
Vulval development*

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C. elegans vulval development is an intensively studied example of animal organogenesis. A network of intercellular signaling, signal transduction, and transcriptional regulation underlies the precise formation of this organ, which is the connection between the hermaphrodite uterus and the outside of the nematode. A single cell of the somatic gonad, the anchor cell, organizes the development of the vulva from epidermal precursors as well as the physical connection of the epidermis with the uterus. WNT signaling acting via the HOX gene *lin-39* renders six epidermal precursor cells competent to respond to other developmental signals. The anchor cell induces nearby epidermal precursor cells to generate vulval cells via an epidermal growth factor (EGF) signaling pathway. The precise pattern of vulval precursor cell fates involves the graded action of the EGF signaling and LIN-12 (Notch) mediated lateral signaling. EGF promotes the 1° fate while LIN-12 promotes the 2° fate. Both EGF and LIN-12 prevent precursor cells from adopting the 3° fate, which generates non-specialized epidermis. EGF-receptor and Notch signaling are antagonistic: EGF-receptor signaling leads to down-regulation of the Notch-like receptor LIN-12, while LIN-12 signaling induces negative regulators of EGF-receptor signaling such as MAP kinase phosphatase LIP-1 and the tyrosine kinase ARK-1. The 1° precursor cell generates vulE and vulF mature vulval cells; the pattern of vulE and vulF cells requires an additional signal from the anchor cell as well as WNT signaling. The two 2° precursor cells generate vulA, vulB1, vulB2, vulC and vulD cells but in mirror symmetric polar patterns: ABCD and DCBA. The reversed polarity of the posterior 2° precursor cell lineage requires WNT signaling mediated by both Frizzled class and Ryk class WNT-receptors LIN-17 and LIN-18, respectively. A network of transcription factors controls the seven mature adult cell types; these include the LIM domain protein LIN-11, the Pax2/5/8 protein EGL-38, the zinc finger protein LIN-29, and the Nkx6.1/6.2 protein COG-1. The anchor cell also patterns nearby uterine cells, via the DSL ligand LAG-2 and LIN-12, to generate the four uv1 cells that form the tight connection with the vulva. This connection is initiated by the anchor cell, which invades between the vulF cells in a process analogous to invasive behavior of metastatic tumor cells. During this invasion process, the basement membranes between the gonad and body wall are degraded. The extensive information about vulval development in *C. elegans* has helped it become a paradigmatic case for identifying and studying a variety of regulatory pathways.

1. Introduction

The *C. elegans* hermaphrodite vulva develops during postembryonic (larval) development from ventral epidermal precursors, and connects the developing uterus to the external environment. In the adult, the vulva is necessary for egg-laying (see [Egg-laying](#)) and for copulation with males (see [Male mating behavior](#)). Vulval development has attracted general interest for three main reasons. First, it serves as a paradigm for organogenesis. In particular, vulva development represents a well-understood case in which invariant development arises from multiple cell-cell interactions. It is also a striking example of tissue remodeling: the formation of a hole at a precise location in an organism. Second, it has been important for the genetic analyses of signaling and signal transduction by epidermal growth factor (EGF)-receptor LET-23 and RAS LET-60; (see [RTKRas/MAP kinase signaling](#)), LIN-12 (see [LIN-12/Notch signaling in C. elegans](#)), and WNT (see [Wnt signaling](#)), as well as the functions of the SynMuv and mediator proteins (see [Transcriptional regulation](#)). Third, it has become a paradigm for examining the interactions among regulatory pathways, notably the antagonism of EGF-receptor (LET-23) and Notch (LIN-12) pathways.

1.1. Steps in vulval development

Vulval development is conveniently thought of as occurring in five steps (Figure 1). **(1) Generation of VPCs:** During the L1 and L2 stages, six vulval precursor cells (VPCs) are specified among the 11 Pn.p cells, which are located in the ventral epidermis. **(2) Vulval Precursor Patterning:** During the L3 stage, a signal from the gonad and signaling among the VPCs specifies three VPCs to generate vulval cells (Figure 2). The vulval lineages are of two types, 1° and 2°, each of which generate distinct sets of progeny. The uninduced VPCs generate a 3° lineage, which make epidermal cells that fuse with the large syncytial epidermis hyp7. In an intact, wild-type hermaphrodite, the VPCs adopt their fates in a precise spatial pattern: 3°-3°-2°-1°-2°-3° (Figure 3). **(3) Generation of the adult cells:** The adult vulva comprises 22 nuclei in cells of seven types, vulA, vulB1, vulB2, vulC, vulD, vulE, and vulF, that differ in their patterns of gene expression and in details of their cell biology. **(4) Anchor cell invasion:** the anchor cell extends process to the center of the vulF cells and forms a hole in the epidermis. **(5) Morphogenesis of the vulva:** the seven types of vulval cells invaginate, and sequentially form seven distinct toroids, connects to the uterus, and everts as the hermaphrodite molts to adulthood. In addition, two aspects of uterine development are crucial to the development of the vulva. **(A) Generation of the anchor cell.** The anchor cell is specified from among two somatic gonadal cells during the end of the L2 stage (see AC/VU decision). **(B) Uterine Patterning.** The anchor cell also patterns the developing uterus, inducing the six pi cells, which generate the utse cell and uv1 cells. The uv1 cells attach to the vulF cells, while the anchor cell ultimately fuses with the utse.

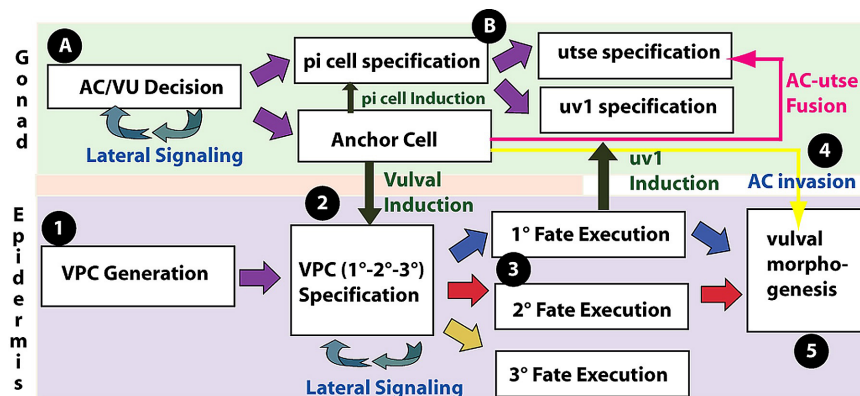


Figure 1. Vulval development is a multi-step process. It involves the coordinated development of the uterine (green, top) and ventral epithelia (purple, bottom) with several interactions between these tissues. 1. The six VPCs are specified from among the ventral epidermal Pn.p cells. 2. The VPCs become specified to 1°, 2° or 3° cell fates by multiple signaling pathways. The anchor cell induces vulval fates (1° and 2°) and lateral signaling among the induced VPCs specifies the 2° VPC fate. 3. The vulval lineages are generated and specific sets of mature cells are produced and specified. 4. The anchor cell breaks down the basement membrane of the gonad and vulval epithelia and invades between the vulF cells. 5. The vulval cells undergo a complex morphogenesis. **A.** The anchor cell becomes specified from a set of two AC/VU cells; the cell that is not the anchor cell becomes one of three VU precursor cells. **B.** The three VU cells generate 12 grandprogeny, six of which are induced by the anchor cell to become pi cells. The six pi cells divide, and four of their progeny are induced by the vulF cells of the vulva 1° lineage to become uv1 cells. The remaining eight cells fuse to form the utse, which ultimately fuses with the anchor cell.

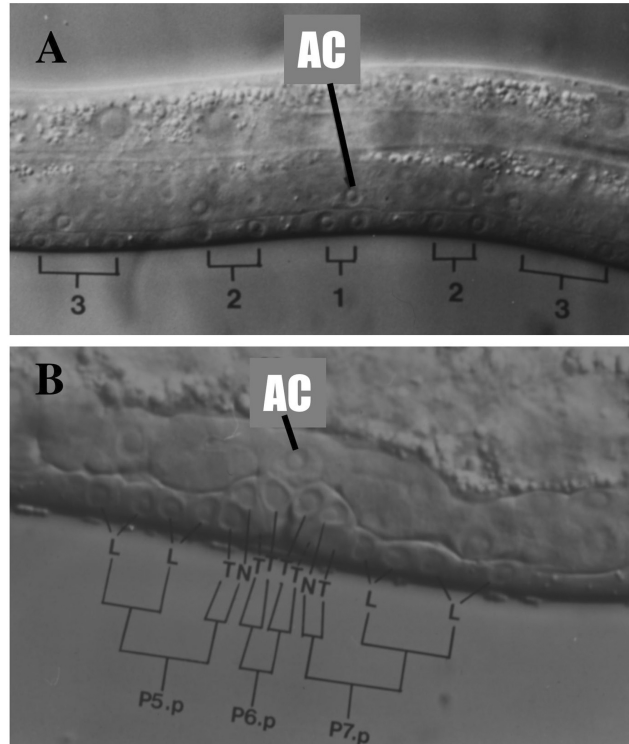


Figure 2. Nomarski images of developing vulva. Left lateral views of larval hermaphrodites (anterior to the left). AC, anchor cell. 3, 3° VPCs (P4.p and P8.p shown here); 2, 2° VPCs (P5.p and P7.p); 1, 1° VPC. (A) mid-L3 stage. At this stage, there are no signs of morphological differentiation but P6.p and its daughters express *egl-17*. B. L3 lethargus. The first two rounds of mitosis of the 2° and 1° vulval precursors are drawn in, with the axis of the third round of division indicated: L, longitudinal (anterior-posterior); T, transverse (left-right); N, no division; S, fuses with *hyp7* epidermal syncytium. In the literature, 1° lineages are referred to as [TTTT] or [EFFE]; 2° lineages are referred to as [LLTN], [NTLL], [ABCD], or [DCBA]; 3° lineages as [S S]; hybrid lineages are [S TN], [NT S], [S TT], [TT S], [LL S] or [S LL]. In abnormal lineages, O refers to an oblique axis of division, and U refers to undivided cell.

