



Morphogenesis of the *Caenorhabditis elegans* vulva

Adam J. Schindler and David R. Sherwood*

Understanding how cells move, change shape, and alter cellular behaviors to form organs, a process termed morphogenesis, is one of the great challenges of developmental biology. Formation of the *Caenorhabditis elegans* vulva is a powerful, simple, and experimentally accessible model for elucidating how morphogenetic processes produce an organ. In the first step of vulval development, three epithelial precursor cells divide and differentiate to generate 22 cells of 7 different vulval subtypes. The 22 vulval cells then rearrange from a linear array into a tube, with each of the seven cell types undergoing characteristic morphogenetic behaviors that construct the vulva. Vulval morphogenesis entails many of the same cellular activities that underlie organogenesis and tissue formation across species, including invagination, lumen formation, oriented cell divisions, cell–cell adhesion, cell migration, cell fusion, extracellular matrix remodeling, and cell invasion. Studies of vulval development have led to pioneering discoveries in a number of these processes and are beginning to bridge the gap between the pathways that specify cells and their connections to morphogenetic behaviors. The simplicity of the vulva and the experimental tools available in *C. elegans* will continue to make vulval morphogenesis a powerful paradigm to further our understanding of the largely mysterious mechanisms that build tissues and organs.

© 2012 Wiley Periodicals, Inc.

How to cite this article:

WIREs Dev Biol 2012. doi: 10.1002/wdev.87

INTRODUCTION

The architect Louis Sullivan famously wrote that ‘form ever follows function’, a principal that is as true in biology as it is in architecture. Organisms have evolved body plans that are uniquely suited to carrying out the tasks necessary for survival and reproduction. In many multicellular organisms, the body is divided into tissues (e.g., muscles, nerves), organs (e.g., skin, lung), and systems (e.g., reproductive, digestive). The formation of such ordered structures, a process termed morphogenesis, remains one of the great mysteries of biology. The inherent difficulty of understanding morphogenesis is that it encompasses a broad range of interconnected cellular activities that occur in precise spatial locations at precise times.¹ Despite the experimental challenges presented by morphogenesis, a greater understanding of how tissues and organs

form holds the promise of improving human health by enhancing our ability to diagnose and treat developmental diseases, engineer tissues to replace diseased body parts,² and arrest the development of tumors that use morphogenetic processes to mimic native tissues.³ A useful experimental system to study morphogenesis would feature three attributes: (1) display a range of conserved cellular behaviors that underlie morphogenesis across species; (2) allow for the examination of the entire morphogenetic process *in vivo*, in real-time, with high visual resolution; and (3) be experimentally tractable so that the system can be manipulated and examined. One model that fits these criteria is development of the *Caenorhabditis elegans* vulva, which offers a number of experimental advantages to the researcher while still featuring complex morphogenetic behaviors. This review provides an overview of the morphogenetic basis of vulval development, highlighting advances in our understanding of oriented cell division, cell

*Correspondence to: david.sherwood@duke.edu

Department of Biology, Duke University, Durham, NC, USA

movement, invagination, cell fusion, cell invasion, and the coordinated attachment of tissues. This array of cellular behaviors results in the formation of the simple, yet elegantly constructed *C. elegans* vulva.

Overview of Vulval Development

The *C. elegans* vulva is a hermaphrodite-specific ectodermal organ that develops post-embryonically and functions to connect the internal reproductive system with the external environment. The vulva is required for mating, as males inject sperm through it, and for deposition of embryos after internal fertilization (Figure 1(a)). Vulval development has emerged as a paradigm of morphogenesis because it offers a simple model of tissue rearrangement in which a tube forms from an epithelial sheet of only 22 cells. The vulva also serves as an ideal system for genetic screens,⁴ as mutations that affect mating and egg-laying do not prevent hermaphrodites from producing viable progeny. Plate-level genetic screens have been conducted to identify vulval development mutants, such as animals that are egg-laying defective (Egl, retain developing embryos in the uterus), have a protruding vulva (Pvl) or have multiple vulvae (Muv). Higher magnification compound microscopic analysis can further determine if these plate-level mutants lack a vulva (Vul), have specific defects in the connection of the vulva with the gonad (Cog), have a squashed vulva (Sqv), or have defects in specific cellular behaviors such as cell fusion, migration, or invasion. A further advantage of studying vulval morphogenesis is that a number of the signaling pathways that specify vulval cell fates have been characterized.^{5,6} This makes the vulva an emerging model to understand how cell specification pathways connect to the morphogenetic processes that shape tissues. These efforts are bringing a more holistic level of understanding to the process of organogenesis.

Vulval Cell Induction and Differentiation

During larval development, six epidermal cells on the ventral surface of the hermaphrodite, P3.p–P8.p, are competent to form vulval tissue. Through a series of inductive interactions involving the LIN-3/epidermal growth factor (EGF) and LIN-12/Notch pathways, P6.p is induced to adopt the 1° vulval precursor cell (VPC) fate, and P5.p and P7.p are induced to the 2° VPC fate (Figure 1(b)). In wild-type animals, P3.p, P4.p, and P8.p are not induced to a vulval fate and contribute to the hypodermis, which generates the cuticle that covers the animal. The 1° VPC produces progeny cell types vulE and vulF, and the 2° VPCs produce progeny cell types vulA, vulB1, vulB2,

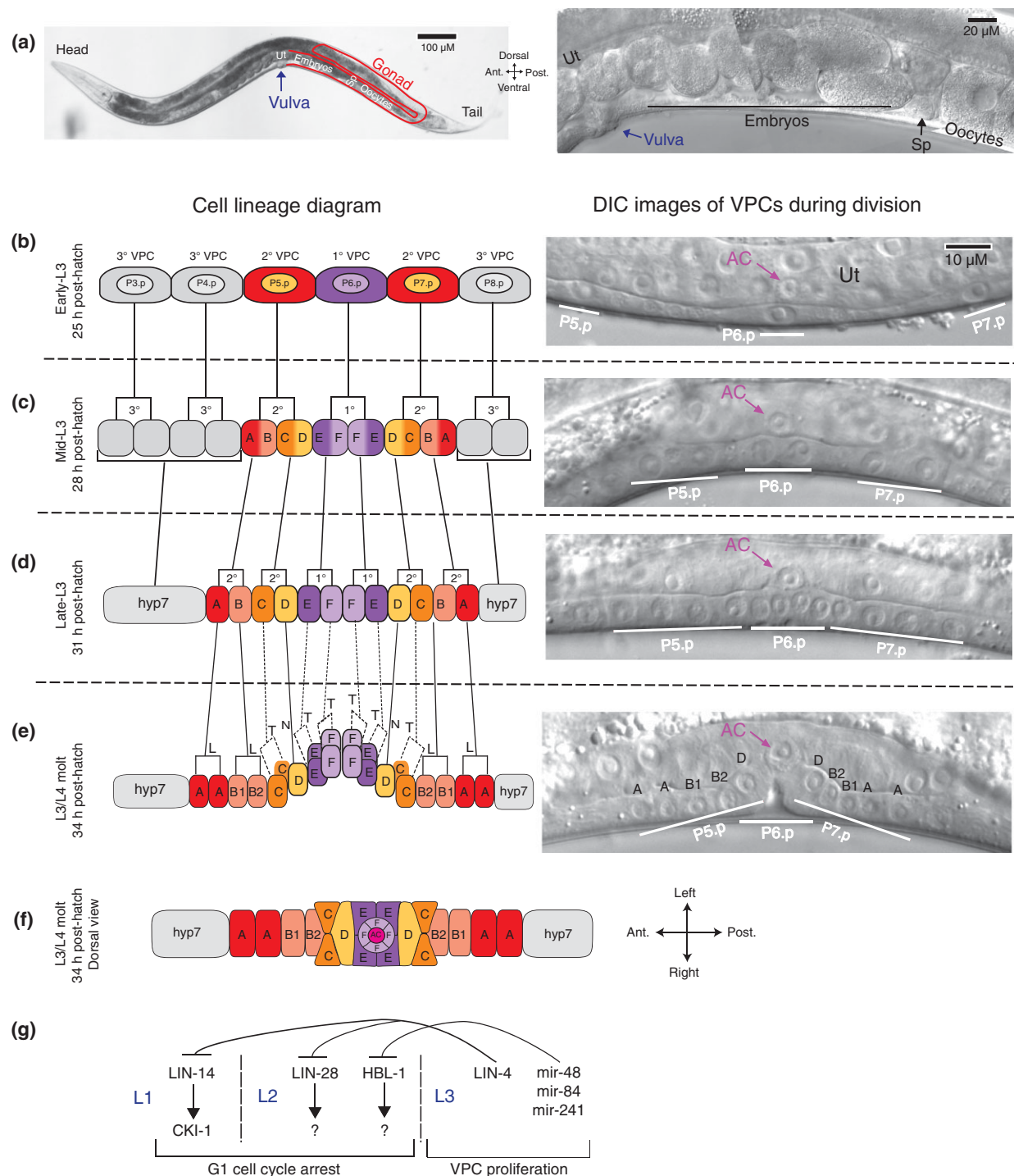
vulC, and vulD (Figure 1(c)–(e)). Each of the different vulval cell types undergoes a distinct morphogenetic program.^{8,9}

Cell Division

Vulval induction determines the subsequent pattern of cell divisions, which occur during the L3 larval stage. The 1° VPC, P6.p, undergoes three rounds of cell division to produce eight cells, and the 2° VPCs, P5.p and P7.p, divide three times to produce seven cells each (Figure 1(c)–(e)). After cell divisions, the vulval primordium has 22 cells displaying mirror-image symmetry, in the sequence AAB₁B₂CCDEEEFFFEEDCCB₂B₁AA (Figure 1(e) and (f)). The first two rounds of cell division occur in the longitudinal (anterior–posterior) orientation. The terminal cell divisions of vulC, vulE, and vulF occur in the transverse (left–right) orientations, and the terminal cell divisions of vulA and vulB occur in the longitudinal direction. vulD does not undergo a third cell division (Figure 1(e) and (f)).

