11
	LLOU	LDDU	**TTTT**	**TTTT**	TTTL	LTOO	1/11
	UUOL	LLTU	**TTTT**	ULLU	TDOO	**DDLD**	1/11
	S **DD**	S ind.	ind.	ind.	ind. S	ind.	1/11
	S OT	ind.	ind.	ind.	ind.	ind.	1/11
	S	S S	ind.	ind.	ind.	ind.	1/11
	S	ind.	ind.	ind.	ind.	ind.	2/11

	ind.	ind.	ind.	ind.	ind.	ind.	1/11
37°C 30 min	S	S	DLUU	LTTT	S S	**DL** S	1/27
	S	S S	**LLTT**	**TDTO**	S LO	S TU	1/27
	S	S S	UULL	LLTU	UTLL	S S	1/27
	S	**TTTT**	UTLL	UUUU	DDTT	**TTTT**	1/27
	DD S	S **TT**	DTLL	LLLU	LDDD	**DDDD**	1/27
	S	S S	**TTTT**	LLDD	**DDDD**	LLTU	1/27
	TTTO	LLTU	ind.	ind.	ind.	TTTT	1/27
	TT S	S ind.	ind.	ind.	ind.	ind.	1/27
	LL S	ind.	ind.	ind.	ind.	ind.	1/27
	ind.	ind.	ind.	ind.	ind.	ind.	2/27
	S	ind.	ind.	ind.	ind.	ind.	10/27
	S	ind.	ind.	ind.	ind.	S S	1/27
	S	S S	ind.	ind.	ind.	ind.	3/27
	S	S	ind.	ind.	ind.	ind.	1/27
	S	S ind.	ind.	ind.	ind. S	ind.	1/27
37°C 60 min	S	LLTU	DDTU	UDLL	TTTT	LLDD	1/17
	S	**LOTT**	LLTT	**TTDD**	LLT?	**?TTL**	1/17
	S S	LLOU	TDDD	**TTTT**	TLLL	**TTTO**	1/17
	S **TT**	LLLL	**LVTT**	**DTTT**	UOLL	**TTTL**	1/17
	OT S	**LOTT**	**TTTT**	DOLO	OTTL	**LTTO**	1/17
	LLLL	LLVU	OTTO	**TTTT**	ULLT	**OOOO**	1/17
	S	**DDDO**	DDTT	ind	DLLL	**DDDD**	1/17
	S	S S	ind.	ind.	ind.	ind.	1/17
	S	**DD** S	ind.	ind.	ind.	ind.	1/17
	S	ind.	ind.	ind.	ind.	ind.	5/17
	ind.	ind.	ind.	ind.	ind.	ind.	3/17
37°C 3-4 hrs	S	LLTV	*L*OTT	**TTTT**	ULLU	**TTTT**	1/9
	S S	LLTL	LOLL	**LTTT**	LLLO	**OOOT**	1/9
	S	ind.	ind.	ind.	ind.	ind.	5/9
	ind.	ind.	ind.	ind.	ind.	ind.	1/9
	S S	S S	S S	S S	S S	S S	1/9

The gonad primordium was ablated in the early L1 stage, and the heat-shock performed in the late L2 / early L3 stage. Bold letters indicate a 1° fate. The 'TT' sublineage of 'LLTT'-like lineages is ambiguous and was scored conservatively as a 2° fate as in Katz et al. (1995).