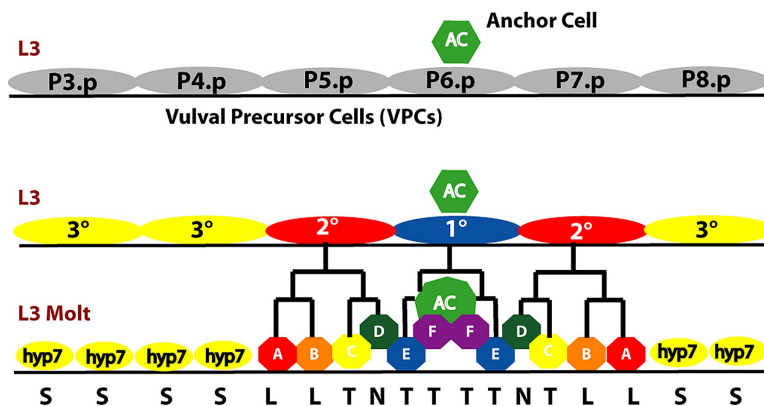


Figure 3. Simple view of vulval lineages. The six multipotent VPCs become specified during the early L3 stage to an invariant pattern of VPC fates. The 1° and 2° VPCs generate distinct sets of progeny cells. *Hyp7*, cell fuses with the large epidermal syncytium *hyp7*. A, generates two vulA nuclei; B, generates a vulB1 and a vulB2 cell; C, generates two vulC cells; D, becomes vulD; E, divides to generate two vulE cells; F, generates two vulF cells. Most of the VPC granddaughters shown L, longitudinal axis of cell division; T, transverse axis of cell division; N, no cell division. Underline indicates adherence to the ventral cuticle. See Sulston and Horvitz (1977) for lineages and Katz et al. (1995) for morphological distinctions between 1° and 2° lineages.

1.2. Phenotypes of vulval development mutants

Mutations that affect vulva development often cause defects that are visible in the dissecting microscope. These phenotypes include the inability of hermaphrodites to lay eggs (EGg-Laying-defective; *Egl*), or morphological defects such as Protruding Vulva (*Pvl*) or abnormal Eversion of the vulva (*Evl*). Specifically, mutants with defective vulval induction, e.g., mutations that decrease activity of the EGF-receptor *let-23* (see below) cause a VULvaless (*Vul*) phenotype. Vulvaless hermaphrodites have fertile eggs that hatch internally and eventually

escape from the corpse of their mother. Hermaphrodites with defects in the vulval lineages or in the vulval-uterine connection have protruding vulvae or are egg-laying defective. Mutations that activate *LET-23*, rendering it constitutive, result in a MUltiVulva (Muv) phenotype. Multivulva hermaphrodites typically have a single functional vulva and additional ventral protrusions, each a pseudovulva formed from vulval tissue. Mutants with reversed polarity of P7.p 2° lineage have a pseudovulva posterior to the normal vulva.

In addition to screens for mutants with vulval defects (Horvitz and Sulston, 1980; Ferguson and Horvitz, 1985; Seydoux and Greenwald, 1993; Eisenmann and Kim, 2000; Palmer et al., 2002), suppressor screens proved useful in identifying new genes and alleles, including members of the *LET-23* pathways and genes involved in other steps in vulval development (e.g., Beitel et al., 1990; Han et al., 1990; Aroian et al., 1991; Clark et al., 1992; Clark et al., 1992; 1993; Han et al., 1993; Clark, 1992).

1.3. Note on phenotypes

The phenotype of a hermaphrodite observed at the dissecting microscope level can sometimes be misleading, and thus the dissecting microscope phenotypes should always be confirmed by observation of anatomy in a compound microscope. For example, *lin-17* mutants often display a vulva (from P5.p and P6.p) and a posterior pseudo-vulva (from P7.p), appearing to be multivulva, but have a normal extent of vulval induction. A number of markers are now available to help distinguish different fates of VPCs (Burdine et al., 1998; Yoo et al., 2004) and of terminal vulval cells (Inoue et al., 2002).

2. Generation of vulval precursor cells

The eleven Pn.p cells (P1.p, P2.p, P3.p, ..., P11.p) are formed during the L1 larval stage as the posterior daughters of the Pn ventral neuro-ectoblasts (P1, P2, P3, ..., P11), the anterior daughters of which generate primarily ventral cord neurons (Sulston and Horvitz, 1977). The Pn cell nuclei migrate into the ventral cord during the L1 stage. The development of the vulva thus depends upon the generation of the Pn.p cells. Action of the Hox genes is responsible for specification of the VPCs P3.p, P4.p, ..., P8.p (P(3-8).p; Figure 4). In particular, *lin-39* is necessary for specification of the VPCs; in the absence of *lin-39* function, P(3-8).p fuse with *hyp7* epidermis in the L1 larval stage (Salser et al., 1993; Clark et al., 1993; Figure 5). In wild-type hermaphrodites, P3.p sometimes fuses with *hyp7* during the L2 stage (Sulston and Horvitz, 1977).

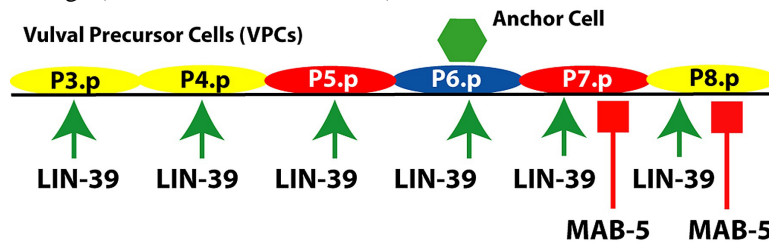


Figure 4. Hox genes and VPC competence group. *lin-39* is expressed in the central body region, including P3.p- P8.p shown. *mab-5* is expressed in more posterior cells including P7.p and P8.p. Arrow, positive regulation of competence; bar, negative regulation of competence. Yellow, 3° fate; red, 2° fate; blue, 1° fate.

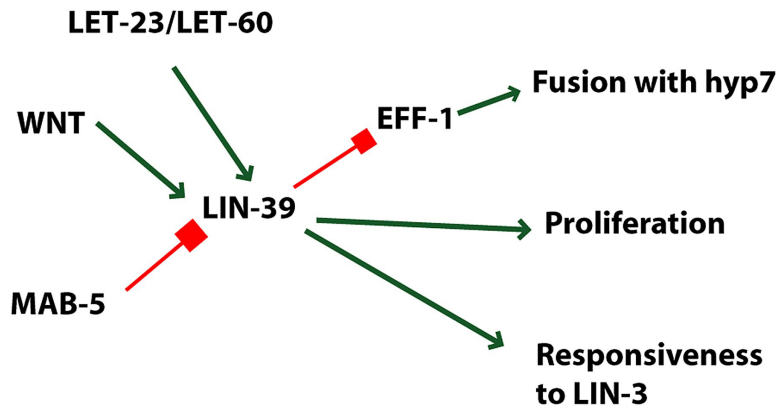


Figure 5. Roles of LIN-39. *lin-39* is positively regulated by WNT and LET-23 signaling, and is proposed to be inhibited by MAB-5 (see Figure 3). LIN-39 regulates proliferation, responsiveness to LIN-3 and inhibits fusion with *hyp7* via inhibition of EFF-1 expression. These events are occurring in the VPCs; transduction of the Wnt signal occurs in the VPCs, but the source of the Wnt signal is unknown. Green arrows, positive regulation; red bars, negative regulation.

2.1. Vulval competence group

In the intact, wild-type hermaphrodite grown under standard conditions, P5.p, P6.p and P7.p generate vulval cells. Ablation experiments indicated that the six cells P3.p, P4.p, P5.p, P6.p, P7.p and P8.p are competent to respond to intercellular signals and generate vulva. (Sulston and White, 1980; Kimble, 1981; Sternberg and Horvitz, 1986). For example, ablation of P6.p may result in P5.p adopting the 1° fate and P4.p adopting the 2° fate, or, P7.p may adopt the 1° fate and P8.p the 2° fate. Ablation of P5.p may result in replacement by P4.p, while ablation of P7.p may result in replacement by P8.p. Ablation of all VPCs but P3.p results in P3.p becoming 1°. There is some variability, especially as a function of time at which the ablation occurs.

Ablation of the gonad during the L1 stage (Sulston and White, 1980), or of the anchor cell prior to the L3 stage, results in all six VPCs becoming 3° (Kimble, 1981; Figure 6). These experiments also indicate that the anchor cell induces the vulva.

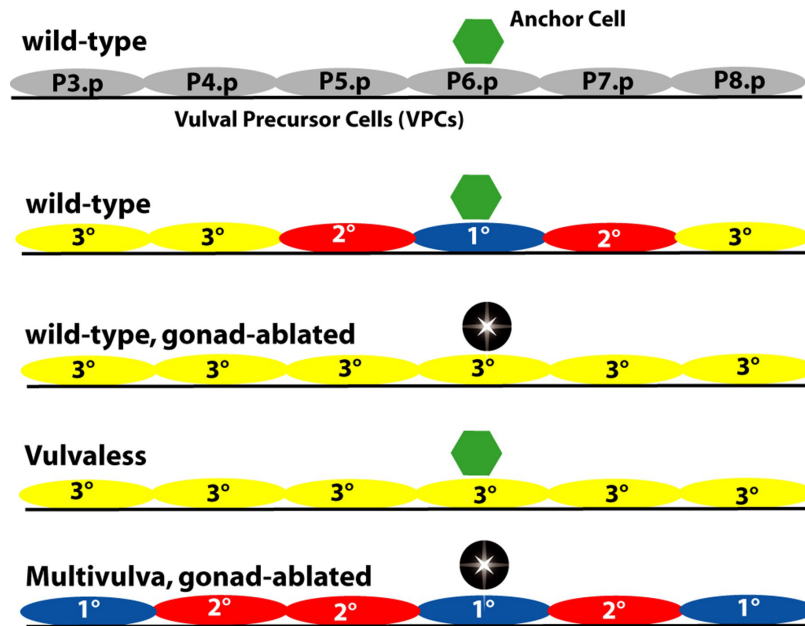


Figure 6. Pattern of VPC fates and main defects in induction. Ablation (indicated by black star) of the gonad or of the anchor cell results in a vulvaless phenotype with all six VPCs having 3° fates. Vulvaless animals have an anchor cell but vulval induction does not occur, resulting in a vulvaless phenotype. Multivulva mutants have more than three VPCs generating vulval progeny. Many have all six VPCs becoming 1° or 2° even in the absence of the anchor cell or gonad. Yellow, 3° fate; red, 2° fate; blue, 1° fate.