Cell Arrangements, Invagination, Toroid Formation, and Cell–Cell Fusion

After the second cell division, the 12 VPCs are arranged as a linear array (Figure 2(a)). As the final cell divisions occur, the central 1° VPCs invaginate by detaching from the ventral cuticle and moving dorsally (Figure 2(b)). In a proximal to distal temporal wave (inner to outermost), cells on each lateral half extend apical membrane processes that form rings with homotypic cells from the other lateral half and undergo short-range cell migrations toward the midline⁸ (Figure 2(c) and (d)). Distal cells migrate along the ventral surface of the inner proximal cell, which displaces cells dorsally and expands the invagination as cells compact around the vulval midline¹⁰ (Figure 2(e)). In addition to short-range migrations, expansion of the vulval lumen also requires VPC expression of glycosaminoglycans (GAGs), which are thought to shape or maintain the lumen formed by invaginating cells (Figure 2(e)).^{11,12} Through the L4 larval stage, ring formation and invagination continue, giving rise to a characteristic ‘Christmas tree’ appearance formed by the dorsal–ventral stack of cells (Figure 2(f)). These ringed structures are termed toroids, circular groups of cells with a central hole, analogous to a bagel (Figure 2(g)). Cell fusions occur during toroid formation to generate multinucleate syncytia. First, sister cells on each half of the midline fuse (i.e., vulA with vulA; Figure 2(b)), then cells of the same subtype from contralateral halves fuse (i.e., anterior vulA with posterior vulA; Figure 2(f)). All

**FIGURE 1** | Legend on next page.

homotypic cells fuse with the exception of vulB1 and vulB2⁸ (Figure 2(e)).

Attachments to Other Tissues

The vulva ultimately forms a connection and a continuous lumen with the uterus to allow for mating

and egg-laying. The connection of these two tissues is initiated by a cell invasion event from the uterine anchor cell (AC), which breaches the basement membrane separating these tissues and contacts the central 1° VPCs¹⁴ (Figure 6(a)). The uterine–vulval attachment matures by further removal of the basement

membrane, allowing direct attachment of vulF cells with the overlying uv1 cells of the ventral uterus¹⁵ (Figure 3). After invasion, the AC fuses with neighboring uterine cells, generating the multinucleated utse cell that forms a thin cellular membrane between the vulval and uterine lumens (Figures 2(f) and 3). The vulva also forms attachments with hypodermal and muscle tissues. vulE cells extend processes that contact hypodermal seam cells on the lateral sides of the animal, which is thought to structurally stabilize the vulva. Eight sex muscle cells, four vm1 and four vm2, contact the vulval lips to control opening during mating and egg-laying (Figure 3). The muscles in turn are innervated by motoneurons that are guided to synaptic locations by cues from the vulva. The final step in vulval formation is eversion, during which the vulva turns partially inside out and the lumen closes into a slit^{7,8} (Figure 1(a)).

VULVAL MORPHOGENESIS

Vulval Cell Induction and Differentiation

Vulval induction has been extensively studied (for reviews, see Refs 17–20), and will be summarized briefly here. During the L1 larval stage, 12 epidermal cells (P1.p–P12.p) are born as the posterior daughters of P ectoblast cell divisions and are positioned along the ventral midline. Six of these cells (P1.p–P2.p, P9.p–P12.p) fuse with the surrounding hypodermal syncytium, hyp7, soon after birth.²¹ The remaining unfused cells, P3.p–P8.p, are termed VPCs, because all possess the potential to be specified as vulval cells.⁹ In wild-type animals, however, only P5.p–P7.p assume vulval identities due to an inductive LIN-3/EGF signal from the AC, which is positioned dorsal

to P6.p (Figure 1(b)). P6.p is thought to receive the highest levels of AC-secreted LIN-3 and through Ras activation assumes the 1° VPC fate. P5.p and P7.p receive moderate LIN-3 and assume the 2° fate. The distal P3.p, P4.p and P8.p cells receive low EGF and assume the non-vulval 3° fate, which is fusion with the hyp7 syncytium.²² Recent work has supported a model in which higher levels of Ras activation in the P6.p lineage result in the engagement of the LIN-45/Raf effector, which promotes the 1° fate, whereas lower Ras activation in the P5.p and P7.p lineages utilizes the RalGEF effector, which signals a pro-2° fate.²³ In addition to a gradient of LIN-3, vulval fates are also patterned by sequential induction in which the 1°-fated VPC is first specified, and as a result of this induction expresses transmembrane and secreted DSL proteins that signal via the LIN-12/Notch receptor on P5.p and P7.p to promote the 2° fate.²⁴ In addition to graded and sequential induction, the 1° and 2° fates are also patterned through pathway quenching. In the 1°-fated VPC, activation of the EGF/Ras/Raf pathway antagonizes the LIN-12/Notch pathway in part by downregulating the LIN-12 receptor,^{23–25} while in the 2°-fated VPCs, activation of LIN-12/Notch upregulates numerous inhibitors of the EGF signaling pathway.^{24,26,27} Other mechanisms appear to further contribute to the precise patterning of the VPCs, including an early role for Hox genes in regulating VPC induction competency, and apparent roles for the Wnt pathway and the ROM-1 protease in VPC induction (reviewed in Ref 18). The extensive levels of redundancy in this well-studied system of VPC induction likely reflects more generally on how cell fate specification systems are wired to ensure robust patterning of tissues during development.

FIGURE 1 | Vulval cell divisions. (a) Adult worm at low (left) and high (right) magnification showing components of the reproductive system. Oocytes in the gonad (outlined in red) move in a distal to proximal direction and are fertilized in the spermatheca (Sp). Embryos pass into the uterus (Ut) and undergo the first rounds of cell division. Muscle contractions push embryos through the vulva to the external environment, where embryogenesis continues until hatching. The posterior gonad is shown; the anterior gonad has an identical arrangement. For additional images of the *Caenorhabditis elegans* reproductive system, see *WormAtlas*.⁷ (b)–(e) Cell divisions in the L3 larval stage. Left is schematic diagram; right is differential interference microscopy (DIC) images of live animals. Lateral view with anterior left and dorsal top. (b) The P6.p cell is induced to the 1° vulval precursor cell (VPC) fate, P5.p and P7.p are induced to the 2° VPC fate, and P3.p, P4.p, and P8.p take on the non-vulval 3° VPC fate. The anchor cell (AC) is located in the uterine epithelium (Ut) and positioned dorsal to P6.p (right DIC image). (c) All six VPCs divide in the mid-L3 larval stage, after which the 3° VPCs arrest cell divisions and initiate fusion with the hyp7 syncytium. The 1° VPCs differentiate to vulE and vulF cell types; the 2° VPCs differentiate to vulA, vulB, vulC, and vulD cell types. (d) The third VPC divisions take place in mid- to late L3 and produce 12 cells in a longitudinal array. (e) The final VPC divisions occur during the L3/L4 molt. vulC, vulE, and vulF cell divisions occur in the transverse (T) orientation, which is indicated by dashed lines in the lineage diagram. vulA and vulB divide in the longitudinal orientation (L), and vulD does not divide (N). In the DIC image, only the longitudinally divided cells and vulD are in the plane of focus. (f) Dorsal view of the vulval primordium following terminal cell divisions, with the AC at the vulval apex. Modeled after Ref 8. For additional schematics of vulval development and a time series of vulval images from the L3/L4 molt to adulthood, see *WormAtlas*.⁷ (g) Heterochronic genes regulate the timing of VPC divisions. LIN-14, acting through the cell-cycle repressor *cki-1*, inhibits cell divisions in L1, and LIN-28 and HBL-1 inhibit L2 cell divisions, acting through unknown targets. In L3, the miRNA *lin-4* represses *lin-14* and *lin-28* mRNA translation to promote cell division. Analogously, the miRNAs *mir-48*, *mir-84*, and *mir-241* redundantly repress *hbl-1* translation.