Table S19. Intact *C. briggsae* bearing *mfIs37[Cel-hsp16-41::Cel-EGF-LIN-3]*

Heat-shock	P3.p	P4.p	P5.p	P6.p	P7.p	P8.p	n
33°C 15 min	S	S S	LLTU	**TTTT**	UTLL	S S	8/10
	S S	S S	LLTU	**TTTT**	UTLL	S S	1/10
	S S	S S	LLTU	**TTTT**	UTLL	LL S	1/10
33°C 30 min	S	S S	LLTU	**TTTT**	UTLL	S S	2/3
	S S	S S	LLTU	**TTTT**	UTLL	S S	1/3
33°C 60 min	S	S S	LLTU	**TTTT**	UTLL	S S	1/5
	S	S S	LLDD	**TTTT**	TTDL	ULLL	1/5
	S	OULL	LLTT	**TTTT**	UTLL	????	1/5
	S S	S S	ind.	ind.	ind.	ind.	1/5
	S	ind.	ind.	ind.	ind.	ind.	1/5
37°C 15 min	S	S S	LLTU	**TTTT**	UTLL	S S	5/13
	S S	S S	LLTU	**TTTT**	UTLL	S S	1/13
	S	S	LLTU	**TTTT**	UTLL	S S	1/13
	S	S S	LLTU	**TTTT**	UTLL	ssLL	1/13
	S	S S	LLTU	**TTTT**	UTLL	UTUL	1/13
	S S	S S	LLTU	**TTTT**	UTLL	UOLL	2/13
	S S	S S	LLTU	**TTTT**	UOLL	LUUT	1/13
	S S	S S	LLTU	**TTTT**	UTLL	DTLL	1/13
37°C 22 min	S	S S	LLTU	**TTTT**	TTLL	UTLL	1/14
	S	S S	LLTU	**TTTT**	UTLL	**TTTT**	1/14
	S	S S	LLTU	**TTTT**	VLLL	**TTOL**	1/14
	S S	LL S	LLTU	**TTTT**	UTLL	ULLL	1/14
	S	LLTU	LLTU	**TTTT**	VLLL	sLTT	1/14
	S S	OUTT	**OOTT**	**TTTT**	TLLL	OLUU	1/14
	S S	LLLU	LVOV	**TTTT**	UTLL	**TOUL**	1/14
	S	OUTU	LLTU	**TTTT**	OLLL	**TOOO**	1/14
	S	LLLU	**OOTT**	**OTTT**	OLOL	**TTTL**	1/14
	S S	LLOO	**OTTT**	**TTTT**	VTLL	**TTDO**	1/14
	S	**LTOT**	LOTT	**TTTT**	TTOL	**OTOL**	1/14
	S	**LOTO**	LDTU	**TTTT**	TOLL	**TODO**	1/14
	S	**LTTT**	LLTU	**TTTT**	UTLL	**LOOT**	1/14
	LLLU	**LOTT**	LLTU	**TTTT**	OLLL	**TTOT**	1/14
37°C 30 min	S	LLLL	LTTT	**TTTT**	TTLL	LLOO	1/12
	S	LLOU	**TTTT**	**TTTT**	OLLL	**OLOT**	1/12
	S	**LTTT**	**TTTT**	**TTTT**	OLLL	LTTO	1/12
	S	LLTT	**TTTT**	**TTTT**	UTLL	LDTT	1/12
	S	LLTT	**TTTT**	**TTTT**	TTTL	TTLL	1/12
	S S	LLTU	TOTL	**TTTT**	UTLL	**TTOT**	1/12
	LLTU	**OLTT**	LTTT	**TTTT**	TTDL	LDDT	1/12
	LLLL	LLOO	**TTTT**	**TTTT**	UOLL	LOLL	1/12
	LOTO	LLOU	**LOTT**	**TTTT**	VOLL	**TTOO**	1/12
	LTOT	LDOD	LDDD	**TTTT**	DTOL	**OTOL**	1/12
	LLTU	**OT**TU	LLTT	**TTTT**	UODL	LDDO	1/12
	LLLU	**OTOO**	LLTT	**TTTT**	**TTOL**	**TTOL**	1/12
37°C 60 min	S	S	LLTU	**TTTT**	TTDL	LTTL	1/8
	S	S **OL**	LLOT	**TTTT**	DTLL	**OOOL**	1/8

S	**LTOL**	O ?TT	**TTTT**	TLLL	LLLL	1/8
S	LLTO	**TTTT**	**TTTT**	TOLL	**OTLL**	1/8
TTOO	LLOU	**OTTT**	**TTTT**	LTDL	S	1/8
LTOL	LLTT	**TTTT**	**TTTT**	ULLL	**OTTL**	1/8
UUUU	**OUOD**	**ODOO**	**TTTT**	OLLL	**LOOL**	1/8
S	**LTOT**	**TDTT**	**TTTT**	**TLLL**	**TLDO**	1/8

Heat-shock was performed in the late L2 / early L3 stage. Bold letters indicate a 1° fate as in the previous table.