Ablation of all six VPCs in *C. elegans* did not result in P2.p or P9.p generating vulval progeny (Sulston and White, 1980; compare to *Panagrellus redivivus* (Sternberg and Horvitz, 1982; see Evolution of development in nematodes closely related to *C. elegans*), suggesting that only P3.p-P8.p form the "vulval equivalence group." Additional support for this conclusion comes from the observation that mutations that activate the LET-23 or LIN-12 signaling pathways (and hence allow VPCs to generate vulval cells independent of a signal from the gonad) only cause P3.p-P8.p but P2.p or P9.p to adopt vulval fates (Greenwald et al., 1983; Ferguson et al., 1987; Sternberg, 1988). Moreover, when the gonad position relative to the VPCs is displaced by a *dig-1* mutation, the position of the 1° cell can shift, no cells other than P3.p-P8.p generate vulval cells (Thomas et al., 1990).

2.2. Hox gene *lin-39*, competence, and fusion

The hox gene *lin-39* is a major determinant of the VPC group (Clark et al., 1993). *lin-39* is expressed in P3.p to P8.p (Salser et al., 1993). Lack of *lin-39* activity results in lack of VPCs because the presumptive VPCs fuse with the hyp7 epidermis. However, overexpression of *lin-39* does not cause additional Pn.p cells such as P2.p or P9.p to become competent. *lin-39* has at least two roles: preventing fusion and stimulating cell division (Figure 5). A mutation in the *eff-1* results in lack of cell fusion (Mohler et al., 2002). Inactivation of both *eff-1* and *lin-39* results in VPCs that fail to fuse and fail to divide (Shemer and Podbilewicz, 2002). WNT signaling via BAR-1/beta-catenin is necessary to maintain expression of *lin-39* in the P(3-8).p (Eisenmann et al., 1998). Expression of *lin-39* is sufficient

to bypass the requirement for *BAR-1* function in generating competent VPCs (Eisenmann et al., 1998). Using a *bar-1* mutant to sensitize the VPCs for loss of competence, Eisenmann et al. (1998) showed that *let-23* and the somatic gonad were involved in establishing competence of VPCs.

2.3. Other genes affecting competence

sem-4, which encodes a zinc finger protein, is necessary for full *lin-39* expression, and overexpression of *lin-39* can rescue the defect of a *sem-4* mutant (Grant et al., 2000). *CEH-20* and *UNC-62* are co-factors of *LIN-39* and as such affect vulval development (Yang et al., 2005). Two redundant GATA factors, encoded by the adjacent *egl-18* and *elt-6* genes, are necessary for vulval competence, and are regulated by *LIN-39* and *CEH-20* (Koh et al., 2002). The translocation *nT1* has a highly penetrant VPC competence defect (Ferguson and Horvitz, 1985; Ferguson et al., 1987). *nT1* disrupts the *egl-18/elt-6* operon, preventing its expression in the vulva (Koh et al., 2004).

2.4. Non-equivalence of the VPCs

More detailed analysis indicates that the VPCs, while multipotential, are not equivalent in their ability to respond to the major inductive signal (Clandinin et al., 1997). In particular *P7.p* and *P8.p* are less sensitive to EGF-R signaling than is *P6.p* (Figure 4). This difference in sensitivity is dependent on the hox gene *mab-5*, which is expressed in *P7.p* and *P8.p* but not *P(3-6).p* (Salser et al., 1993). Also, *sem-4* is preferentially required in *P7.p*, and this effect is in part due to *mab-5* (Grant et al., 2000). The details of fate specification may differ between *P5.p* and *P7.p*. These differences are relatively subtle, but very intriguing in light of vulval development in other nematode species (see Evolution of development in nematodes closely related to *C. elegans*).

3. Overview of VPC 1°-2°-3° pattern formation

The 1°, 2°, and 3° fates occur in a precise spatial pattern. Formation of the pattern of VPC fates involves several key elements. An inductive signal from the anchor cell acts via *LET-23* to induce *P6.p* and to a lesser extent *P5.p* and *P7.p*. Lateral signaling among induced VPCs via *LIN-12* that specifies 2° fates and inhibits 1° fates. Cross inhibition exists between the two pathways: *LIN-12* signaling turns on inhibitors of *LET-23* signaling, and *LET-23* signaling up-regulates ligand for *LIN-12* and down-regulates the receptor *LIN-12* (Figure 7). Inhibitors also act as if they are noise suppressors to ensure that fate specification is dependent upon signals. Figure 8 shows the key signaling pathways involved in vulval patterning. The current view of vulval precursor cell patterning is that the two systems cooperate to give a precise pattern (reviewed by Sternberg, 2004; Sundaram, 2004). Vulval induction apparently involves both a graded signal (*LIN-3* acting via *LET-23*) as well as a sequential, subordinate signal (*DSL* ligands acting via *LIN-12*; see *LIN-12/Notch* signaling in *C. elegans*).

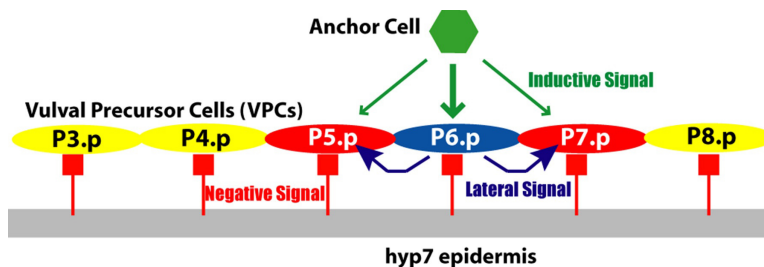


Figure 7. Three-signal model for vulval patterning. The invariant pattern of VPC fates is proposed to arise from the combined action of three signaling pathways. Yellow, 3° fate; red, 2° fate; blue, 1° fate. Green arrows, positive regulation; red bars, negative regulation. The inductive signal from the anchor cell (*LIN-3*; green) is necessary for non-3° fates. The Lateral signal among the induced VPCs (blue) promotes the 2° fate (via *LIN-12*). The negative signal is inferred from the non-autonomy of some class B synMuv genes. Adapted from Herman and Hedgecock (1990); Horvitz and Sternberg (1991).

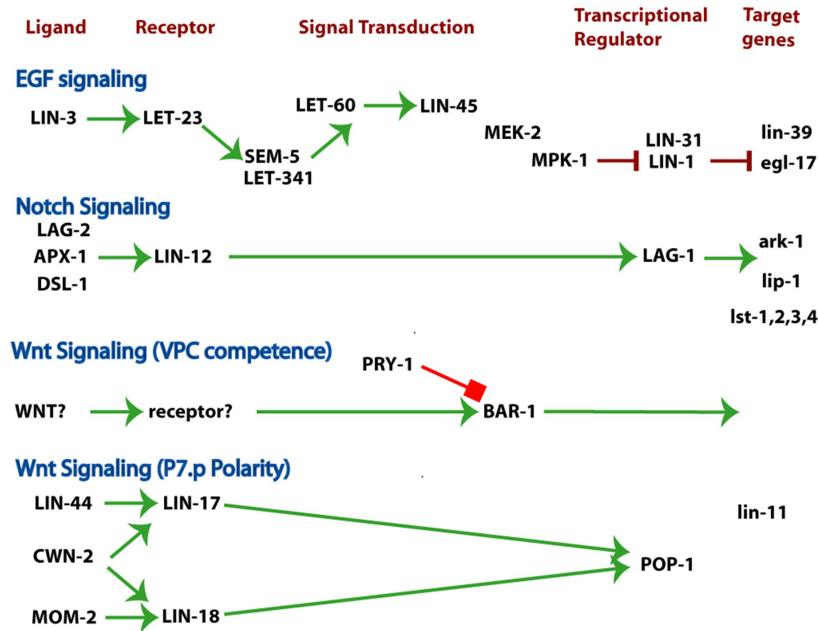


Figure 8. Three major signaling pathways patterning the vulva and uterus. EGF signaling. Notch signaling. WNT signaling. The ligand, receptor(s), signal transducers, nuclear targets and regulated genes for each pathway are indicated. Wnt signaling plays several roles during vulval development, including specification of VPC competence and polarity of P7.p. See RTK/Ras/MAP kinase signaling, LIN/Notch signaling in *C. elegans*, and Wnt signaling. Green arrows, positive regulation; red bars, negative regulation.

3.1. Specification and determination of the VPC fates

VPC patterning is conveniently and appropriately thought of in terms of VPC level specification (Sternberg and Horvitz, 1986). A specified VPC generates a characteristic 1°, 2° or 3° lineage, and expresses markers that are characteristic of each fate.

3.2. Commitment to fates

An open question in *C. elegans* development concerns fate commitment. I use specification to mean a cell knows what to do but is not irreversibly committed to that fate; commitment is irreversible (within a reasonable set of perturbations). The rapidity of *C. elegans* development, and the lack of long-term culture have precluded extensive tests of commitment. One can argue that cells are specified and then development proceeds without any need for long-term stability. One experiment with VPCs revealed that the 2° fate VPCs are not committed (Wang and Sternberg, 1999). Expression of high levels of LIN-3 after the VPCs have divided in a LIN-12 activated mutant alters their fate such that they express 1° lineage markers and display the morphological hallmarks of 1° lineages, such invagination; thus cells specified to become 2° are not committed even after they divide. The 3° fate is irreversible after fusion with hyp7, about an hour after their cell division. The 1° fates so far have not been altered after their division (Ambros, 1999; Wang and Sternberg, 1999). Ambros (1999) found that VPCs could become specified to become 1° prior to the end of their S phase but specification of 2° fates required the end of S phase. In this view, the cell cycle helps prioritize the specification process.

Hybrid lineages arise by too little induction (for example ablation of the anchor cell before the VPCs are fully induced) or by late induction acting on the daughter cells (for example if LIN-3 expression is induced after the VPCs divide; Wang and Sternberg, 1999). Fusion of VPC daughters (P3.pa, P3.pp, P4.pa, P4.pp, P8.pa, and P8.pp) occurs about an hour after they are formed, which eliminates their competence to respond to LIN-3.

3.3. The cell cycle and vulval development

Vulva development involves cell cycle in two distinct ways. Cell cycle regulation is one important consequence of vulval cell fate specification, and conversely, the cell cycle influences fate specification. One of the major differences between the uninduced 3° and the induced vulval (1° and 2°) VPC fates is in the extent of cell division. Cyclin E, encoded by *cye-1* is necessary for proliferation of the VPCs (Fay and Han, 2000). In *cye-1* mutants, there are fewer VPC divisions, but differentiation, assayed by expression of *egl-17::gfp* occurs at the

normal time. Moreover, Boxem and van den Heuvel (2002) found that cell division could be uncoupled to some extent from differentiation.