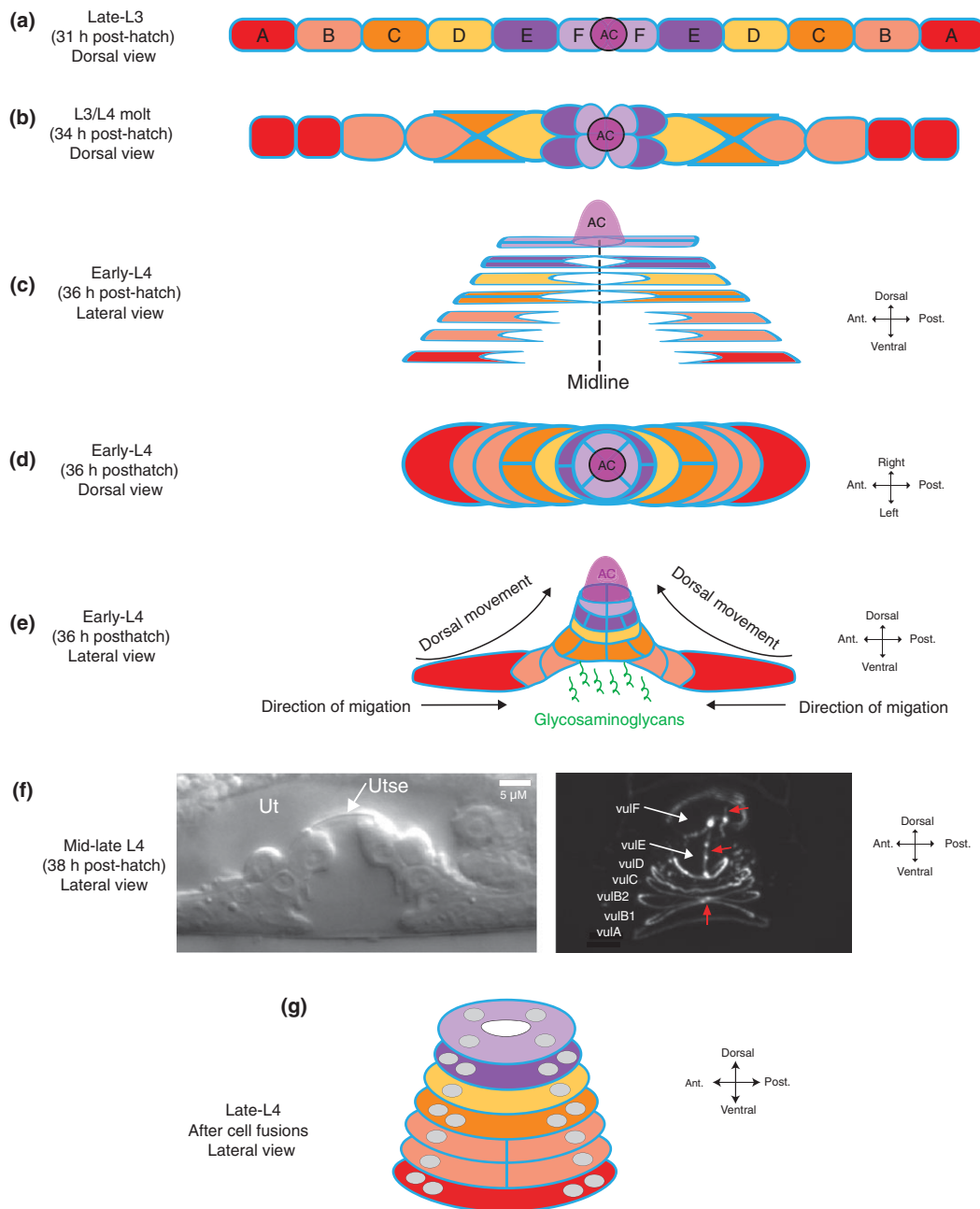


FIGURE 2 | Vulval invagination and toroid formation. (a) After the second cell divisions, the vulval precursor cells (VPCs) are arranged in a longitudinal array. The anchor cell (AC) is positioned at the junction of the vulF cells at the vulval midline. Apical domains are in blue here and in other panels. (b) Concurrent with the final cell divisions, the 1^o VPCs invaginate by moving dorsally. The vulF occupies the most dorsal position, with vulE positioned ventral to it. (c) VPCs extend apical membrane processes toward the midline and establish junctions with homotypic cells from the contralateral half, forming rings that encircle a tube in the center of the vulval primordium. In this schematic, vulC–vulF have formed rings. Sister vulA cells on each lateral half fuse shortly after the final cell division,⁸ shown as a single-cell boundary. (d) Dorsal view of early-L4 VPCs, showing the lateral migration of vulval cells toward the midline. (e) Invagination is driven by distal VPCs migrating toward the midline along the ventral surface of inner cells, which forces cells to move dorsally.¹⁰ Glycosaminoglycans are expressed by the VPCs and are thought to maintain and shape the forming lumen.^{11,12} (f) As the L4 stage continues, vulval cells continue to migrate and homotypic cells undergo fusions. A uterine lumen forms, and the uterine and vulval epithelia become separated by the thin membrane of the utse syncytium (left differential interference microscopy image). The right image shows labeling of apical junctions with the apical membrane marker AJM-1::GFP.¹³ At this stage, the sister cells on each lateral half have fused, and contralateral vulA and vulD cells have fused at the midline. Red arrows indicate apical borders between vulB1, vulB2, vulC, vulE, and vulF cells. (g) Schematic of vulval toroids in late-L4 after fusion has completed. Gray circles represent nuclei. All homotypic cells fuse except vulB1 and vulB2. For additional images, see Refs 8 and 10 and WormAtlas.⁷

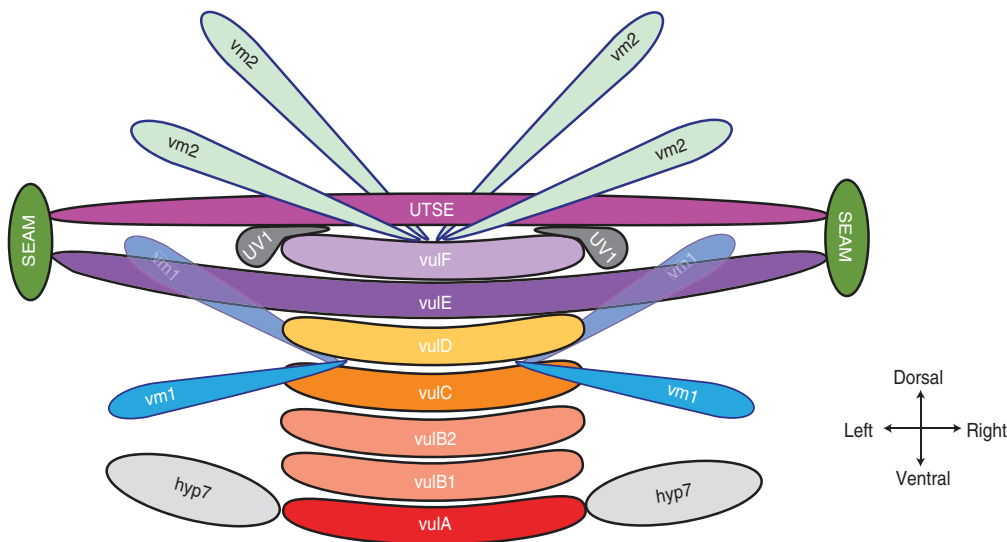


FIGURE 3 | Attachments of the vulva to other tissues. (a) The vulA cells on the ventral surface contact the hyp7 hypodermal syncytium; vulE cells extend laterally to contact the hypodermal seam cells; and vulF cells contact the uv1 cells and are overlaid by the utse (see Figure 2(f)). Eight vulval muscles, four vm1 and four vm2, control vulval opening. vm1 cells contact the vulva between vulC and vulD toroids, and vm2 cells contact between vulF and the uterus. Modeled after Refs 8 and 16. For additional images of late vulval development, see *WormAtlas*.⁷

Cell Division

During the L1 larval stage, P3.p–P8.p VPCs are born and immediately arrest in an extended G₁ phase.²⁸ The VPCs remain in this quiescent state until the mid-L3 stage, when they resume division. Proper vulval formation requires the precise control of cell division timing, cell-cycle number, spindle orientation, and polarity. Many classes of genes and genetic pathways have been found to regulate these distinct aspects of vulval cell divisions and are discussed below.

Heterochronic Genes Regulate Vulval Cell Division Timing

Genes that regulate developmental timing are referred to as heterochronic, as their loss or gain of activity leads to changes in the timing of specific developmental events in relation to development as a whole.²⁹ Heterochronic genes that regulate the timing of VPC divisions include the transcription factors LIN-14 and HBL-1,³⁰ the RNA binding protein LIN-28,³¹ and the microRNA *lin-4* (Figure 1(g)).³² LIN-14 functions to promote L1 larval stage cell fates, whereas LIN-28 specifies aspects of L2 fates.^{29,31} Loss of *lin-14* or *lin-28* leads to an early G₁ to S transition and precocious VPC divisions, presumably as a result of skipping the L1 or L2 stages, respectively. Although the vulva is precociously induced in *lin-14* and *lin-28* mutants, it displays normal lineage patterns and marker gene expression. The vulva fails to connect properly to the uterus, however, likely because of mistiming in the development of the two tissues.^{14,28,29} One downstream effector of LIN-14 is

cki-1, a *C. elegans* p21/p27 cyclin-dependent kinase inhibitor, which maintains a G₁ state and inhibits cell-cycle progression.³³ *cki-1* is first expressed in the late L1 stage and is absent in the L3 stage upon initiation of cell divisions. *lin-14* is required for the early larval expression of *cki-1*, and the loss of *cki-1* results in precocious VPC divisions similar to *lin-14* mutants.³³ During VPC proliferation in L3, LIN-14 and LIN-28 protein levels are reduced through binding of *lin-4* miRNA to the 3' untranslated regions of *lin-14* and *lin-28* mRNAs, leading to their translational repression (Figure 1(g)).^{28,31,34} In *lin-4* loss-of-function mutants, the first VPC divisions occur at the correct time in mid-L3, but subsequent cell divisions are missing or delayed.^{28,35} *lin-4* also has a role in specifying vulval fates, as P5.p and P7.p fail to adopt 2° VPC identities in *lin-4* mutants. This is because the continued presence of LIN-14 protein inhibits the activity of LIN-12, which is necessary for 2° fate specification.³⁶

Similar to *lin-28*, the transcription factor *hbl-1*/Hunchback also regulates timing of VPC divisions by promoting L2 cell fates (Figure 1(g)). A mutant allele of *hbl-1* or RNAi depletion of the gene causes precocious VPC divisions at or before the L2/L3 molt, leading to Pvl and Egl phenotypes.³⁷ *hbl-1* is negatively regulated in a redundant manner by the *let-7* family microRNAs *mir-48*, *mir-84*, and *mir-241*.³⁸ The mechanisms by which inhibition of HBL-1, LIN-14, and LIN-28 lead to promotion of VPC divisions in L3 is not well understood and cannot be accounted for solely by *cki-1* inhibition, as the phenotype of *cki-1*

RNAi reduction of function is a single extra VPC cell division in L2.³³ Activation of the cell cycle in L3 thus appears to involve other targets that function downstream of heterochronic gene repression.

General Cell-Cycle Machinery and Control of Vulval Cell Divisions

The *C. elegans* cell-cycle machinery, built around the regulation of expression and activity of cyclin-dependent kinases (Cdks) and cyclins, is highly conserved with other metazoans.³⁹ Many general regulators of the cell cycle have been found to influence the number and timing of vulval cell divisions. As mentioned above, *cki-1* is a key regulator of the G1 quiescent state of VPCs and its expression is positively regulated by the phosphatase *cdc-14*.^{40,41} The *C. elegans* retinoblastoma (Rb) gene ortholog *lin-35* functions redundantly with the putative SCF complex regulator *lin-23* and the Cdh1/Hct1/FZR ortholog *fzr-1* to limit VPC cell division in the G1 quiescent state in the L1/L2 larval stages, possibly by reducing cyclin levels.^{42,43}

Regulators of the cell cycle during the proliferative L3 stage have also been identified. The CUL-4/DDB-1 E3 ubiquitin ligase complex, which downregulates CKI-1 protein expression in hypodermal seam cells, also likely downregulates CKI-1 in VPCs. *ddb-1::GFP* is expressed in all vulval cells, and a *ddb-1* null mutant has a reduced number of VPC progeny, consistent with its loss leading to the continued expression of CKI-1.⁴⁴ An additional general regulator of vulval cell divisions is *cye-1*/Cyclin E, which promotes the G₁- to S-phase transition.⁴⁵ In *cye-1* mutant animals, the VPC cell cycle in the L3 stage is lengthened and the third cell division frequently does not occur. *cul-1* is an ortholog of mammalian cullins, which targets Cyclin E for degradation as part of the SCF E3 ubiquitin ligase complex.⁴⁶ Consistent with a role in limiting cell divisions through degradation of CYE-1 protein, *cul-1* mutants display vulval hyperplasia, producing an average of 82 vulval cells.⁴⁷ Similar to *lin-14* and *lin-28* mutants, perturbations in cell cycle do not affect vulval cell differentiation, suggesting that cell-cycle regulation and differentiation can be uncoupled.^{33,43,45,47} One exception to this finding is the overproliferation defects associated with loss of *lin-35/Rb*, in which vulval fate patterning and morphogenesis are also aberrant.⁴² This suggests that *lin-35/Rb* might have additional cell-cycle-independent roles in vulval differentiation,⁴⁸ similar to the roles of Rb in vertebrate muscle and bone differentiation.⁴⁹

Vulval-Specific Regulators of Cell Division

How developmental pathways regulate precise cell-cycle progression during tissue formation and

organogenesis remains largely unknown.⁵⁰ Although some transcriptional regulators of vulval cell divisions have been identified, the precise links between these genes and the cell cycle remain unclear. During VPC quiescence in L1 and L2, the transcription factors LIN-1/Ets and LIN-31/FoxB promote the expression of CKI-1 to prevent precocious VPC divisions.⁴¹ Upon exit from quiescence in the L3 stage, the Hox gene *lin-39* promotes the initiation of VPC division.⁵¹ Still other transcription factors regulate the number of cell divisions upon initiation of cell division. Loss-of-function mutants of *cog-1*, an Nkx6 homeodomain transcription factor, display defects in which the final cell divisions of vulB, vulC, and vulF sometimes do not occur, and the vulD cells undergo a third cell division.⁵² *cog-1* also regulates vulval gene expression and the proper connection of uterine and vulval tissues, indicating that it is required for several aspects of vulval differentiation.^{5,52} Mutations in the zinc-finger transcription factor *bed-3* cause a cell division defect similar to *cog-1*, suggesting that the two transcription factors form part of a regulatory network required for differentiation of a subset of vulval cells.⁵³ The LIM homeodomain transcription factor *lin-11* also regulates multiple aspects of vulval identity, including cell division arrest of vulD.⁵⁴ A future challenge will be to understand how these vulva-specific transcriptional regulators direct the general cell-cycle machinery in specific cell lineages.