Table S20. Anchor cell ablations in *C. briggsae* strain (JU616) bearing *mfIs11[Cbr-lin-3]*

Ablation time	P3.p	P4.p	P5.p	P6.p	P7.p	P8.p	n
L2 lethargus			3-cells	indu	ced		2/2
early L3	S	S S	LLTU	TTTT	UTLL	S S	1/4
	S S	S S	LLTU	TTTT	UTLL	S S	1/4
	S S	S S	LLTU	TOUT	UTLL	S S	1/4
	S	S	S DU	DDDD	S ss	S S	1/4
DU dividing	S	S S	LLTU	TTTT	UTLL	S S	2/2
DU divided	S	S S	LLTU	TTTT	UTLL	S S	1/3
	S S	S S	LLTU	TTTT	UTLL	LLLL	1/3
	S S	S S	LLTU	TTUT	UTLL	UTLL	1/3
VU divided	S	S	LLTU	TTTT	UTLL	S S	1/4
	S	S S	LLTU	DDDD	UTLL	S S	1/4
	S S	S S	LLTU	TTUT	UTLL	S S	1/4
	S S	LLTU	LDTU	TTTT	UTLL	S S	1/4
3° dividing	S	S S	LLTU	TTTT	UTLL	S S	1/3
or divided	S S	S S	LLTU	TTTT	UTLL	S S	1/3
	S S	LLTU	ind.	ind.	ind.	UTLL	1/3
P(5-7).p	S	S S	LLTU	TTTT	UTLL	S S	2/4
dividing	S S	LLDU	DDDD	DDDD	UTLL	S S	1/4
	S	LLTU	ind. 7	DDDD	UTLL	S S	1/4
Pn.p	S S	LLLU	DDDD	DDTT	UTLL	UOLL	1/5
2-cell stage	S	LLTU	DDDT	TTTT	UTLL	S S	1/5
	S	LLLD	DLTT	TTTT	UTLL	S S	1/5
	S	S S	LLTU	TDOT	UTLL	S S	1/5
	S	S S	LLTU	TTTT	UTLL	S S	1/5

Table S21. Anchor cell ablations in *C. briggsae* strain (JU617) bearing *mfIs12[Cbr-lin-3]*

Ablation time	P3.p	P4.p	P5.p	P6.p	P7.p	P8.p	n
L2 lethargus	S	S S	S S	S S	S S	S S	2/7
	S S	S S	S S	S S	S S	S S	1/7
	S S	S S	LlsU	S S	S S	S S	1/7
	S S	S S	LLLL	S S	S LL	S S	1/7
	S S	S S	LLTU	TTUT	UTLL	S S	1/7
	S	S S	LLTU	TTTT	UTLL	S S	1/7
early L3	S S	S S	LLDU	S S	UULL	S S	1/11
	S	S	LLLU	D*DD*D	UTLL	S S	1/11
	S	S S	LLTU	TUUT	UTLL	S S	1/11
	S S	S S	LLTU	TUUT	UTLL	S S	2/11
	S	S S	LLTU	TTUT	UTLL	S S	1/11
	S S	S S	LLTU	TTUT	UTLL	S S	1/11
	S	S S	LLTU	TTTT	UTLL	S S	2/11
	S S	S S	LLTU	TTTT	UTLL	S S	2/11
DU divided	S S	S S	LLTU	TTTT	UTLL	S S	2/2
VU dividing	S	S S	LLTU	TTTT	UTLL	S S	2/4
	S S	S S	LLTU	TUTT	UTLL	S S	1/4
	S	S S	LLTU	TUUT	UTLL	S S	1/4
3° dividing	S	S S	LLTU	TTTT	UTLL	S S	2/8
or divided	S S	S S	LLTU	TTTT	UTLL	S S	2/8
	S	S S	LLTU	TUUT	UTLL	S S	1/8
	S S	S TU	LLTU	TTTT	UTLL	S S	1/8
	S S	LLTU	LLTU	TTTT	UTLL	S S	1/8
	S S	LLTU	LLTU	TTTT	UTLL	U LL	1/8

Table S22. Anchor cell ablations in the *Cel-lin-45(n1018)* mutant (strain JU646)