The heterochronic genes *lin-4*, *lin-14*, and *lin-28* control the G1 to S transition of VPCs (Euling and Ambros, 1996). The cell cycle inhibitor *cki-1* is one target of this heterochronic pathway (Hong et al., 1998). *cki-1::GFP* is expressed in the VPCs during their G1 phase. Inactivation of *cki-1* results in VPC division during the L2 stage, and expression of *cki-1* in P6.p under control of *egl-17* transcriptional regulatory sequences blocks its mitosis (Hong et al., 1998). *cki-1* expression in VPCs is reduced in the absence of *lin-14*, which results in precocious development of the VPCs.

4. Induction of the vulva by the anchor cell

The anchor cell (AC) induces vulval formation directly by expressing the epidermal growth factor (EGF)-like ligand LIN-3. The action of LIN-3 is graded and acts at a distance. The induction by the anchor cell can also be indirect, with induction of LIN-12-mediated lateral signaling and possibly additional *lin-3* signaling.

4.1. The anchor cell is necessary and sufficient

Ablation of the anchor cell or its ancestors prior to the L3 stage completely blocks vulval development, and the VPCs have 3° fates (3°-3°-3°-3°-3°-3°). Ablation of all gonadal cells but the anchor cell results in full induction of the vulva (Kimble, 1981). Males will copulate with these vulvae but not transfer sperm (M. Barr, K. Liu and P. Sternberg, unpublished observations).

lin-3 encodes the inductive signal from the anchor cell that induces the VPCs (Hill and Sternberg, 1992; Figure 9). *lin-3* is necessary for vulval induction (Horvitz and Sulston, 1980; Sulston and Horvitz, 1981; Ferguson and Horvitz, 1985; Ferguson et al., 1987; Hill and Sternberg, 1992). *lin-3* is expressed in the anchor cell during the time induction takes place. Expression of the EGF domain of LIN-3 directed by a heat shock (*hsp-16*) promoter/enhancer is sufficient to induce the vulva in the absence of the gonad and hence the anchor cell (Hill and Sternberg, 1992).

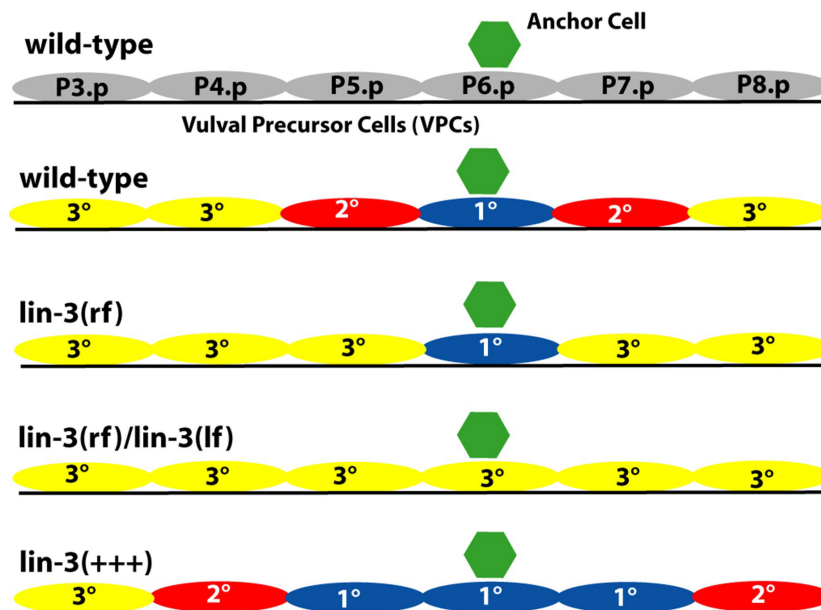


Figure 9. Level of LIN-3 directs pattern of VPC cell fates. In wild-type, the six VPCs adopt the 3°-3°-2°-1°-2°-3° pattern in each case. Animals with reduced *lin-3* function (*lin-3* reduction of function; *lin-3(rf)*) display a range of phenotypes including only P6.p induced to 1°. A further decrease in *lin-3* function results in a highly penetrant vulvaless phenotype. Excessive *lin-3* in the anchor cell (*lin-3(+++)*) results in an expansion of the pattern, including cases as shown here. Yellow, 3° fate; red, 2° fate; blue, 1° fate.

4.2. Action at a distance

The anchor cell signals at a distance from the VPCs. Cell ablation studies reveal that a distal VPC can be induced to a 1° or 2° fate (or some intermediate; Sternberg and Horvitz, 1986; Katz et al., 1995; Sherwood and

Sternberg, 2003). In addition, in *dig-1* mutants, a dorsally located anchor cell can induce patterns such as 1°-2°-1°, 1°-2°-1°-2° or 2°-2°-2°-2° (Thomas et al., 1990). The dorsally located anchor cell can extend a process ventrally, but this occurs after induction, and is fully consistent with the anchor cell invasion of the vulval epidermis (Sherwood and Sternberg, 2003).

4.3. Graded action of LIN-3

The anchor cell signal is able to act at a distance and appears graded based on a correlation of distance from the anchor cell and the fate of individual VPCs (Sternberg and Horvitz, 1986). An individual VPC, after ablation of other VPCs, can have a 2° fate. Also, in a *dig-1* mutant there can be 2° VPCs without 1° VPCs (Thomas et al., 1990). In addition, molecular genetic experiments (Katz et al., 1995; Katz et al., 1996) strongly support the possibility of a graded signal. The dose of LIN-3 was controlled in two ways. The EGF domain of LIN-3 along with a synthetic signal sequence was expressed under the transcriptional control of a heat-shock enhancer/promoter. By varying the duration and temperature of heat shock, different apparent doses of LIN-3 could be produced. A high dose results in 1° fates; an intermediate dose in 2° fates; and a low dose in 3° fates. In most cases there is a distribution of fates as well as intermediate fates. A distinct set of experiments with partially activated LET-23 gave similar results (Katz et al., 1996).

The graded expression of *egl-17*, the best-known target of LET-23 signaling in the 1° VPC (Burdine et al., 1998), provides a molecular correlate for a graded inductive signal. *egl-17-CFP-LacZ*, is initially expressed in P5.p, P6.p and P7.p with higher expression in P6.p, thereby indicating a graded signal (Yoo et al., 2004). Its expression is increased in P6.p and decreased in P5.p and P7.p due to LIN-12 signaling as described below.

4.4. Non-graded action of LIN-3: sequential induction

In addition to the strong support for a graded signal, there is also strong support for a sequential signaling mechanism in which activation of LET-23 in P6.p leads to activation of LIN-12 in P5.p and P7.p. Certain genetic mosaics lacking *let-23* activity suggest that direct induction by the AC is not necessary for the 2° fate: if *let-23(+)* is present in all cells, there is a wild-type vulva; if all cells are *let-23(-)* there is no vulva (Figure 10; Simske and Kim, 1995; Koga et al., 1995). If P6.p is *let-23(+)* but P5.p and P7.p are *let-23(-)*, then in almost all cases there was a normal vulva. Thus, a cell can become 2° without receiving LIN-3. This key result is consistent with the observations that activation of LIN-12 can specify a 2° fate in the absence of an anchor cell (Greenwald et al., 1983) or of LET-23 signaling (Sternberg and Horvitz, 1989; Han et al., 1990), and strongly makes the point that the direct induction of 2° by the anchor cell is not necessary. LIN-12 is expected to be the receptor for this 2°-inducing signal: *lin-12* activity is necessary and sufficient for the 2° fate (Greenwald et al., 1983), and ligands for LIN-12 are up-regulated in P6.p in response to the inductive signal (Chen and Greenwald, 2004).

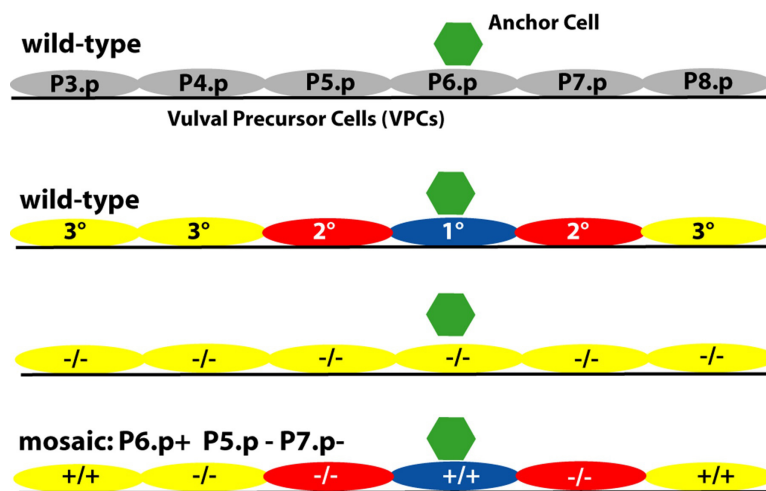


Figure 10. Sequential signaling inferred from mosaic analysis of *let-23*. yellow, 3° fate; red, 2° fate; blue, 1° fate. Genotype of VPCs in key mosaic animal shown. *-/-*, lacking *let-23* activity; *+/+*, *let-23(+)* activity present. In the absence of *let-23*, all VPCs become 3°. In mosaic animals which lack *let-23* in P5.p and P7.p but have *let-23* in P6.p, the vulva is usually wild-type. This observation indicates that *let-23(+)* is not required in presumptive 2° VPCs. Results of Simske and Kim (1995) and Koga and Ohshima (1995).

The converse mosaic animals had a striking phenotype: if P6.p is *let-23(-)* but P5.p and P7.p are *let-23(+)*, then additional cells are induced (Simske and Kim, 1995; also see Hajnal et al., 1997). Hajnal et al. argue that LET-23 might be sequestering LIN-3 and preventing its spread to more distal cells.

4.5. Possible relay induction by LIN-3

Dutt et al. (2004) have suggested that *lin-3* expression in induced VPCs extends the range of induction, and that this role of *lin-3* depends on ROM-1. *rom-1* encodes a protease related to *Drosophila* Rhomboid, which cleaves Spitz, a *Drosophila* protein related to LIN-3. *lin-3* encodes multiple isoforms that differ in the region between the EGF domain and the transmembrane domain (Hill and Sternberg, 1992; Dutt et al., 2004), a part of the protein that would likely be cleaved for LIN-3 in a relay mechanism. The B but not the A isoform of LIN-3 is ROM-1 dependent (Dutt et al., 2004).