Oriented Cell Divisions

Oriented cell division is a common morphogenetic mechanism to shape organs.⁵⁵ After the first two rounds of longitudinal cell divisions, 6 of the 10 VPC granddaughters that divide a third time (vulC, vulE, and vulF precursors) change their mitotic spindle orientation to divide in the transverse direction (Figure 1(e)). Our current understanding of the mechanisms that regulate mitotic spindle reorientation during these cell divisions is rudimentary. Combined loss of the Rac-like GTPases *ced-10* and *mig-2* alters the terminal cell divisions of vulC and vulE, such that they occur longitudinally or obliquely rather than transversely. Interestingly, the vulF cell divisions are still transverse after *mig-2* and *ced-10* loss, suggesting that vulF may be regulated differently from vulC and vulE.⁵⁶ Mutations in the guanine nucleotide exchange factor (GEF) *unc-73*/Trio produce a similarly penetrant defect to combined loss of *ced-10* and *mig-2*, indicating that *unc-73* is likely the GEF for both genes.⁵⁶ Mutations in *lin-40*/MTA, a component of the NuRD transcriptional co-repression complex, also transform transverse to longitudinal divisions, suggesting that changes in gene expression may be

required to properly orient the mitotic spindle.⁵⁷ It will be important in the future to determine whether these Rac genes or *lin-40* ultimately influence spindle orientation through conserved extrinsic (e.g., planar cell polarity, Fat/Dachsous) or intrinsic (e.g., LGN/Numa/Gai) pathways known to regulate spindle orientation.⁵⁵

An evolutionary comparison of 51 rhabditid species showed that the terminal cell divisions of distal VPCs (vulA and vulB) always occur longitudinally, and terminal cell divisions of proximal VPCs (vulE and vulF) always occur transversely. Terminal vulC divisions are variable: transverse in *Caenorhabditis* species but longitudinal in diplogastrids such as *Pristionchus pacificus*.^{58,59} An analysis of ring formation in 12 nematode species by Kolotuev and Podbilewicz⁶⁰ found that longitudinal divisions generate two rings (e.g., vulB1 and vulB2 in *C. elegans*), whereas one ring forms from transverse or no cell divisions (e.g., vulC, vulD, vulE, vulF). While VulA is an apparent exception to this rule as it forms one ring despite dividing longitudinally,⁶⁰ the divided VulA cells fuse with each other in most species (including *C. elegans*) prior to toroid formation, thus precluding two rings from forming. Blocking vulA fusions in *C. elegans* results in the formation of an additional toroid ring. Further, an additional ring forms in *lin-11* mutants in which vulC undergoes a longitudinal division.⁶⁰ Thus, the number of toroid rings and the structure of the mature vulva appear to be dictated by the orientation of cell divisions and the number of dividing cells.

Establishing Mirror-Image Symmetry

The final order of cell types in the vulva has mirror-image symmetry around the vulF cells (Figure 1(e)). Although the molecular mechanism is unclear, the AC is required for patterning of E-F-F-E fates in the 1° VPC lineage (Figure 6(c)). Ablation of the AC at the two- or four-cell 1° VPC stage causes mispatterning of vulE and vulF fates. A similar mispatterning defect is observed in *dig-1* mutants in which the AC is displaced dorsally, demonstrating that proximity of the AC with the 1° VPCs is required for proper patterning.⁶¹ The Wnt pathway is required to polarize the 2° VPC cell fates (P5.p and P7.p) and establish the mirror-image symmetry of these lineages (A-B-C-D/D-C-B-A) (Figure 1(c)). A Wnt ligand, EGL-20, is expressed in posterior cells and acts at a distance as a directional cue through a Ror tyrosine kinase receptor, CAM-1, and the planar cell polarity core pathway component VANG-1/Van Gogh, to generate a posterior-oriented polarity in VPC cell divisions.^{62,63} A second group of Wnt ligands refines the EGL-20-induced posterior polarity to orient VPC cell divisions toward the

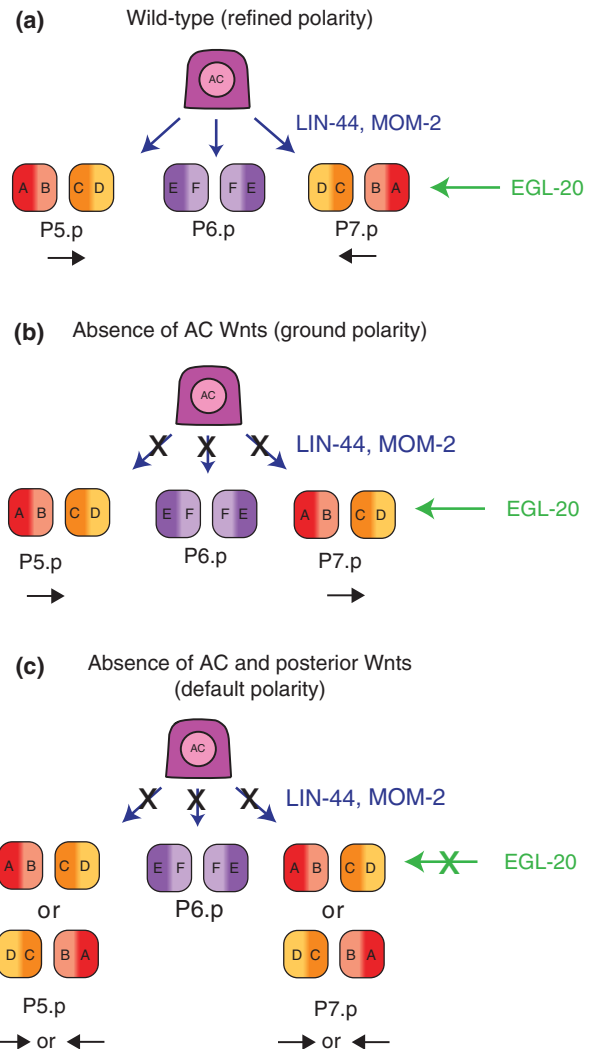


FIGURE 4 | Wnt signaling determines the polarity of vulval precursor cell (VPC) cell divisions. (a) In the presence of posterior-expressed Wnt ligand *egl-20* and AC-expressed Wnt ligands *lin-44* and *mom-2*, P5.p and P7.p have mirror-image symmetry in their cell division patterns, which is termed 'refined polarity'.⁶³ (b) When *lin-44* and *mom-2* are absent, *egl-20* directs a posterior-oriented 'ground polarity', such that both P5.p and P7.p divide in the same orientation. (c) In the absence of *lin-44*, *mom-2*, and *egl-20*, P5.p and P7.p divide with random polarity. This is considered the 'default polarity'.⁶³

center of the vulval primordium, thus reversing P7.p polarity. This second group of Wnt ligands, LIN-44 and MOM-2, are expressed in the AC and function redundantly via their putative VPC-expressed receptors, LIN-17/Frizzled and LIN-18/Ryk, respectively (Figure 4(a)). A third Wnt, CWN-2, also appears to have a modest, redundant function with LIN-44 and MOM-2.^{64–66} The phenotype of combined mutants of *lin-44* and *mom-2* is that VPCs divide normally and appear to be properly specified, but do so with reversed P7.p polarity (Figure 4(b)), producing one

P5.p and P6.p-derived vulva and a second P7.p-derived pseudo-vulva.^{63–65} Reduction of all Wnts, including posterior localized EGL-20, leads to randomized P5.p and p7.p polarity, which is the apparent default state (Figure 4(c)). The precise time of action and downstream effectors used by LIN-17/LIN-18 and CAM-1/VANG-1 to influence the orientation of P5.p and P7.p remain an area for future study.

Cell Rearrangements, Invagination, Toroid Formation and Cell–Cell Fusion

Cell Rearrangements and Invagination

Invagination initiates in the late-L3 stage after the AC has invaded and just prior to the terminal VPC divisions. The vulF cells detach from the ventral cuticle and move dorsally to generate a small invagination at the midline (Figure 2(b)). In a temporal order starting with vulE, vulval cells extend unidirectional apical membrane processes that make homotypic contacts with their contralateral partner cells (i.e., vulD with vulD) at the midline, which results in ring formation around a central lumen⁸ (Figure 2(c)). As they form rings, vulval cells migrate laterally and ventrally toward the midline relative to proximal cells, which pushes inner cells dorsally and expands the invagination (Figure 2(d) and (e)). Ring formation has been proposed as the driving force for invagination, moving the vulva dorsally as cells migrate toward the midline.^{8,10} The cellular behaviors of vulval invagination are notably similar to those of ventral enclosure in *C. elegans* embryogenesis, in which the coordinated movement and extension of cellular processes toward a contralateral partner at the ventral midline is key to forming an epithelial monolayer that covers the embryo.⁶⁷ Initial studies suggest that a coordinated action of guidance signaling pathways and cytoskeletal components regulates both processes.

Two reports have shown roles in vulval morphogenesis for the guidance molecule SMP-1/Semaphorin and its receptor PLX-1/Plexin.^{68,69} Following the cell divisions in L1 that produce the Px.p cells, the VPCs extend longitudinally until establishing contacts with neighboring cells. In *smp-1* and *plx-1* mutant animals, VPCs sometimes extend past their neighbors and make contact on their lateral sides rather than at their anterior–posterior ends. This causes mispositioning of VPCs and later defects in vulval morphogenesis.⁶⁹ These results suggest an inhibitory effect of SMP-1 on the outgrowth of PLX-1 expressing VPCs, which is analogous to the role played by the semaphorin MAB-20 in inhibiting ectopic contacts between adjacent cells during ventral enclosure.⁷⁰

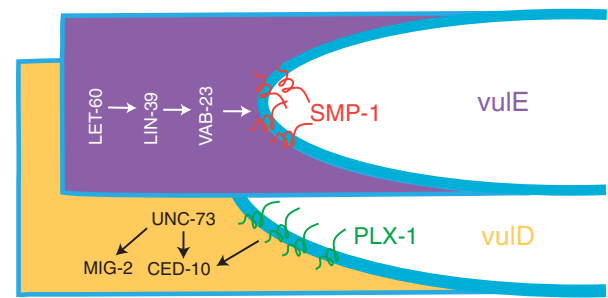


FIGURE 5 | Molecular pathways of vulval cell migration. Cells that are proximal to the midline (vulE in this example) express SMP-1 on the apical membrane, downstream of LET-60/Ras, LIN-39/Hox, and the zinc-finger transcription factor VAB-23. More distal cells (vulD) express PLX-1, which regulates cell migrations in part through the Rac GTPase CED-10 and its GEF UNC-73. A second pathway involving MIG-2 also regulates migration. Modeled after Ref. 68.