Ablation time	P3.p	P4.p	P5.p	P6.p	P7.p	P8.p	n
L2 lethargus	S	S S	S S	S S	S S	S S	2/4
	S S	S S	LLOU	TUTT	DL S	S S	1/4
	S	S S	LLOU	TTTT	UTLL	S S	1/4
early L3	S	S S	S S	S S	S S	S S	1/4
	S S	S S	S S	S S	S S	S S	3/4
DU dividing	S	S S	S S	S S	S S	S S	3/5
	S S	S S	S S	S S	S S	S S	1/5
	S S	S S	S LL	S S	UD S	S S	1/5
DU divided	S	S S	S S	S S	S S	S S	2/6
	S S	S S	S S	S S	S S	S S	2/6
	S	S S	S DL	S S	S S	S S	1/6
	S	S S	LLTU	TUUT	UTLL	S S	1/6
3° dividing	S S	S S	S ?L	S S	S S	S S	1/7
	S	S S	LLLU	S S	S S	S S	1/7
	S	S S	LLOU	S S	S S	S S	1/7
	S S	S S	LLLU	S UO	UTLL	S S	1/7
	S S	S S	S TU	OTUL	S S	S S	1/7
	S	S S	LLOD	LLOU	UTLL	S S	1/7
	S S	S S	LLLL	LLDT	UTLL	S S	1/7
3° divided	S	S S	LLLL	S UU	LLLL	S S	1/3
	S	S S	LLTU	LLLL	LLLL	S S	1/3
	S S	S S	LLTU	TUUT	UTLL	S S	1/3
P(5-7).p	S	S S	LLTU	DDUT	UTLL	S S	1/2
dividing	S S	S S	LLTU	TUUT	UTLL	S S	1/2
Pn.p	S S	S S	LLTU	TOTT	UTLL	S S	1/8
2-cell stage	S	S S	LLTU	DDDD	UTLL	S S	1/8
	S	S S	LLTU	OLOT	UTLL	S S	1/8
	S S	S S	LLOO	LLTT	UTLL	S S	1/8
	S	S S	LLTU	TTTT	UTLL	S S	2/8
	S S	S S	LLTU	TTTT	UTLL	S S	2/8

Table S23. Anchor cell ablations in the *Cel-bar-1(ga80)* mutant (strain EW15)

Ablation time	P3.p	P4.p	P5.p	P6.p	P7.p	P8.p	n
early L3	S	S	S S	S	S S	S S	1/4
	S	S	S S	S S	S S	S S	1/4
	S	S	LLDD	S	S S	S S	1/4
	S	S	S	DDDD	S S	S S	1/4
DU dividing	S	S	S S	S S	S S	S S	1/1
VU dividing	S	S	S	S S	S S	S S	2/7
	S	S	S S	S ss	S S	S S	1/7
	S	S S	S S	S DD	S S	S S	1/7
	S	S	S ss	LL?U	S S	S S	1/7
	S	S S	LLTU	UTLL	ssss	S S	1/7
	S	S	LLLL	LLUL	LLLL	S S	1/7
VU divided	S	S	S	LLDD	S LL	S S	1/2
	S	S	LLLU	LLDD	DLLL	S S	1/2
P8.p dividing	S	S	LLDU	S	S S	S S	1/1
P8.p	S	S	S	LDLL	ULLL	S S	1/2
divided	S	S	LLDU	DUUT	LLLL	S S	1/2
P(5-7).p	S	S	S	DLDD	UDLL	S S	1/2
dividing	S	S	LLLU	LDDD	UTLL	S S	1/2
Pn.p	S	S	LLUU	DDDD	UTUL	S S	1/2
2-cell stage	S	S	S S	LLTU	UTLL	LLLL	1/2

Table S24. Anchor cell ablations in the *Cel-sel-10(n1077)* mutant (strain MT2244)