4.6. Nuclear targets of the LET-23-LET-60-MPK-1 pathway

Two transcription factors, LIN-31 and LIN-1 are likely targets of the MAPK pathway (Tan et al., 1998). LIN-31 and LIN-1 can both be phosphorylated by MAP kinase MPK-1. Inactivation of either *lin-1* or *lin-31* leads to a Multivulva phenotype. LIN-1 and LIN-31 form a complex that is disrupted by LIN-31 phosphorylation (Tan et al., 1998). Increased expression of LIN-31 or a non-phosphorylatable LIN-31 protein causes a vulvaless phenotype. During the L3 stage, *lin-31* mutants display both vulvaless and multivulva phenotypes (Ferguson and Horvitz, 1985). *lin-31* mutants display inappropriate divisions of Pn.p cells during the L2 stage (Ferguson et al., 1987; Miller et al., 2000), suggesting also a role in the proper specification of VPCs.

lin-39 is upregulated by LET-23 signaling (Maloof and Kenyon, 1998) and is an excellent candidate for a direct target. Since overexpression of *lin-39* does not result in gonad-independent vulval differentiation, it is unlikely to be the only target (Figure 5). *egl-17* is induced early in the induction process (Burdine et al., 1998; Ambros, 1999; Wang and Sternberg, 1999; Cui and Han, 2003; Yoo et al., 2004) and could be an early target of RAS-stimulated transcription. Romagnolo et al. (2002) used RNA from whole animals hybridized to microarrays to search for RAS-regulated genes and found 718; among these might be some that are physiologically relevant during vulval development.

Mediator complex proteins, which link site-specific transcription factors to RNA polymerase, also are necessary for vulval development. *lin-25* and *sur-2* (*mdt-23*) are defective in vulval induction and also have defects earlier and later during vulval development (Ferguson et al., 1987; Tuck and Greenwald, 1995; Singh and Han, 1995). *mdt-6* is positive-acting (Kwon and Lee, 2001). It is striking that other Mediator components have negative effects, e.g., *dpy-22* (*mdt-12*; Moghal et al., 2003; H. Sawa, unpublished observations). There are two extreme interpretations of this observation. First, there might be differential requirements for mediator components on distinct transcriptional targets. Second, there might be one main target for Mediator and there is a regulatory pathway acting among the Mediator components with some components acting in a positive and others in negative manner.

4.7. WNT signaling and induction

In addition to its role in VPC competence, activation of WNT signaling can increase the extent of vulval induction if the LET-23 pathway is compromised (Gleason et al., 2002). It is not known whether WNT signaling can compensate for the complete absence of LIN-3 signaling.

5. Physiological inputs to vulval development

The invariance of vulval development is not only due to the growth of *C. elegans* under standardized laboratory conditions; however, when vulval development is partly compromised, a number of physiological effects are evident. Ferguson and Horvitz (1985) observed that certain vulvaless mutations are suppressed by starvation during larval growth while others are suppressed by passage through the dauer phase. The suppression does not appear to correlate with genetic pathways, and might be due to some type of informational suppression (see Genetic suppression). Recently several other potentially physiological inputs (Battu et al., 2003; Moghal et al., 2003), and an important role for zinc have been identified (Bruinsma et al., 2002; Yoder et al., 2004).

5.1. G protein regulation

The heterotrimeric G protein Gq (EGL-30; see [Heterotrimeric G-proteins in *C. elegans*](#)) stimulates vulval induction under certain growth conditions, in particular growth in liquid (Moghal et al., 2003). Expression of activated EGL-30 in neurons stimulates vulval induction. The EGL-30 effect requires the voltage-gated calcium channel encoded by *egl-19*. The site of action of *egl-19* for this effect is in muscle cells. These observations lead to the hypothesis that neuronal and muscle excitation can somehow affect vulval development. The positive signal from Gq acts in parallel to or downstream of RAS. Because the effect is of the magnitude of WNT signaling via BAR-1 β -catenin, it is possible that this effect is via the WNT pathway (Moghal et al., 2003).

A G protein coupled receptor, SRA-13, acts negatively on vulval development via the G protein GPA-5 (Battu et al., 2003). Starvation decreases vulval induction, and this effect depends upon SRA-13. *sra-13* is expressed in chemosensory neurons as well as body-wall muscle (Battu et al., 2003) and *gpa-5* is also expressed in chemosensory neurons. The sites of action of SRA-13 and GPA-5 for vulval induction effects are not known. The relationship of the positive EGL-30 (Gq) effect and negative GPA-5 effects is intriguing but not yet known. These findings highlight the importance of nematode physiology, revealed by growth conditions, on vulval development. The effect on vulval induction of growth in liquid (Moghal et al., 2003) and of starvation (Battu et al., 2003) were only revealed by using sensitized genetic backgrounds. I believe that there could be much plasticity lurking beneath the surface, a surface that can be scratched away by careful genetic analysis.

5.2. Zinc regulation

Two genes identified by loss-of-function mutations that suppress activated LET-60 Ras encode cation diffusion facilitators that appear to regulate zinc. CDF-1 is a cation diffusion regulator, and has sites of action in the intestine as well as in the VPCs, consistent with its expression in both tissues (Bruinsma et al., 2002). Exogenous zinc negatively regulates LET-23 signaling (Bruinsma et al., 2002). SUR-7 encodes a distinct cation diffusion facilitator (Yoder et al., 2004). Yoder et al. (Yoder et al., 2004) argue that zinc regulation is at the level of KSR-1 and PAR-1, which somehow regulate LIN-45 Raf signaling (see [RTKRas/MAP kinase signaling](#)).

6. Negative regulation of induction

A variety of negative regulators of vulval induction have been identified. Their roles in signal transduction by LET-23 are described in [RTKRas/MAP kinase signaling](#).

6.1. Nuclear negative regulators

The synMuv genes are three classes (A, B and C) of broadly expressed nuclear proteins that in various combinations inhibit VPCs from adopting vulval fates. Ferguson and Horvitz (1989) described two redundant sets of genes, class A and B, where hermaphrodites defective in both A and B function have a Multivulva phenotype. Mutants defective in only A, or in only B, have normal vulval development. Molecular cloning revealed that many Class B synMuv genes encode proteins with mammalian orthologs that have been well-characterized as transcriptional regulators, including the synMuv genes *lin-35* Rb, *efl-1* E2F, and *hda-1* histone deacetylase (Lu and Horvitz, 1998), as well as a zinc finger protein (Melendez and Greenwald, 2000) and components of the NuRD and SWI/SNF complexes (Lu and Horvitz, 1998; Solari and Ahringer, 2000; Ceol and Horvitz, 2001; Cui et al., 2004). Other synMuv genes encode novel nuclear proteins.

Epistasis experiments suggest that synMuv genes inhibit LET-23 pathway output (Ferguson et al., 1987; Huang et al., 1994). Site of action studies with synMuv genes suggest that they control a pathway of intercellular signaling from *hyp7* to the VPCs, although no signal has been identified (Figure 11; Figure 12). *lin-15AB* and *lin-37B* mosaics suggested action not in the VPCs, and possibly in *hyp7* (Herman and Hedgecock, 1990; Herman and Hedgecock, 1995); Mosaic analysis and tissue-specific rescue experiments indicate that the focus of *lin-35* is *hyp7* (Myers and Greenwald, 2005). However, *lin-36B* mosaics suggest a site of action in the VPCs (Thomas and Horvitz, 1999), and *lin-15A* mosaics and tissue-specific rescue experiments also suggest a VPC focus (A. Gonzalez-Serricchio and P. W. Sternberg, unpublished observations).

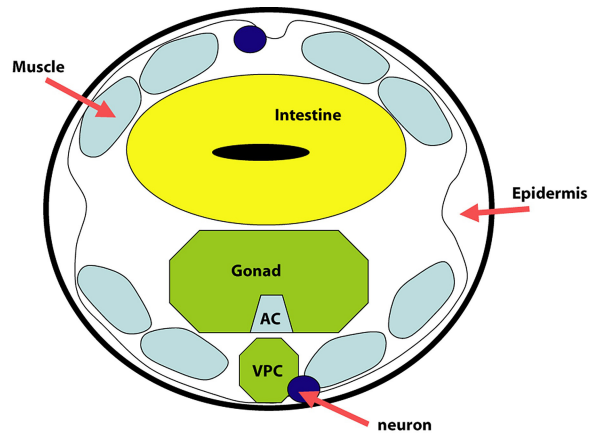


Figure 11. Schematic transverse section through the developing vulva. The major tissues of the worm at the position of the vulva are shown in this schematic transverse section. VPC, vulval precursor cell (P6.p); AC, anchor cell. The VPCs are separated from the AC by the basement membranes of both the gonad and the epidermis, and the pseudocoelom (body cavity). See *WormAtlas* for detailed description of the anatomy.

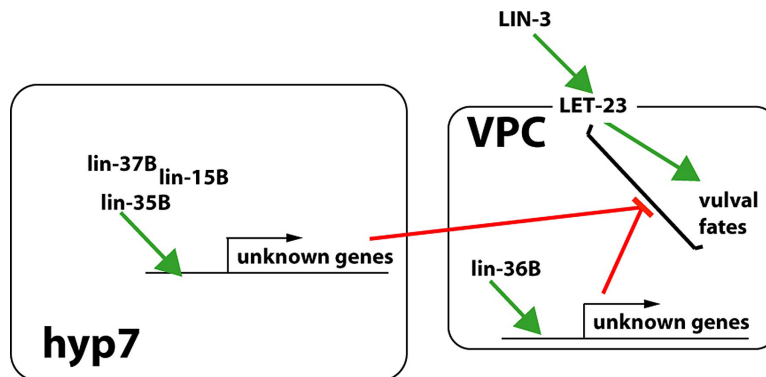


Figure 12. Synthetic multivulva mutants suggest signaling from hyp7 to VPCs. *lin-15b*, *lin-35* and *lin-37* have been argued to act in hyp7 while *lin-36* has been argued to act in the VPCs. These proteins where known are nuclear and presumably regulate gene expression (green arrows), although the physiological targets are unknown (unknown genes) as is the sign of the regulation. Epistasis analysis suggests that the synMuv genes somehow act negatively on LET-23 signaling (red bars) thereby inhibiting the specification of 1° and 2° VPC fates.