There are instances in *Drosophila* and mouse in which semaphorin molecules attract plexin-expressing cells.^{71,72} Dalpe et al.⁶⁸ examined the role of the SMP-1/PLX-1 pathway during later stages of vulval development and identified an attractive role during vulval cell migrations in the L4 stage.⁶⁸ SMP-1 is initially expressed on the lumen-facing surface of proximal VPCs, and attracts distal cells expressing PLX-1 on their apical membranes⁶⁸ (Figure 5). As distal cells migrate into the lumenal area, they switch on the expression of SMP-1 to attract the PLX-1-expressing cell ventral to it. SMP-1 expression is downstream of LET-60/Ras, LIN-39/Hox, and the zinc-finger transcription factor VAB-23⁷³ (Figure 5). In the absence of either *smp-1* or *plx-1*, VPCs fail to migrate and sometimes detach from neighboring cells, causing Egl, Pvl, and Muv phenotypes.⁶⁸ The two described roles for the SMP-1/PLX-1 signaling pathway during vulval development demonstrate that a ligand–receptor interaction can be both attractive and repulsive within the same cell lineage during distinct temporal events.

The *ced-10* and *mig-2* Rac-like GTPases and their GEF *unc-73*, which are required to orient VPC division planes (see above), also regulate VPC migration.^{56,68} *ced-10* and *mig-2* function redundantly: animals with single gene mutations have negligible vulval cell migration defects, whereas *ced-10;mig-2* double mutants are 77% defective. Genetic evidence suggests that *ced-10* regulates VPC migration exclusively in a pathway with *plx-1*, whereas *mig-2* has roles outside the *plx-1* pathway, as a *mig-2;plx-1* double mutant enhances the migration defect of a *plx-1* null mutant. The *unc-73* mutants also enhance the *plx-1* defect, consistent with it serving as the GEF for *mig-2*⁶⁸ (Figure 5). There is therefore at least one

parallel pathway of vulval cell migration involving *unc-73* and *mig-2* that has yet to be elucidated.

Toroid Formation

Cell–cell adhesion plays a critical role in morphogenesis by generating changes in cell shape.⁷⁴ Highly regulated adhesions occur during the development of the vulva, as homotypic cells form toroid rings (Figure 2(b)). The mechanisms that mediate these complex morphogenetic behaviors are poorly understood. Only a single gene has been identified that is specifically required for the midline adhesion of vulval cells: *par-1*, a membrane-associated EMK-family serine/threonine kinase.⁷⁵ In *par-1* RNAi-treated animals, vulval cell specification, invagination, and migration are normal, but cells fail to make contact with or maintain attachments to their homotypic partners. This results in a gap between rings from each half-vulva and a Pvl phenotype. Fusions between sister cells on each lateral half are normal, demonstrating that the ability to fuse is not compromised by the reduction of *par-1*.⁷⁵ It is currently unclear how *par-1* functions to regulate adhesion of vulval cells. Additional insight will come from the identification of *par-1* downstream targets.

The seven vulval cell types form only homotypic adhesions, despite the close proximity of differently fated cells. Even in *cye-1* mutants with fewer than the correct number of vulval cells or in *plx-1* and *smp-1* mutants with defects in migration, ring formation between nonhomologous cells is not known to occur.^{45,68,69} This cell-type matching is also observed in ventral enclosure in *C. elegans* and in *Drosophila* dorsal closure, in which two epithelial sheets adhere at the midline, with only similarly patterned cells forming adhesions. Filopodia extension has been observed in leading edge cells during these processes and also in vulval ring formation.^{10,68,76,77} It is thought that filopodia sample the environment until encountering a match, although the mechanisms of matching are not well understood.⁷⁷

Cell–Cell Fusions

Cell–cell fusions occur within five of the seven vulval toroids during the L4 stage (Figure 2(f) and (g)). Sister vulA, vulC, vulE, and vulF cells first fuse on each lateral half, then homotypic cells from each half-vulva fuse at the midline, in the order vulD, vulA, vulC, vulF, and vulE.⁸ vulB1 and vulB2 toroids remain unfused. Fusion is a common event in *C. elegans*—300 of 1090 somatic cells undergo fusion, generating multinucleate syncytia in the hypodermis, uterus, and vulva.⁷⁸ All *C. elegans* fusions are executed by two proteins, EFF-1 and AFF-1, which function as

homotypic fusogens (i.e., mediate fusion between two cells expressing the same fusogen). EFF-1 and AFF-1 are type I transmembrane proteins, and appear to be the first identified members of a divergent, ancient family of fusogens.⁷⁹ Most vulval cell fusions require *eff-1*, although fusions of vulA and vulD require *aff-1* instead of or in addition to *eff-1*.^{80,81} In *eff-1* mutant animals, vulval specification, cell division, migration, and midline attachment are normal, but membranes fail to fuse, causing exploded or protruding vulva phenotypes.⁸⁰ The *aff-1* mutants also appear normal for vulva formation but are Egl, possibly due to a failure to properly connect the uterus with the vulva (see below).⁸²

Ectopic expression of *eff-1* or *aff-1* in cultured insect cells is sufficient to induce cell–cell fusions.^{82,83} Given this intrinsic fusogenic capability, expression of *eff-1* and *aff-1* is thought to be tightly controlled to prevent premature fusion. In P3.p–P8.p VPCs, the Hox gene *lin-39* inhibits the expression of *eff-1* prior to LIN-3 induction of VPC fate. After induction, *lin-39* is downregulated in 3°-fated VPCs, which results in *eff-1* expression and fusion to the neighboring hyp7 cell.⁵¹ In the 1° and 2°-fated VPCs, LET-60/Ras acting downstream of LIN-3 maintains the expression of *lin-39*, which through the expression of the zinc-finger protein *vab-23* suppresses *eff-1* expression.⁷³ The transcription factor *nhr-67*/Tailless also represses *eff-1* expression, and RNAi reduction of *nhr-67* causes inappropriate fusions between vulE and vulF cells.⁸⁴ An interesting question for future studies is how the pathways that repress *eff-1* expression are themselves shut down to allow expression of *eff-1* during fusions.

Role of *Sqv* Mutants in Invagination and Lumen Formation

In a screen for mutants with defective vulval morphogenesis, Herman et al.¹¹ identified eight genes whose mutants have a squashed vulva (*Sqv*) phenotype, as well as apparent defects in oogenesis and early embryogenesis.¹¹ In the *sqv* mutants, VPCs detach from the cuticle and form an invagination, but it is narrower and shorter than in wild-type animals. Other aspects of VPC differentiation, including cell division, ring formation, and cell fusion, are normal in *sqv* mutants, indicating that the defect is limited to the invagination process.¹¹ Characterization of the *sqv* genes revealed that all eight encode enzymes that function to produce chondroitin and heparan sulfate GAGs—large, unbranched oligosaccharides that covalently modify proteins in the Golgi apparatus.^{11,85–89} The modified proteins are secreted into the extracellular matrix or, if transmembrane, located at the plasma membrane with glycosylated

regions on the extracellular face⁹⁰ (Figure 2(d)). Chondroitin appears to be the specific modification necessary for vulval morphogenesis, as *sqv-5* encodes a glycosyltransferase specific for the production of chondroitin,⁸⁹ and a mutant allele of *rib-2*, a gene required for initiation and elongation of heparan sulfate, does not have a Sqv phenotype.⁹¹ The ways in which GAGs regulate vulval morphogenesis have not been ascertained, but a possible explanation is that the presence of glycosylated proteins in the extracellular space between the plasma membrane and cuticle generates hydrostatic forces that allow expansion of the forming lumen. GAGs are negatively charged molecules that attract cations and water, generating turgor pressure to resist external force.⁹⁰ By analogy, chondroitin sulfate is secreted into the extracellular space during sea urchin gastrulation, and blocking secretion by drug treatment impaired the timing and extent of invagination.⁹² Other explanations have been posited for the role of GAGs in invagination, including the regulation of cell–cell adhesiveness, extracellular matrix remodeling during migration, and apical constriction.^{90,93}

The expression pattern of SQV proteins is consistent with a cell-autonomous role in vulval invagination. SQV-1, SQV-5, and SQV-7 proteins are expressed in cells throughout the vulva, and SQV-4 is expressed in vulC–vulF cells.^{86,87,89} Upstream regulators of *sqv* gene expression in the vulva have not been identified to date. Interestingly, a Sqv phenotype was observed in combined mutants of *lin-35*/Retinoblastoma and *spr-1*, an ortholog of the CoREST transcriptional repressor.⁹¹ The cause of the Sqv phenotype in *lin-35;spr-1* is not clear, as mutant animals have wild-type levels of chondroitin in the vulval cells.⁹¹ Thus, there appears to exist at least one pathway that functions in parallel to the *sqv* genes during vulval invagination.

Attachments to Other Tissues

Organogenesis requires functional connections between different tissues in the body. The vulva connects with three different tissues: (1) the uterine epithelium, to generate a continuous lumen between the tissues; (2) hypodermal cells, to structurally stabilize the vulva by physically tethering it to the body wall; and (3) muscle cells, to move embryos through the vulva to the outside environment (Figure 3).