Ablation time	P3.p	P4.p	P5.p	P6.p	P7.p	P8.p	n
L2 lethargus	S S	S S	S S	S S	S S	S S	1/3
	S S	S S	LLDL	S S	LLLL	S S	1/3
	S	S S	LLLL	S S	UTDL	S S	1/3
early L3	S S	S S	S S	S S	S S	S S	2/4
	S	S S	LLUU	S S	LTLL	S S	1/4
	S S	LLDD	ssLL	UTLL	S S	S S	1/4
DU dividing	S	S S	LLTU	S S	UTLL	S S	1/2
	S S	S S	LDDD	UL S	UDLL	S S	1/2
DU divided	nd	S S	LLTU	ssss	UTLL	S S	1/5
	S S	S S	LLDU	S LL	UTLL	S S	1/5
	S S	S S	LLDU	ssUU	UTLL	S S	1/5
	S S	S S	LLTU	UTLL	UTLL	S S	1/5
	S	S S	LLTU	DDUD	UTLL	S S	1/5
VU dividing	S	S S	LLTU	TTTL	UTLL	S S	1/4
	S S	S S	LLTU	LUDD	UTLL	S S	1/4
	S S	S S	LLTU	TTUT	UTLL	S S	1/4
	S S	LLDD	LLTU	UTLL	UTLL	S S	1/4
3° dividing	S S	S S	LLTU	S DD	UTLL	S S	1/9
	S S	LLOO	S TU	UTLL	S S	S S	1/9
	S S	S S	LLTU	LLDU	UDLL	S S	1/9
	S S	S S	LLTU	UTDD	ULLL	S S	1/9
	S S	S S	LLTU	LUUT	UTLL	S S	1/9
	S S	S S	LLTU	TUTT	UTLL	S S	1/9
	S S	S S	LLTU	DUUD	UTLL	S S	1/9
	S S	S S	LLTU	TUUT	UTLL	S S	2/9
3° divided	S S	S S	LLTU	TUUT	UTLL	S S	2/4
	S	S S	LLTU	TUOT	UOLL	S S	1/4
	S S	S S	LLTU	TTDT	UOLL	S S	1/4
P(5-7).p	S S	S S	LLLU	TULL	UOLL	S S	1/2
dividing	S S	LLUU	TTTT	UTLL	S S	S S	1/2
Pn.p	S	S S	LLTU	TTTT	LLLL	S S	1/4
2-cell stage	S	S S	LLOU	TTTT	ULLL	S S	1/4
	S S	S S	LLLU	TTTT	ULLL	S S	1/4
	S S	LLOU	TTTT	UTLL	S S	S S	1/4

Table S25. Anchor cell ablations in the *Cel-sel-10(bc243)* mutant (strain MD1290)

Ablation time	P3.p	P4.p	P5.p	P6.p	P7.p	P8.p	n
L2 lethargus	S S	S S	S S	S S	S S	S S	2/4
	S S	S S	LLLL	S S	OOLL	S S	1/4
	S S	S S	LDDD	S S	DLLL	S S	1/4
early L3	S S	S S	LLOO	S S	S LL	S S	1/2
	S S	S S	LLOU	S S	UOLL	S S	1/2
DU dividing	S S	S S	LLOU	LLOU	UTLL	S S	1/1
DU divided	S S	S S	ssss	ssss	LOLL	S S	1/5
	S S	S S	LLLL	S S	U*sss*	S S	1/5
	S S	S S	LLLL	ssss	LOLL	S S	1/5
	S S	S S	LLTU	ssss	UOLL	S S	1/5
	S S	S S	LLTU	OLLL	UOLL	S S	1/5
VU dividing	S S	S S	LLTU	L*L*OU	UOLL	S S	1/3
	S S	S S	LLTU	LLTU	UTLL	S S	1/3
	S S	S S	LLTU	LUUT	UTLL	S S	1/3
3° dividing	S S	S S	LLTU	OUDD	ULLL	S S	1/5
	S S	S S	LLTU	OOTL	UOLL	S S	1/5
	S S	S S	LLOU	LTUT	UTLL	S S	1/5
	S S	S S	LLTU	TUUT	UTLL	S S	1/5
	S S	S S	LLTU	TTTT	UTLL	S S	1/5
3° divided	S S	S S	LLTU	ODDT	UTLL	S S	1/2
	S S	S S	LLTU	TTTO	UOLL	S S	1/2
P(5-7).p dividing	S S	S S	LLTU	TTTT	UTLL	S S	1/1
Pn.p	S S	S S	LLLU	OTLT	ULLL	S S	1/2
2-cell stage	S S	S S	LLTU	TTTT	UTLL	S S	1/2

Table S26. Anchor cell ablations in *Cel-lin-45(n1018); Cel-sel-10(n1077)* (strain JU972)