6.2. Signal transduction regulators

Other negative regulators were identified as suppressors of hypomorphic mutations in LET-23-RAS pathway components. These genes do not interact strongly with the synMuv genes, but do interact with each other. *sl-1*, *unc-101*, *ark-1*, *gap-1* and *lip-1* (Jongeward et al., 1995; Lee et al., 1994; Yoon et al., 1995; Hajnal et al., 1997; Berset et al., 2001; Hopper et al., 2000) are of this class. In addition, Yoo et al. (2004) found four additional negative regulators, *dpy-23*, *lst-1*, *lst-2*, *lst-3*, and *lst-4*. These four regulators, and *lip-1* (Berset et al., 2001) are targets of LIN-12 lateral signaling.

unc-101 and *apm-1* each encode medium chains of the AP-1 adaptin, and these are inhibitory on vulval induction (Lee et al., 1994; Shim, 2000); while LET-23 is a plausible target for these membrane trafficking proteins, the targets are not known. *dpy-23* encodes a medium chain of the AP-2 adaptin and is induced by LIN-12 (Yoo et al., 2004; see below).

7. LIN-12-mediated lateral signaling

The VPCs signal each other via a LIN-12-mediated lateral signaling pathway. This pathway has two related roles: to prevent adjacent induced VPCs from both becoming 1°, and to induce VPCs that flank a 1° VPC to become 2° (Figure 13).

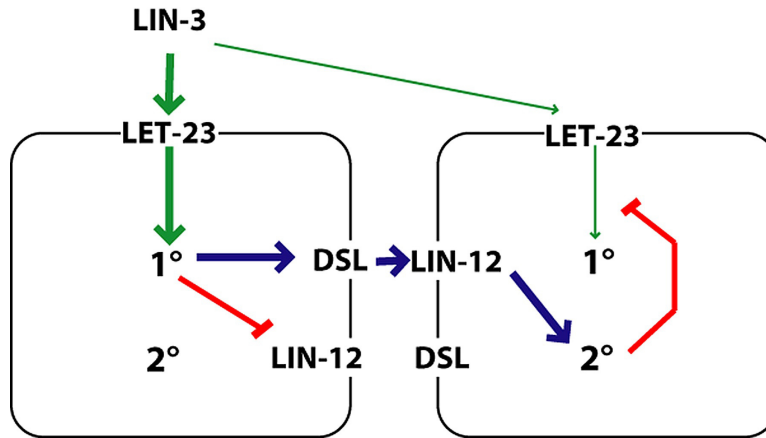


Figure 13. Simple model of antagonism between 1° and 2° VPCs. Signals for 1° specification (green) compete with signals for 2° specification (blue). In this example, the left cell is winning the competition to become 1° because it receives more LIN-3. LIN-3 signaling via LET-23 promotes the 1° fate, which includes the production of DSL ligands and downregulation of the receptor LIN-12. DSL ligands activate LIN-12 on neighboring cells, thereby promoting the 2° fate, which inhibits LET-23 signaling. Red bars, inhibition. Model based on Sternberg and Horvitz (1989); Berset et al. (2001); Shaye and Greenwald (2002); and Yoo et al. (2004).

7.1. *lin-12* genetics

lin-12 is necessary and sufficient for specification of the 2° VPC fate (Greenwald et al., 1983). Null alleles of *lin-12* lack 2° VPCs. Gain of function *lin-12* mutants cause all six VPCs to generate 2° lineages, even in the absence of inductive signaling. As described in LIN-12/Notch signaling in *C. elegans*, *lin-12* encodes a Notch-type transmembrane receptor (Yochem et al., 1988).

lin-12(lf) hermaphrodites have additional anchor cells, and this complicated somewhat the analysis of vulval induction (Greenwald et al., 1983). With two (or more) anchor cells, P5.p, P6.p and P7.p are 1° and P4.p and P8.p occasionally are 1° or one daughter is 1° like (Sternberg and Horvitz, 1989).

7.2. Lateral signal

The lateral signal involves at least three redundant genes, *apx-1*, *lag-2* and *dsl-1* (Chen and Greenwald, 2004; see LIN-12/Notch signaling in *C. elegans*). APX-1 and LAG-2 are transmembrane ligands for LIN-12. DSL-1 is a secreted ligand for LIN-12. Expression of *lag-2* occurs in all VPCs prior to induction and is upregulated in P6.p. Expression of *apx-1* and *dsl-1* appears to be initiated by induction of P6.p. The main effect is to induce neighbors to become 2°, thereby inhibiting them from becoming 1°. The redundancy of *lag-2*, *dsl-1* and *apx-1* for VPC patterning appears to have prevented the discovery of their role by genetic screens. The targets of LIN-12 include a number of redundant negative regulators of LET-23 signaling (Yoo et al., 2004; Figure 14).

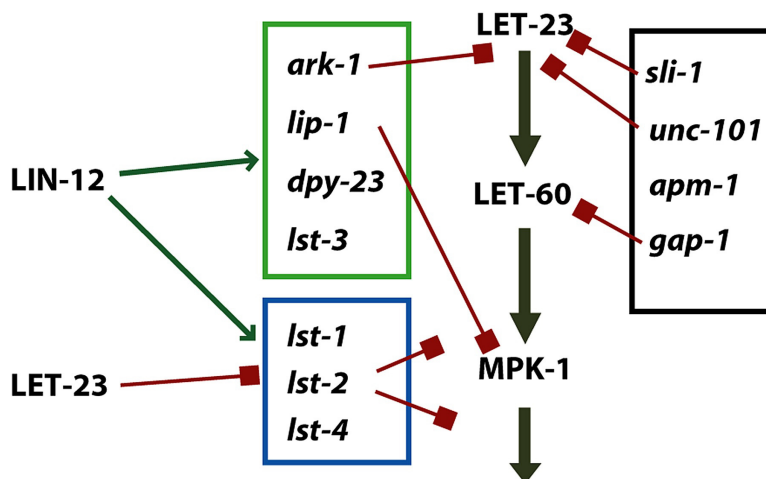


Figure 14. Lateral signaling targets and negative regulation of LET-23 signaling. Green box, targets whose expression is induced by LIN-12; blue box, targets whose expression is inhibited by LET-23 but induced by LIN-12; Black box, targets whose expression has not been analyzed or that are not regulated by these signaling pathways. See Yoo et al. (2004) for details.

The pattern of VPC fates in a *lin-15* multivulva mutant ($1^\circ-2^\circ-2^\circ-1^\circ-2^\circ-1^\circ$; Figure 15) is reminiscent of spacing patterns such as that of bristles in the insect abdomen and thus suggests a lateral inhibition mechanism whereby a specialized cell type (1° VPC) inhibits adjacent cells from also becoming of that type and instead differentiating as some alternative (2° VPCs). While adjacent 2° VPCs are common, adjacent 1° VPCs are rare. In a *lin-15* strain, which appears to have activated LET-23 signaling, a single VPC (e.g., P7.p) will become 1° in the absence of the gonad and of its neighbors (Sternberg, 1988). If there are two adjacent VPCs (P7.p and P8.p), they will become $1^\circ-2^\circ$ or $2^\circ-1^\circ$, with approximately equal probability. Thus, these cells signal each other.

In the presence of the gonad, P7.p becomes 1° and P8.p becomes 2° , indicating that the inductive signal can bias the stochastic lateral signaling among induced VPCs (Sternberg, 1988). Double mutant combinations among inductive signaling pathway genes and *lin-12* mutations support this view: a *lin-12* loss-of-function mutation eliminates the lateral inhibition in a *lin-15* mutant resulting in all 1° VPCs (Sternberg and Horvitz, 1989).

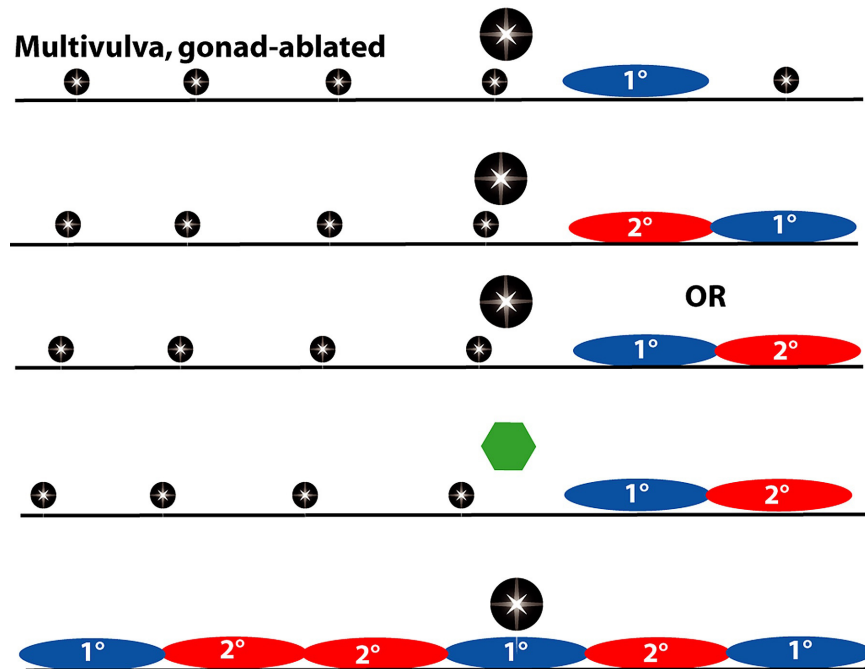


Figure 15. Lateral signaling. In the absence of the gonad and other VPCs, P7.p becomes 1° in a *lin-15* multivulva mutant. If P8.p is also present, the two cells adopt a $1^\circ-2^\circ$ or $2^\circ-1^\circ$ pattern at random. In the presence of the anchor cell, P7.p becomes 1° and P8.p 2° (Sternberg, 1988).

8. Coupling of LET-23 and LIN-12 signaling

The antagonism of LET-23 and LIN-12 is fundamental to the specification of 1° and 2° fates (Figure 16). As described above, LET-23 activation leads to 1° fates at high levels and 2° at low levels. The genetic requirements for this 2° specification has not been elucidated. LIN-12 lateral signaling is able to prevent adjacent cells from becoming 1° (Sternberg, 1988), and can induce a cell to become 2° (Greenwald et al., 1983; Simske and Kim, 1995; Koga and Ohshima, 1995). A presumptive 1° cell downregulates LIN-12 protein (Shaye and Greenwald, 2002). Conversely, a presumptive 2° cell downregulates LET-23 signaling (Berset et al., 2001; Yoo et al., 2004). *C. elegans* vulva development was the first case where the interplay of growth factor receptor and Notch signaling integration was discovered.