The Anchor Cell and the Uterine-Vulval Connection

The uterine epithelium initially develops independently of the vulval epithelium and is separated from

it by gonadal and epidermal basement membranes. During the mid-L3 stage, the AC, a specialized ventral uterine cell, breaches the basement membrane separating the tissues and contacts the vulval cells (Figure 6(a)).¹⁴ The AC is a striking example of a single cell performing pleiotropic functions during development: in the late-L2 larval stage, the AC induces VPC fates via LIN-3 expression²²; in L3, the AC expresses Wnt ligands to orient VPC planar polarity (Figure 4),⁶³ expresses the LIN-12/Notch ligand LAG-2/Delta to induce the surrounding ventral uterine cells to adopt specific fates,⁹⁴ patterns the 1° VPC lineage,⁶¹ and breaches the basement membrane (Figure 6). The critical role for the AC in vulval morphogenesis was appreciated by Judith Kimble and David Hirsh, who named the cell for its ability to ‘anchor’ the gonad to the vulva.⁹⁵

Cell invasion across basement membrane occurs during several developmental events, and is also a key cell biological process in tumor metastasis and leukocyte trafficking.⁹⁹ AC invasion integrates a stimulatory signal from the developing vulva with a cell-autonomous invasion program (Figure 6(b)). The 1° VPCs secrete a diffusible chemotactic cue that triggers invasion and guides the invading AC to centrally located 1° VPCs. The presence of a diffusible cue was demonstrated using genetic backgrounds in which the 1° VPCs are displaced from the AC, which results in the AC extending membrane processes toward the ectopic VPCs and through the basement membrane.¹⁴ Although the vulval cells are required for complete invasion, the AC still invades 20% of the time in Vul animals, demonstrating that AC invasion through basement membrane may be triggered by other cues or driven cell autonomously.¹⁴

The AC initiates its invasion program at around the L2 molt by concentrating F-actin and other cytoskeletal components at the basal (ventral) surface in contact with the basement membrane.⁹⁶ Among the genes involved in cytoskeletal polarization are *unc-6*/netrin, a guidance molecule expressed in the ventral nerve cord (VNC), its receptor *unc-40*/DCC, and *pat-3/ina-1*, whose protein products heterodimerize to form the integrin–receptor complex (Figure 6(b)). In the mid-L3 stage, the basal membrane of the AC extends actin-enriched membrane protrusions that penetrate the basement membrane at a location between the two vulF cells (Figure 6(a)).^{96,100} AC invasion requires the transcription factor *fos-1a*, an ortholog of vertebrate Fos transcription factors that are expressed in invasive tumor cells.¹⁰¹ A downstream target of FOS-1A in the AC is *zmp-1* (Figure 6(b)),¹⁰² a member of the matrix metalloprotease (MMP) family of genes that have

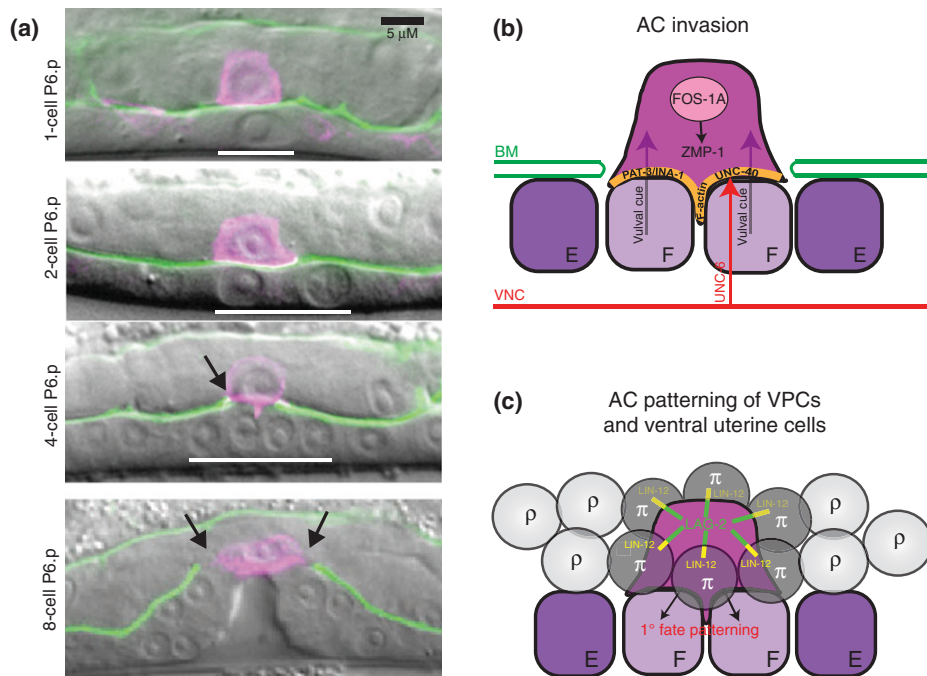


FIGURE 6 | The anchor cell (AC) connects the uterine and vulval tissues and patterns cell fates. (a) AC invasion into the vulval epithelium. At the one-cell P6.p stage (top panel), the AC is positioned dorsal to P6.p (indicated with white bar) and separated from it by basement membrane (green). Following the first vulval precursor cell (VPC) division (second panel), the AC initiates invasion across the basement membrane. By the four-cell P6.p stage (third panel), a breach is evident in the basement membrane (indicated with arrow), and the AC has contacted the 1° VPCs. The AC remains at the vulval apex following the final VPC division (bottom panel) and the gap in the basement membrane continues to expand beyond the border of the AC membrane (arrows). The AC expresses a reporter for the plextrin homology (PH) domain of phospholipase C δ fused to mCherry under an AC-specific *cdh-3* promoter.⁹⁶ The basement membrane expresses GFP-tagged laminin β subunit under its own promoter.⁹⁷ (b) Molecular pathways regulating AC invasion. The transcription factor FOS-1A is required for invasion, and regulates the expression of the matrix metalloprotease ZMP-1. The basal (ventral) AC membrane is enriched for cytoskeletal components F-actin and the INA-1/PAT-3 integrin–receptor complex. The UNC-6/netrin ligand is released by the ventral nerve cord (VNC) and regulates invasion through its receptor UNC-40/DCC. A vulval cue from the 1° VPCs is also required for complete invasion. Modeled after Ref 98 (c) The AC patterns uterine and vulval fates. The AC expresses LAG-2/Delta ligand, which signals to the LIN-12/Notch receptor on six surrounding ventral uterine cells to induce the π fate rather than the default ρ fate. The presence of the AC between the vulF cells is also required for 1° fate patterning.

been implicated in proteolytic breakdown of basement membrane and are expressed downstream of Fos proteins during tumor cell invasion.¹⁰³ Analogous to the situation in vertebrates, in which single or double knockdown of MMPs results in only minor developmental defects,¹⁰⁴ a null allele of *zmp-1* has no invasion defect.¹⁰² The simplified genetic architecture of *C. elegans*, with only six MMPs, makes the AC model of invasion particularly useful for achieving greater clarity on the involvement of proteases during cell invasion. More broadly, studies on AC invasion are facilitating the identification of novel genes that regulate the process of cell invasion. A recent whole-genome RNAi screen identified 99 genes that promote invasion, most of which were newly identified regulators of this process. Knockdown of human orthologs of two novel regulators, the NEMO-like kinase *lit-1* and the CCT chaperonin complex component *cct-5*, reduced invasiveness in metastatic

human carcinoma cells.⁹⁸ Thus, AC invasion is an effective model to understand mechanisms that regulate invasion and to identify new therapeutic targets to block invasive behavior in cancer.

Following basement membrane invasion, the AC remains at the vulval apex, where its basal region contacts and moves between the central vulF cells to complete the invasion process.^{8,16} Direct contact of the AC with the vulF cells appears to be required to pattern of E-F-F-E fates in the 1° VPC lineage⁶¹ (Figure 6(c)). Morphogenesis of the vulva also requires the complete invasion of the AC between the vulF cells.¹⁰⁵ Animals mutant for *fos-1* or *unc-6* have defective AC invasion through the basement membrane and incomplete AC contact with vulF, which results in a closed dorsal vulval lumen, suggesting that AC invasion between the vulF cells is required for dorsal lumen formation.¹⁰⁵ Lumen formation in the vulF cells also depends on

the NlpC/P60 family protein EGL-26, which may function as a palmitoyltransferase.^{106,107} EGL-26 is expressed in the vulE lineage and localizes to the apical membrane where it might modify a lipid signaling molecule required for proper vulF morphology.

The AC also patterns the ventral uterine (VU) cells surrounding it, all of which are involved in establishing the uterine–vulval connection.¹⁵ During the L3 larval stage, the AC expresses LAG-2/Delta, which signals via LIN-12/Notch on six adjacent VU cells, inducing them to adopt the π (pi) cell fate rather than the default ρ (rho) cell fate⁹⁴ (Figure 6(c)). Specification of π fate is required for proper uterine–vulval connection, as ablation of all π cells causes a completely penetrant Egl phenotype, as do gene mutations that result in the failure of VU cells to adopt the π fate.^{94,108–110} Following specification, the 6 π cells divide to generate 12 cells, 8 of which fuse to form the uterine-seam (utse) syncytium, which stabilizes the uterus through attachment to the lateral epithelial cells¹⁶ (Figure 3). The remaining four π cells are specified as uv1 cells, which contact the underlying vulF cells through adherens junctions and stabilize the uterine–vulval connection (Figure 3). LIN-3/EGF signaling from vulF cells via LET-23 receptor tyrosine kinase on uv1 cells is required for adoption of uv1 fate.¹¹¹ *egl-38*, a homolog of Pax5 transcription factor, is also required for the adoption of uv1 fate, functioning in both vulF and uv1 cells.^{111–113}

A question that until recently remained unsolved was how the uv1 cells and vulF cells make direct contact with each other, as basement membrane initially separates these cells. A recent study revealed that the gap in the basement membrane widens quickly beyond the cell boundaries of the AC during the mid- and late L3 larval stages (Figure 6(a)), thus allowing uv1 and vulF cells to make direct contact.¹¹⁴ To understand how the basement membrane is removed, animals expressing the tagged basement membrane components laminin::Dendra and type IV collagen::Dendra were examined. Dendra is a highly stable, photoconvertible fluorescent protein that changes irreversibly from a green to a red fluorescent state following exposure to short-wavelength light.¹¹⁵ Optical highlighting of laminin::Dendra and type IV collagen::Dendra revealed that laminin and type IV collagen are removed through a novel mechanism of basement membrane sliding, and that this shift is dependent on the dividing and invaginating vulval cells. Furthermore, an RNAi expression screen identified the integrin receptor complex INA-1/PAT-3 and VAB-19, a homolog of the tumor suppressor Kank, as regulators of basement membrane opening. Both concentrate within vulval cells at the basement

membrane gap boundary and halt expansion of the shifting basement membrane.¹¹⁴ Basement membrane sliding followed by targeted adhesion represents a newly identified mechanism for creating precise basement membrane breaches that is likely used by cells to break down compartment boundaries not only in tissue morphogenesis, but also in disease states such as cancer.