Ablation time	P3.p	P4.p	P5.p	P6.p	P7.p	P8.p	n
L2 lethargus	S	S S	S S	S S	S S	S S	2/4
	S S	S S	S S	S S	S S	S S	1/4
	S S	S S	S S	S S	LL S	S S	1/4*
early L3	S S	S S	DDDU	S S	DDLL	S S	1/2
	S S	S S	LLLL	LLDU	UDLL	S S	1/2*
DU dividing	S	S S	DDDU	S S	DDLL	S S	1/1
DU divided	S	S S	S S	S S	S S	S S	1/4
	S	S S	LLLL	S S	S S	S S	1/4
	S	S S	LDDD	S S	S LL	S S	1/4
	S S	S ss	LLTU	DDLL	LLLL	S S	1/4*
VU dividing	S S	S S	LLLL	LULL	LLLL	S S	1/1
VU divided	S	S S	LLTU	DDDD	DDLL	S S	1/2*
	S	S S	LL??	??LL	??LL	S S	1/2*
3° dividing	S S	S S	LLOU	LLLU	UDLL	S S	1/3
	S S	S S	????	???L	ssss	S S	1/3
	S S	LLLL	LLTU	TTLL	UTLL	S S	1/3*
3° divided	S S	S S	LLLL	LLUL	DDDL	S S	1/2
	S S	LLDU	UDLU	UTLL	S S	S S	1/2*#
P(5-7).p dividing	S	S S	????	LDDL	LLLL	S S	1/1
Pn.p	S	S S	LLTU	TTTT	UTLL	S S	1/2
2-cell stage	S	S S	LLTU	TTTT	DDLL	S S	1/2*

*: these animals were cultured at 25°C before ablation.

Table S27. Anchor cell ablations in the *Cel-gap-1(ga133)* mutant (strain AH12)

Ablation time	P3.p	P4.p	P5.p	P6.p	P7.p	P8.p	n
L2 lethargus	S S	S S	S S	S S	S S	S S	3/4
	S S	S S	S S	S OU	S S	S S	1/4
early L3	S	S S	S S	S S	S S	S S	1/3
	S S	S S	S S	S S	S S	S S	1/3
	S S	S S	S LO	UULO	S S	S S	1/3
DU dividing	S	S S	S S	S S	S S	S S	1/3
	S S	S S	S S	S S	S S	S S	1/3
	S	S S	LLTU	TTTT	UTLL	S S	1/3
DU divided	S	S S	S S	S S	S S	S S	2/9
	S S	S S	S S	S S	S S	S S	1/9
	S S	S S	S S	ssss	S S	S S	1/9
	S	S S	S S	OOOL	S S	S S	1/9
	S S	S S	S S	OUUT	UTLL	S S	1/9
	S	S S	S TU	TTTT	UTLL	S S	1/9
	S S	S S	S DD	TTTT	UTLL	S S	1/9
	S	S S	LLTU	TTTT	UTss	S S	1/9
VU dividing	S S	S S	S OU	LLOU	UTLL	S S	1/6
	S S	S S	LLOU	TUDD	UTLL	S S	1/6
	S S	S S	LLTU	TUUT	UTLL	S S	1/6
	S S	S S	LLTU	TTUT	UTLL	S S	1/6
	S S	S S	LLTU	TTTT	UTLL	S S	1/6
	S	S	LLOU	TDDT	UTLL	S S	1/6
VU divided	S	S S	LLTU	TUUT	UTLL	S S	1/3
	S S	S S	LLTU	TUTT	UTLL	S S	1/3
	S	S S	LLTU	TTTT	UTLL	S S	1/3
3° dividing	S	S S	LLTU	TUUT	UTLL	S S	1/4
	S S	S S	LLTU	TUTT	UTLL	S S	2/4
	S S	S S	LLTU	TTUT	UTLL	S S	1/4
3° divided	S	S S	LLTU	TUUT	UTLL	S S	1/1
P(5-7).p dividing	S	S S	LLTU	TTTT	UTLL	S S	1/4
	S S	S S	LLTU	TTTT	UTLL	S S	2/4
	S S	S S	LLTU	TUUT	UTLL	S S	1/4
Pn.p 2-cell stage	S	S S	LLTU	TTTT	UTLL	S S	4/5
	S S	S S	LLTU	TTTT	UTLL	S S	1/5

Table S28. Anchor cell ablations in the *Cel-ark-1(sy247)* mutant (strain PS1461)