Activation of LIN-12 bypasses the requirement for the gonad or for LET-23 (Greenwald et al., 1983; Sternberg and Horvitz, 1989). However, a strong LIN-3 signal can override the effect of activated LIN-12, if a VPC is near the anchor cell (Sternberg and Horvitz, 1989), if *lin-1* is removed (Beitel et al., 1995), or if LIN-3(EGF) is delivered by a heat shock construct (Wang and Sternberg, 1999). Validated targets of LIN-12 in presumptive 2° cells are *lip-1*, encoding MAPK phosphatase (Berset et al., 2001), *dpy-23* and the *lst* genes (Yoo et al., 2004). *lst-1*, *lst-2* and *lst-4* also appear to be inhibited by LET-23 (Figure 14), which creates the potential for interesting dynamics in the cross-talk between LIN-12 and LET-23 signaling pathways.

8.1. Multiple redundancies program precision

C. elegans VPC patterning involves multiple sources of information each of which would be capable, in principle, of generating a normal pattern. These include: the graded action of *lin-3* from the anchor cell, sequential signaling from P6.p via LIN-12, sequential ROM-1-dependent LIN-3 signaling; and non-equivalence of the VPCs due to HOX gene action. The fact that WNT signaling can increase vulval induction (Gleason et al., 2002) and that WNTs are expressed in the anchor cell (Inoue et al., 2004) raises the possibility that WNT signaling is another way in which the vulval might be induced. These pathways might act in combination to generate an exquisitely precise pattern. In *C. elegans* the graded anchor cell LIN-3 signal and LIN-12-mediated lateral signaling are the major players. In other species, the other pathways might predominate.

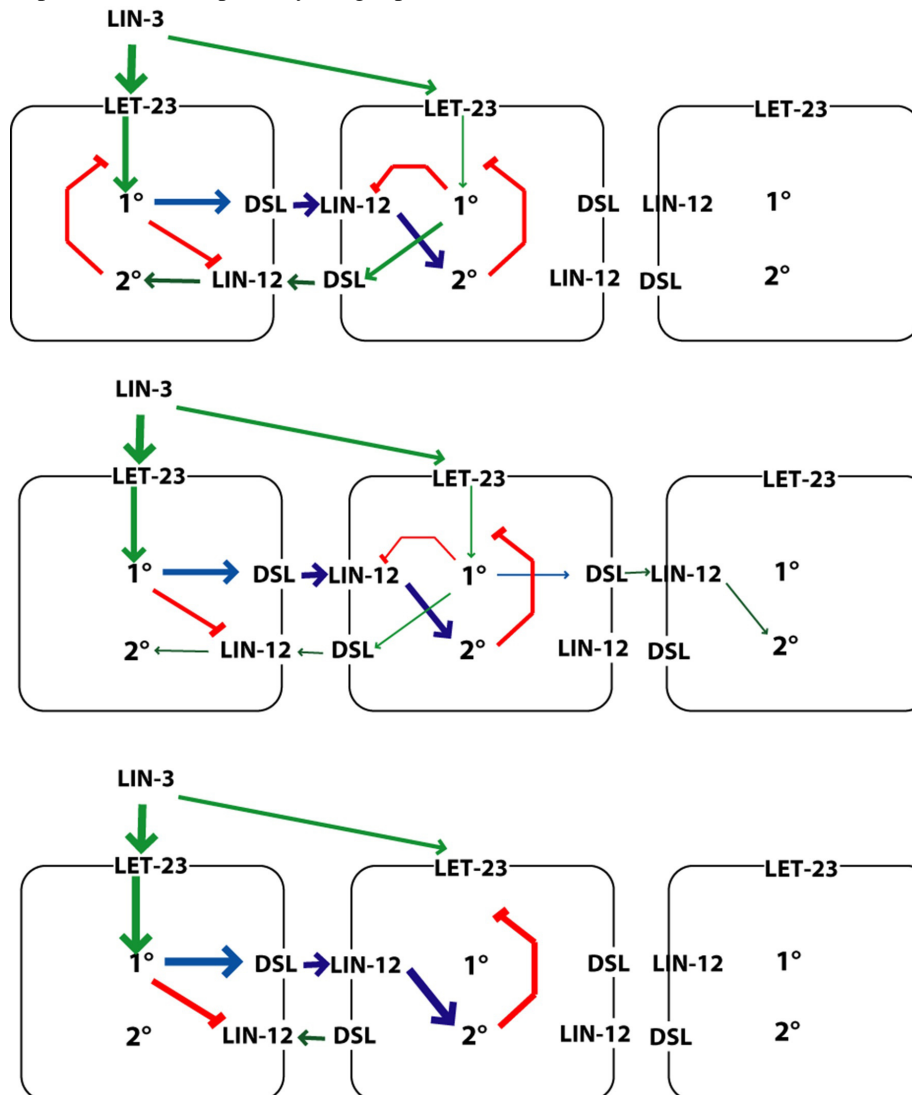


Figure 16. Model of VPC pattern formation. Three of the six VPCs (P6.p, P7.p and P8.p) are shown in this simplified diagram to illustrate the critical aspects of the 1°-2° patterning mechanism. LIN-3 from the anchor cell acts in a graded fashion with P6.p receiving more signal than P7.p. LET-23 activation promotes the 1° fate and production of DSL ligands for LIN-12, and inhibits LIN-12 protein levels. LIN-12 activation promotes the 2° fate and inhibits the response to LET-23 activation. The three panels give a flavor of the type of dynamics that might underlie this process. Model from Sternberg and Horvitz (1989); Berset et al. (2001); Shaye and Greenwald (2002); Yoo et al. (2004) and Sternberg (2004).

9. Patterning of adult cell types

There are seven vulval cell types formed from the fusion of defined cells produced by vulval lineages: vulA, vulB1, vulB2, vulC, vulD, vulE and vulF. They each have distinct functions and patterns of gene expression (Burdine et al., 1998; Sharma-Kishore et al., 1999; Inoue et al., 2002; Kiroauc and Sternberg, 2003; Inoue et al.,

2005). For example, the vulval muscles vm1 insert between the vulE and vulF cells, while the vm2 muscles insert between the vulC and vulD cells. The vulF cells are the main contact with the anchor cell and then uv1 (Newman et al., 1996; Sherwood and Sternberg, 2003).

Transcription factors encoded by *egl-38* (Chamberlin et al., 1997), *cog-1* (Palmer et al., 2002), and *lin-11* (Freyd et al., 1990) are responsible in part for the identities of specific vulval cell types. *egl-38* is necessary for expression of *lin-3* in vulF cells (Chang et al., 1999), and is involved in repressing *egl-17* and *zmp-1* in these same cells (Inoue et al., 2005). *cog-1*, which is expressed in all the terminal vulval cells but with different levels and dynamics, regulates gene expression in vulB1, vulB2, vulC, vulD and vulE (Palmer et al., 2002; Inoue et al., 2005). *lin-11*, which is more highly expressed in the vulC and vulD cells than in vulA and vulB cells, regulates gene expression in mature vulval cell types (Freyd et al., 1990; Gupta et al., 2003). The transcription factor LIN-29 has been proposed to regulate vulval cell-type genes in a temporal window (Newman et al., 2000; Inoue et al., 2002; Inoue et al., 2005).

9.1. 1° lineage

The 1° lineage generates two cell types, vulE and vulF, in a mirror symmetric pattern EFFE around the anchor cell (Figure 17). The anchor cell is necessary for this pattern after it induces the 1° VPC (Wang and Sternberg, 2000). WNT receptor LIN-17 is necessary as well, but the phenotype of a *lin-17* mutation has only been seen in the absence of the anchor cell. Transcription factor EGL-38 is necessary for *lin-3* expression in the vulF cells (Chang et al., 1999), which signals to the uterine uv1 cells (Figure 18). The peptidase-like protein EGL-26 is expressed in the membranes of vulE cells and is necessary for vulF morphogenesis (Hanna-Rose and Han, 2002). Transcription factor COG-1, expressed in most vulva cells, has a related phenotype to EGL-26 (Palmer et al., 2002), but the relationship among these genes is not known.

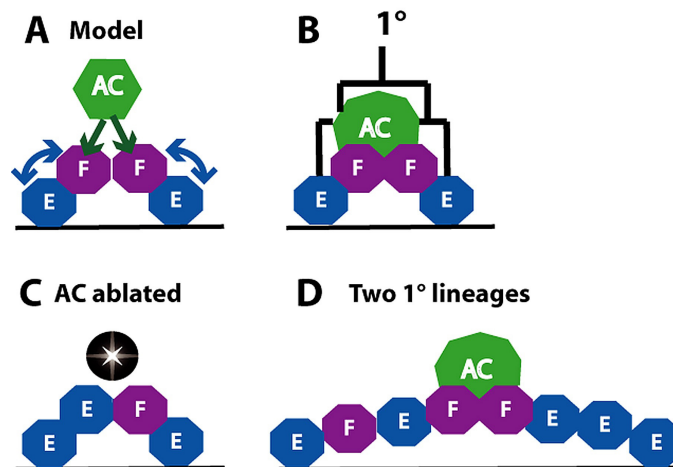


Figure 17. Patterning of the 1° lineage. A. Model posits a signal via RAS to specify vulF cells near the anchor cell. B. Intact, wild-type 1° lineage has two F cells near the anchor cell and two E cells more distal. C. After AC ablation, the patterns are variable. D. In a *lin-12(lf)* mutant in which all ventral uterine cells but one anchor cell have been ablated, and in which P7.p has been ablated to keep only two 1° lineages, there is a normal pattern at the anchor cells, but a variable pattern more distal. Data and model summarized from Wang and Sternberg (2000).

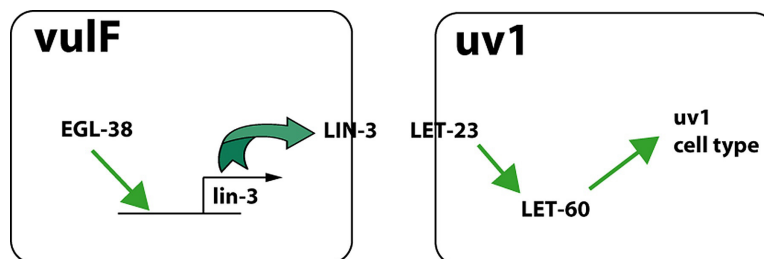


Figure 18. uv1 specification. The vulF cell expresses LIN-3 and signals the presumptive uv1 cells via LET-23 and LET-60. The site of EGL-38 action is not known, but it is necessary for *lin-3* expression in the vulF cell (Chang et al., 1999). Activated LET-23 bypasses this requirement for EGL-38 function.