Following its invasion, the AC is positioned at the vulval apex (Figure 6(a)), from which it must be removed to form a continuous lumen between uterine and vulval tissues. Removal of the AC is achieved through fusion with the uterine utse cell during the early L4 stage. After fusion, the AC nucleus and cytoplasm are cleared, leaving a thin plasma membrane between the uterine and vulval lumens that is thought to be broken by first embryo passage¹⁵ (Figures 2(f) and 3). *aff-1* is expressed in the AC and utse and required for fusion. Its expression is downstream of the FOS-1A transcription factor, which is expressed throughout the uterine tissue with strongest expression in the AC.^{82,102} Also required for AC–utse fusion are the AC-expressed ATPase NSF-1¹¹⁶ and the π -cell expressed LIM domain transcription factor LIN-11.¹¹⁷ LIN-11 expression levels and retention in the nucleus are regulated in π cells by the small ubiquitin-like modifier SMO-1.¹¹⁸

Vulval Cell Attachments to Hypodermal Cells and Muscles

In addition to attaching to uterine cells, the vulva also contacts hypodermal and muscle cells, which provides structural stability and controls the opening of the vulva during mating and egg-laying, respectively (Figure 3). The hypodermal connections occur through vulA cell contact with the hyp7 syncytium at the ventral surface of the vulva, and vulE contact with lateral seam cells near the dorsal surface. The transcription factor *lin-29* is expressed in seam cells, and mosaic analysis suggests that the loss of *lin-29* in the subset of seam cells closest to the vulva causes vulval morphology defects, possibly through failure to attach to vulE cells.¹¹⁹ Little else is known about the vulE–seam cell connection, such as the molecular pathways that induce vulE membrane extension and the mechanisms of cell–cell attachment.

More is known about the mechanisms guiding muscle attachment to the vulva. The vulval musculature is derived from two sex myoblasts (SM), which are born in the posterior of the animal during the L1 stage and migrate anteriorly during L2 and L3 to flank the developing gonad near the vulva region. Following migration, the SM cells each divide three times in the L3 stage to generate 16 total cells. Eight

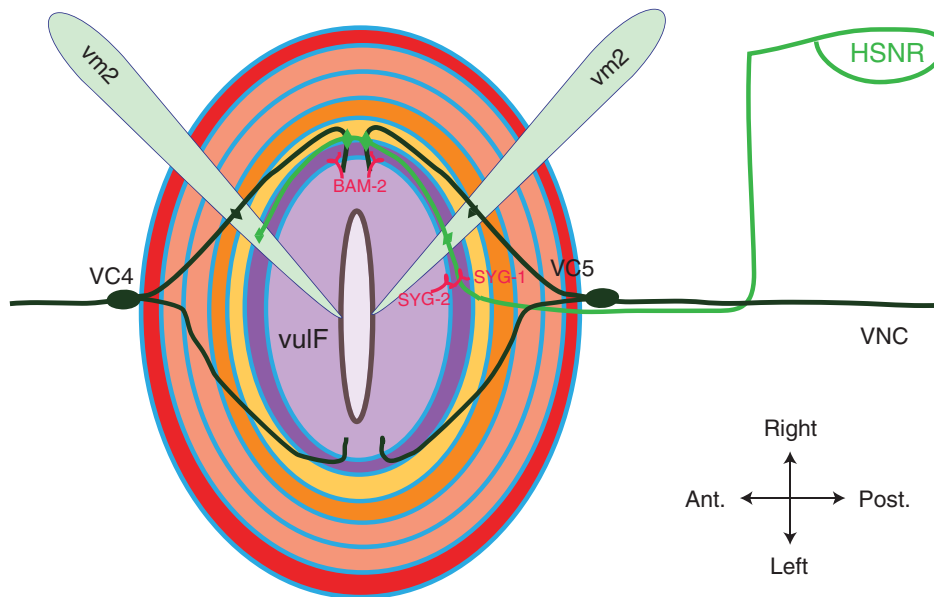


FIGURE 7 | Patterning of neuronal connections in the vulval region. Dorsal view of the everted vulva. The two hermaphrodite-specific (HSN) neurons (shown is the right HSN in green) extend axons ventrally to the nerve cord, then anteriorly to the vulva. The interaction between HSN-expressed SYG-1 and vulF-expressed SYG-2 serves to guide the future sites of synaptic contact, which occur on vm2 muscle cells (shown are two of four vm2 cells, light blue) and ventral cord (VC) neurons. VC4 and VC5 have cell bodies at the anterior and posterior of the vulval epithelium, respectively, and extend axons around the vulva and dorsally to synapse on vm2 cells. vulF-expressed BAM-2 is required to terminate axon branches. Modeled after Refs 123 and 124.

of these, the um cells, induce contractions that move eggs through the uterus. The other eight cells, four vm1 and four vm2, extend processes in a diagonal configuration that contact the vulval lips and control opening during mating and egg-laying. The four vm2 cells connect between the uterus and vulF; the four vm1 cells connect between vulC and vulD toroids^{8,15} (Figure 3).

The muscles that control vulval opening are innervated by six ventral cord (VC) motorneurons and two hermaphrodite-specific neurons (HSN). vm2 muscles are directly contacted by neurons, whereas vm1 muscles are electrically linked to vm2.¹²⁰ The six VC neurons, VC1–6, run anterior–posterior along the VNC and extend branches dorsally at the vulval opening. VC4 and VC5 have cell bodies closest to the vulva and extend the longest projections into the vulval epithelium (Figure 7). The HSN cell bodies are dorsal and posterior to the vulva, with axons that extend ventrally to the VNC, run anterior toward the head, defasciculate dorsally into the vulval region, and branch to synapse onto vm2, VC4 and VC5¹²⁰ (Figure 7). Branching of VC and HSN neuronal processes and their correct synapse formation requires signals from the vulva. In Vul animals, VC neurons do not send dorsal processes, and HSN axon extension toward the VNC and branching at the vulva are defective. These defects are not observed in animals

lacking the gonad, SM cells, or other neurons, demonstrating a singular role for the vulval cells in regulating neuronal outgrowth branching and synapse formation.^{121–123}

Genetic screens have begun to provide insight into the mechanisms by which the vulval cells direct VC and HSN neurite outgrowth. VC4 and VC5 axonal processes extend around the vulva without crossing the midline. This pattern of outgrowth is dependent on BAM-2, a neuroligin-like transmembrane protein that is expressed in vulF (Figure 7). In *bam-2* mutants, axon branches cross the midline, demonstrating that BAM-2 functions in vulF as a signal to terminate axon branching.¹²⁴ HSN axon guidance and synapse formation are also established by signals from the 1° VPCs. One 1° VPC-expressed signal is UNC-6/netrin, which appears to combine with VNC-expressed UNC-6 to coordinate the complex trajectory of HSN axon growth. Specifically, 1° VPC-derived UNC-6 is the main determinant of HSN dorsal growth from the VNC.¹²⁵ The 1° VPCs also direct HSN synapse formation onto muscles and neurons, which occurs near the dorsal region of the vulva. SYG-2, a transmembrane protein of the immunoglobulin (Ig) superfamily, is expressed in 1° VPCs and acts as a receptor for SYG-1, a second Ig transmembrane protein expressed on HSN axons. SYG-2 serves as a guidepost to localize SYG-1, which establishes sites of

synaptic vesicle clustering (Figure 7). In *syg-1* and *syg-2* mutants, HSN synaptic vesicle clusters are located in ectopic anterior locations.^{123,126} These findings highlight the key role of the vulva in organizing both the outgrowth and neuronal contacts necessary for egg-laying.

Bridging the Gap between Cell Fate Specification and Morphogenesis

How cell fate specification is linked to morphogenetic behaviors that shape tissues remains largely unknown.¹²⁷ Five transcription factors have been identified as major regulators of a gene regulatory network (GRN) that helps to specify the fates of the seven vulval cell types. These are the LIM homeodomain protein LIN-11, the C2H2-type zinc-finger protein LIN-29, the Nkx6 homeoprotein COG-1, the Pax2/5/8 ortholog EGL-38, and the nuclear hormone receptor NHR-67.^{5,6,84} How this GRN is regulated by VPC induction and whether these transcription factors control the effectors of vulval morphogenesis are unclear. Recently, a transcription factor, the C4H2 zinc-finger protein VAB-23, was found to bridge the gap between cell fate specification and the effectors of morphogenesis.⁷³ During vulval induction, the Hox gene *lin-39* is upregulated in the induced VPCs by EGF/Ras signaling.¹²⁸ Pellegrino et al.⁷³ found that LIN-39 and its conserved cofactor CEH-20¹²⁹ directly control VAB-23 expression in the vulval cells, with highest levels in the 1° lineage where EGF/Ras signaling is strongest. VAB-23 functions to repress *eff-1* expression in vulE and vulF cells and promotes *smp-1* expression in vulF, vulE, and vulD cells.⁷³ The *vab-23* mutants have ectopic *eff-1* expression, inappropriate cell–cell fusions, a reduction in toroid number, and defects in migration. VAB-23 is also required for the correct differentiation of the vulF and vulE cell fates, as determined by reporter gene expression.⁷³ These results demonstrate the connection between vulval induction (EGF/Ras), cell fate differentiation (LIN-39/VAB-23), and morphogenetic effectors (EFF-1/SMP-1), providing a paradigm for understanding how cells link specification with morphogenesis.

CONCLUSION

The vulva has served as a model of organogenesis for more than two decades, providing insight into developmental mechanisms of tissue patterning. Studies on the vulva have expanded our knowledge of important cellular processes, including oriented cell division, migration, invasion, adhesion, and fusion. The experimental advantages of the vulva—limited

number of cells, *in vivo* visual accessibility, and ease of genetic and transgenic analysis—can now be combined with advanced microscopic techniques such as live cell imaging, convertible fluorophores, force sensors, and optogenetics to provide insight into cellular dynamics. The vulva is also amenable to the systems-level approaches available in *C. elegans*, including interactome, localizome, expression profiling, modEncode, and RNAi databases.¹³⁰ Thus, future studies on vulval development will yield greater knowledge about fundamental mechanisms underlying morphogenesis. Among the interesting future subjects to explore are:

Molecular Basis of Lumen Formation

Lumen formation allows movement of materials between different tissues and the outside environment. How lumens achieve their correct size and shape is poorly understood.¹³¹ Vulval lumen formation involves cell shape changes and migrations that generate an invagination, and proteoglycan secretion that is thought to create and maintain lumen shape. Several questions remain unanswered about the mechanisms of lumen formation, including the cues that trigger the initial invagination, the upstream regulators of *sqv* gene expression, and the identity of the proteins modified by oligosaccharide chains.