Ablation time	P3.p	P4.p	P5.p	P6.p	P7.p	P8.p	n
L2 lethargus	S	S S	ssLL	TTTT	UTLL	S S	1/1
early L3	S	S S	S S	S ss	UU S	S S	1/10
	S	S S	ssOU	DDDs	UO S	S S	1/10
	S	S S	ssLL	TTTT	UTLL	S S	1/10
	S	S S	LLTU	TTTT	UTUs	S S	1/10
	S	S S	LLTU	TUTT	UTLL	S S	1/10
	S	S	LLTU	TTTT	UTLL	S S	1/10
	S	S S	LLTU	TTTT	UTLL	S S	3/10
	S S	S S	LLTU	TTTT	UTLL	S S	1/10
DU dividing	S	S S	LLTU	TTTT	UTLL	S S	1/1
DU divided	S	S S	LLOU	DUUO	UTLL	S S	1/11
	S	S S	LLOU	TTTT	UULL	S S	1/11
	S	S S	LLTU	TTTT	UTLL	S S	7/11
	S S	S S	LLTU	TTTT	UTLL	S S	2/11
VU dividing	S S	S S	S LL	TTTT	UTLL	S S	1/1
VU divided	S S	S S	LLTU	TTTT	UTLL	S S	1/1
3° dividing	S	S S	LLTU	TTTT	UTLL	S S	6/11
	S S	S S	LLTU	TTTT	UTLL	S S	5/11
3° divided	S S	S S	LLTU	TUUT	UTLL	S S	1/1
P(5-7).p	S	S S	LLTU	TTTT	UTLL	S S	1/2
dividing	S S	S S	LLTU	TTTT	UTLL	S S	1/2
Pn.p	S	S S	LLTU	TTTT	UTLL	S S	1/4
2-cell stage	S S	S S	LLTU	TTTT	UTLL	S S	3/4

Table S29. Anchor cell ablations in *Cel-ark-1(sy247); Cel-gap-1(ga133)* (strain JU703)

Ablation time	P3.p	P4.p	P5.p	P6.p	P7.p	P8.p	n
L2 lethargus	S	S S	S TU	TTTT	UT S	S S	1/2
	S S	S S	LLTU	TTTT	TTLL	S S	1/2
early L3	S S	S S	S S	S S	S S	S S	2/7
	S S	S S	S S	S UU	S S	S S	1/7
	S S	S S	S *ss*	OTUO	S S	S S	1/7
	S S	S S	LLOU	TTTT	ULLL	S S	1/7
	S S	S S	LLTU	TTTT	UTLL	S S	2/7
DU dividing	S S	S S	S S	S S	UD S	S S	1/2
	S S	S S	LLTU	TDDT	UTLL	S S	1/2
DU divided	S S	S S	S S	LTTL	S S	S S	1/6
	S S	S S	S DU	S S	LOLL	S S	1/6
	S	S S	ssOU	TTTT	S S	S S	1/6
	S S	S S	LLTU	TTTT	UTLL	S S	3/6
VU dividing	S S	S S	S TU	TDTT	S S	S S	1/5
	S	S S	LDDD	DDDD	UO S	S S	1/5
	S	S S	LLTU	TTTT	UTLL	S S	1/5
	S S	S S	LLTU	TTTT	UTLL	S S	1/5
	S S	S S	LLTT	TTTT	UTLL	S S	1/5
VU divided	S	S S	LLTU	TTTT	UTLL	S S	2/5
	S	S S	LLTU	TTOT	UTLL	S S	1/5
	S S	S S	LLTU	TTTT	UTLL	S S	1/5
	S	S S	LLTU	TLTL	UOLL	S S	1/5
3° dividing	S	S S	LLTU	TTTT	UTLL	S S	2/6
or divided	S S	S S	LLTU	TTTT	UTLL	S S	1/6
	S S	S S	LLTT	TTTT	UTLL	S ss	1/6
	S S	S S	LLTU	TTTT	UTLL	UOss	1/6
	S S	S S	LLTU	TUUT	UTLL	S S	1/6
P(5-7).p	S	S S	LLTU	TTTT	UTLL	S S	1/2
dividing	S S	S S	LLLU	TOTT	UTLL	S S	1/2
Pn.p	S	S S	LLTU	TTTT	UTLL	S S	1/4
2-cell stage	S S	S S	LLTU	TTTT	UTLL	S S	1/4
	S S	S S	LLTU	TTTT	UTLL	UL S	1/4
	S S	S S	LLTU	TTTT	TTLL	TTTL	1/4

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