9.2. Polarity of 2° lineages

The cell division pattern and cell fates of the P5.p and P7.p lineages are mirror images, ABCD and DCBA respectively (Figure 19). The asymmetry within in each of these lineages is independent of the gonad, as for example, a single VPC in a gonad-ablated *let-23(gf)* mutant has asymmetry (Katz et al., 1996). WNT signaling, likely from the gonad, reverses polarity of P7.p (Inoue et al., 2004). The WNT protein MOM-2 is expressed in the anchor cell (Inoue et al., 2004) and LIN-44 might be expressed in the anchor cell or uterus based on weak GFP signal (Inoue et al., 2004) and in situ hybridization (Y. Kohara, personal communication). There are two parallel genetic pathways: one involves the WNT protein LIN-44 and the Frizzled type receptor LIN-17; the second involves the WNT MOM-2 and the Ryk type receptor LIN-18 (Ferguson et al., 1987; Sternberg and Horvitz, 1988; Sawa et al., 1996; Inoue et al., 2004). The levels of TCF/LEF protein POP-1, a WNT target, correlates with WNT signaling: P5.p daughters express POP-1 in a High-Low (HL) pattern, and P5.p granddaughters express in an HLHL pattern, while P7.p has a LH then a LHLH pattern of protein level (Deshpande et al., 2005). Thus, the pattern of POP-1 expression in the P7.p neage is reversed from the default by WNT signaling through LIN-17 and LIN-18.

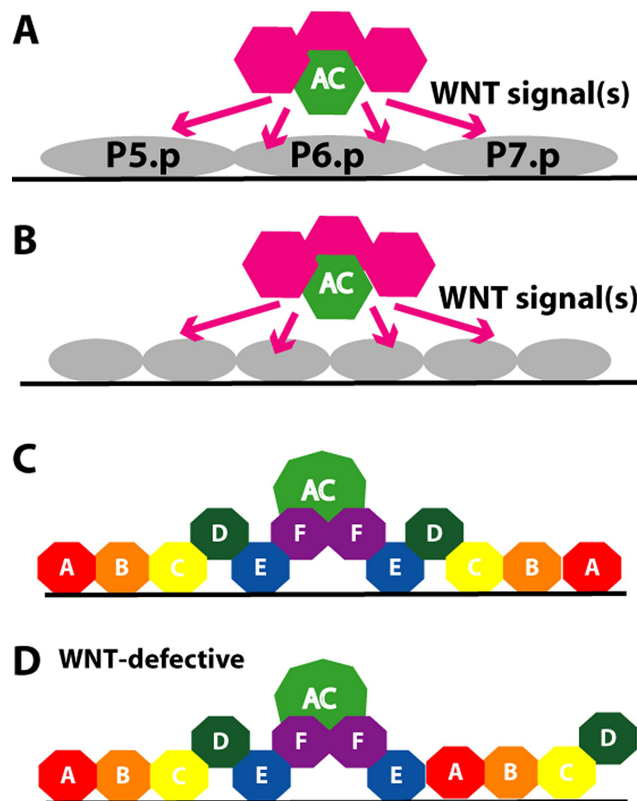


Figure 19. Polarity of P7.p 2° lineage. Multiple WNT signals from the anchor cell and probably uterus reverse the polarity of the P7.p lineage such that it has an DCBA pattern rather than the default ABCD pattern as does P5.p. A. early L3. B. mid-L3. C. late L3. D. late L3 in animals defective in *lin-17* and *lin-18* WNT-receptor pathways. Model from Inoue et al. (2004) and Deshpande et al. (2005).

10. The vulval-uterine connection

The anchor cell is the key organizer of the vulva and the connection to the somatic gonad (Newman et al., 1996; Figure 1). Once specified during the L2 molt (see Hermaphrodite cell-fate specification), the anchor cell induces and patterns the vulva via LIN-3 and LET-23. The anchor cell then patterns the ventral uterus, inducing the uterine pi cells via LAG-2 and LIN-12 signaling (Newman et al., 1995; Cinar et al., 2003; Figure 20).

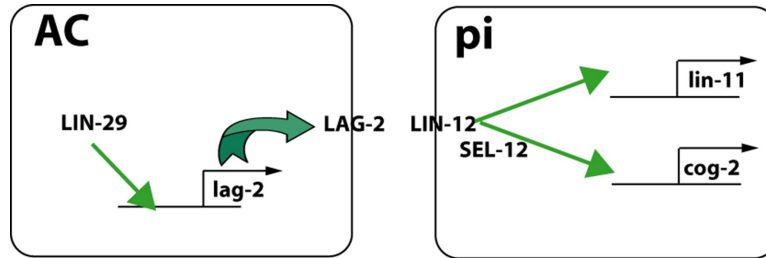


Figure 20. Uterine pi cell induction. The anchor cell expresses DSL-type ligand LAG-2 in a LIN-29-dependent manner and signals presumptive pi cells via LIN-12. LIN-12 activation leads to expression of *lin-11* and *cog-2*, two transcription factors necessary for utse development, including fusion of the anchor cell with utse. Model adapted from Newman et al. (1999).

10.1. pi cell induction and function

egl-13 (a.k.a. *cog-2*), which encodes Sox domain transcription factor expressed in the uterine pi cells, is necessary for the fusion of the anchor cell and the utse (Hanna-Rose and Han, 1999; Cinar et al., 2003). *lin-11*, another transcription factor, is also required for differentiation of pi cells progeny and is regulated by LIN-12 signaling in pi cells (Newman et al., 1999). Sumoylation of LIN-11 is necessary for its function in pi cell specification (Broday et al., 2004).

10.2. uv1 induction

The anchor cell induces six of 12 VU granddaughters to become pi cells, which are distinguished from the uninduced VU.xx cells by the lineage they generate. The pi cell undergo only one round of cell divisions while the uninduced cells undergo two rounds of cell division. The pi cells generate the four uv1 cells that connect to the vulF cells. Specification of the uv1 cells requires vulF cells, which express *lin-3* (Chang et al., 1999). The anchor cell apparently responds to cues from vulF (and possibly negative cues from vulE) to locate the vulF cells (Sherwood and Sternberg, 2003). Meanwhile, the anchor cell helps pattern the vulE and vulF cells via LET-60 RAS (Wang and Sternberg, 2000). Moreover, a WNT signal, likely from the anchor cell reverses the polarity of the P7.p lineage (Inoue et al., 2004; Deshpande et al., 2005). The anchor cell then fuses with the utse syncytium (Newman et al., 1996). The relative timing of the signaling involved in these events has not been fully resolved.

10.3. AC invasion

The anchor cell breaks down the basement membranes, secreted by the gonad and the epidermis, that separates these two tissues. The anchor cell then extends a process towards the developing 1° lineage. This invasion requires the vulva; although in ~20% of vulvaless animals, the anchor cell will nonetheless invade the basement membrane (Sherwood and Sternberg, 2003). The anchor cell can pass by the vulE cells and locate the vulF cells. The signal from the vulval to the invading AC acts at a distance because a distant 1° VPC, indicated by expression of *egl-17::yfp* during the L3 stage, can attract extension of the anchor cell.

11. Morphogenesis

During the L4 larval stage, the vulval cells move towards the anchor cell, invaginate, fuse into seven toroids (see Cell fusion), and during the L4-to-adult molt evert. The epidermal cells of the vulva organize the egg-laying system.

11.1. Invagination

The ability of the vulval invagination to form is regulated by eight *sqv* genes (*sqv-1*, *sqv-2*, *sqv-3*, *sqv-4*, *sqv-5*, *sqv-6*, *sqv-7*, and *sqv-8*; Herman et al., 1999:). Hermaphrodites with decreased *sqv* function have only a rudimentary invagination (SQuashed Vulva phenotype). The *sqv* genes encode enzymes of a glycosaminoglycan biosynthesis (Herman and Horvitz, 1999; Berninsone et al., 2001; Hwang and Horvitz, 2002a; Hwang and Horvitz, 2002b; Hwang et al., 2003a; Hwang et al., 2003b). The *sqv* genes are expressed broadly in the vulva with the exception of *sqv-4*, which is expressed in vulC, D, E and F (Hwang and Horvitz, 2002b).

Morphogenesis also involves cytoskeletal reorganization, and proteins such as the ARL2 ortholog EVL-20 (Antoshechkin and Han, 2002). The Rac proteins encoded by *mig-2* and *ced-10* (Kishore and Sundaram, 2002) are

necessary for appropriate morphogenesis of the vulva. The different vul cells extend processes toward the developing vulval lumen, contact each other and fuse to form toroidal adult vulval cells (Sharma-Kishore et al. 1999). A plexin homolog (a semaphorin receptor) is expressed sequentially from vulF to vulA as the toroids fuse (Dalpe et al., 2005).

11.2. Organization of neurons and muscles

The vulval epithelium organizes the development of the muscles and neurons that mediate egg-laying (Li and Chalfie, 1990). This is most obviously seen in *dig-1* mutants in which the gonad is shifted anteriorly and the vulva, vulval muscles and neurons are all similarly shifted anteriorly (Thomas et al., 1990). Specific gene products expressed in subsets of vulval cells direct details of development of individual neurons. *bam-2* is necessary for axonal branch termination of the VC motoneurons, and is expressed in the vulF cells (Colavita and Tessier-Lavigne, 2003). *syg-1* and *syg-2* are required for synapse formation by the HSN neurons (Shen and Bargmann, 2003; Shen et al., 2004). *syg-2* is expressed in vulE cells and *syg-1* in the HSNs.

12. Concluding remarks

There is now a reasonable conceptual model for understanding how the pattern of VPCs is established, but many components remain to be identified and the precise relationship among many of the genes has not been established. By contrast, there is only a rudimentary model for how the pattern of the seven vulval cell types are established, and even less of a picture of how the vulval cells undergo morphogenesis to form the adult structure. Similarly, our understanding of the control of timing of vulval development is also incomplete, and the relationship of the cell cycle to fate specification remains intriguing. In spite of these limitations, the extensive knowledge of *C. elegans* vulval development makes it an interesting case for computational modeling studies (e.g., Fisher et al., 2005). The precision of the wild-type pattern of VPC fates stands in stark contrast to the variable phenotypes of many mutants and perturbations; I view these phenotypes as providing a rich source of information about the underlying mechanisms and their dynamics.

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