Regulation of Adhesion and Fusion

VPCs extend membrane processes that meet their matching partners at the midline to form adhesions, but whether this recognition is based on cell-type specific receptor complexes or different mechanisms has not been determined. Fusions between homotypic cells (other than vulB) follow their adhesion at the midline. It is not well understood how the expression and subcellular localization of EFF-1 and AFF-1 fusogens are regulated to control the precise timing of fusions between matching cells.¹³²

Anchor Cell Invasion

AC invasion into the vulval epithelium offers a single-cell, *in vivo* model to study the cellular processes of invasion. Genetic screens and candidate gene approaches have led to the identification of more than 100 genes required for normal AC invasion.^{96,98,100,102,133,134} Many of the genes are uncharacterized, and understanding how they function in AC invasion should provide insights into this conserved biological process. Among the areas of focus are the involvement of proteases, transcriptional regulation of the invasive program, the identity of exogenous stimulatory cues that target membrane

processes to specific sites for invasion, and the mechanisms by which a cell shifts from cell–basement membrane to cell–cell attachments.

Axon Branching and Synapse Formation

During development of the egg-laying system, the vulva serves as an organizer of neuronal circuitry without receiving direct innervations itself. It terminates VC4 and VC5 axon branches by expression of the transmembrane protein BAM-2, but the VC-expressed binding partner of BAM-2 has not been identified. The SYG-1/SYG-2 interaction between 1° VPCs and the HSN neuron is an example of guidepost signaling, in which a cell acts as a scaffold for synapse formation. The downstream effectors of the SYG-1/SYG-2 signaling pathway that regulates guidepost signaling have yet to be determined.

Gene Regulatory Networks of Vulval Cell Specification and Morphogenesis

The GRNs that connect the adoption of cellular identity with gene expression and cell biological behaviors sit at the heart of understanding developmental mechanisms. Only a few developmental GRNs are understood at even a rudimentary level.¹³⁵ The two-step patterning of vulval cell fates—induction by the gonad followed by differentiation into seven different cellular subtypes—is a model for how cell specification connects with gene expression and morphogenesis. Future work can build on the knowledge of vulval specification pathways and the genes involved in morphogenesis to make connections between gene expression and morphogenesis.

ACKNOWLEDGMENTS

We thank members of the Sherwood Lab, Judith Kimble and Sunita Kramer for advice and suggestions. This work was supported by an American Cancer Society Postdoctoral Fellowship to AJS and Basil O'Connor Scholars Research Award, The Pew Scholars Program in the Biomedical Sciences, and NIH Grants GM079320 and GM100083 to DRS.

REFERENCES

- Gilbert SF, Singer SR, Tyler MS, Kozlowski RN. *Developmental Biology*. 8th ed. Sunderland, MA: Sinauer Associates; 2006.
- Langer R, Vacanti JP. Tissue engineering. *Science* 1993, 260:920–926.
- Egeblad M, Nakasone ES, Werb Z. Tumors as organs: complex tissues that interface with the entire organism. *Dev Cell* 2010, 18:884–901.
- Jorgensen EM, Mango SE. The art and design of genetic screens: *Caenorhabditis elegans*. *Nat Rev Genet* 2002, 3:356–369.
- Inoue T, Wang M, Ririe TO, Fernandes JS, Sternberg PW. Transcriptional network underlying *Caenorhabditis elegans* vulval development. *Proc Natl Acad Sci U S A* 2005, 102:4972–4977.
- Ririe TO, Fernandes JS, Sternberg PW. The *Caenorhabditis elegans* vulva: a post-embryonic gene regulatory network controlling organogenesis. *Proc Natl Acad Sci U S A* 2008, 105:20095–20099.
- Lints R, Hall DH. Reproductive system, egg-laying apparatus. *WormAtlas* 2009. doi:10.3908/wormatlas.1.24.
- Sharma-Kishore R, White JG, Southgate E, Podbilewicz B. Formation of the vulva in *Caenorhabditis elegans*: a paradigm for organogenesis. *Development* 1999, 126:691–699.
- Sternberg PW, Horvitz HR. Pattern formation during vulval development in *C. elegans*. *Cell* 1986, 44:761–772.
- Shemer G, Kishore R, Podbilewicz B. Ring formation drives invagination of the vulva in *Caenorhabditis elegans*: Ras, cell fusion, and cell migration determine structural fates. *Dev Biol* 2000, 221:233–248.
- Herman T, Hartwig E, Horvitz HR. sqv mutants of *Caenorhabditis elegans* are defective in vulval epithelial invagination. *Proc Natl Acad Sci U S A* 1999, 96:968–973.
- Herman T, Horvitz HR. Three proteins involved in *Caenorhabditis elegans* vulval invagination are similar to components of a glycosylation pathway. *Proc Natl Acad Sci U S A* 1999, 96:974–979.
- Mohler WA, Simske JS, Williams-Masson EM, Hardin JD, White JG. Dynamics and ultrastructure of developmental cell fusions in the *Caenorhabditis elegans* hypodermis. *Curr Biol* 1998, 8:1087–1090.
- Sherwood DR, Sternberg PW. Anchor cell invasion into the vulval epithelium in *C. elegans*. *Dev Cell* 2003, 5:21–31.

15. Newman AP, Sternberg PW. Coordinated morphogenesis of epithelia during development of the *Caenorhabditis elegans* uterine-vulval connection. *Proc Natl Acad Sci U S A* 1996, 93:9329–9333.
16. Newman AP, White JG, Sternberg PW. Morphogenesis of the *C. elegans* hermaphrodite uterus. *Development* 1996, 122:3617–3626.
17. Sundaram MV. The love-hate relationship between Ras and Notch. *Genes Dev* 2005, 19:1825–1839.
18. Sternberg PW. Vulval development. *WormBook* 2005:1–28.
19. Fay DS, Yochem J. The SynMuv genes of *Caenorhabditis elegans* in vulval development and beyond. *Dev Biol* 2007, 306:1–9.
20. Wang M, Sternberg PW. Pattern formation during *C. elegans* vulval induction. *Curr Top Dev Biol* 2001, 51:189–220.
21. Sulston JE, Horvitz HR. Post-embryonic cell lineages of the nematode, *Caenorhabditis elegans*. *Dev Biol* 1977, 56:110–156.
22. Katz WS, Hill RJ, Clandinin TR, Sternberg PW. Different levels of the *C. elegans* growth factor LIN-3 promote distinct vulval precursor fates. *Cell* 1995, 82:297–307.
23. Zand TP, Reiner DJ, Der CJ. Ras effector switching promotes divergent cell fates in *C. elegans* vulval patterning. *Dev Cell* 2011, 20:84–96.
24. Yoo AS, Bais C, Greenwald I. Crosstalk between the EGFR and LIN-12/Notch pathways in *C. elegans* vulval development. *Science* 2004, 303:663–666.
25. Levitan D, Greenwald I. LIN-12 protein expression and localization during vulval development in *C. elegans*. *Development* 1998, 125:3101–3109.
26. Berset T, Hoier EF, Battu G, Canevascini S, Hajnal A. Notch inhibition of RAS signaling through MAP kinase phosphatase LIP-1 during *C. elegans* vulval development. *Science* 2001, 291:1055–1058.
27. Berset TA, Hoier EF, Hajnal A. The *C. elegans* homolog of the mammalian tumor suppressor Dep-1/Sccl inhibits EGFR signaling to regulate binary cell fate decisions. *Genes Dev* 2005, 19:1328–1340.
28. Euling S, Ambros V. Heterochronic genes control cell cycle progress and developmental competence of *C. elegans* vulva precursor cells. *Cell* 1996, 84:667–676.
29. Ambros V, Horvitz HR. Heterochronic mutants of the nematode *Caenorhabditis elegans*. *Science* 1984, 226:409–416.
30. Ruvkun G, Giusto J. The *Caenorhabditis elegans* heterochronic gene *lin-14* encodes a nuclear protein that forms a temporal developmental switch. *Nature* 1989, 338:313–319.
31. Moss EG, Lee RC, Ambros V. The cold shock domain protein LIN-28 controls developmental timing in *C. elegans* and is regulated by the *lin-4* RNA. *Cell* 1997, 88:637–646.
32. Lee RC, Feinbaum RL, Ambros V. The *C. elegans* heterochronic gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14*. *Cell* 1993, 75:843–854.
33. Hong Y, Roy R, Ambros V. Developmental regulation of a cyclin-dependent kinase inhibitor controls postembryonic cell cycle progression in *Caenorhabditis elegans*. *Development* 1998, 125:3585–3597.
34. Olsen PH, Ambros V. The *lin-4* regulatory RNA controls developmental timing in *Caenorhabditis elegans* by blocking LIN-14 protein synthesis after the initiation of translation. *Dev Biol* 1999, 216:671–680.
35. Chalfie M, Horvitz HR, Sulston JE. Mutations that lead to reiterations in the cell lineages of *C. elegans*. *Cell* 1981, 24:59–69.
36. Li J, Greenwald I. LIN-14 inhibition of LIN-12 contributes to precision and timing of *C. elegans* vulval fate patterning. *Curr Biol* 2010, 20:1875–1879.
37. Abrahante JE, Daul AL, Li M, Volk ML, Tennesen JM, Miller EA, Rougvie AE. The *Caenorhabditis elegans* hunchback-like gene *lin-57/hbl-1* controls developmental time and is regulated by microRNAs. *Dev Cell* 2003, 4:625–637.
38. Abbott AL, Alvarez-Saavedra E, Miska EA, Lau NC, Bartel DP, Horvitz HR, Ambros V. The *let-7* MicroRNA family members *mir-48*, *mir-84*, and *mir-241* function together to regulate developmental timing in *Caenorhabditis elegans*. *Dev Cell* 2005, 9:403–414.
39. Kipreos ET. *C. elegans* cell cycles: invariance and stem cell divisions. *Nat Rev Mol Cell Biol* 2005, 6:766–776.
40. Saito RM, Perreault A, Peach B, Satterlee JS, van den Heuvel S. The CDC-14 phosphatase controls developmental cell-cycle arrest in *C. elegans*. *Nat Cell Biol* 2004, 6:777–783.
41. Clayton JE, van den Heuvel SJ, Saito RM. Transcriptional control of cell-cycle quiescence during *C. elegans* development. *Dev Biol* 2008, 313:603–613.
42. Fay DS, Keenan S, Han M. *fzr-1* and *lin-35/Rb* function redundantly to control cell proliferation in *C. elegans* as revealed by a nonbiased synthetic screen. *Genes Dev* 2002, 16:503–517.
43. Kipreos ET, Gohel SP, Hedgecock EM. The *C. elegans* F-box/WD-repeat protein LIN-23 functions to limit cell division during development. *Development* 2000, 127:5071–5082.
44. Kim Y, Kipreos ET. The *Caenorhabditis elegans* replication licensing factor CDT-1 is targeted for degradation by the CUL-4/DDB-1 complex. *Mol Cell Biol* 2007, 27:1394–1406.
45. Fay DS, Han M. Mutations in *cye-1*, a *Caenorhabditis elegans* cyclin E homolog, reveal coordination

- between cell-cycle control and vulval development. *Development* 2000, 127:4049–4060.
46. Winston JT, Chu C, Harper JW. Culprits in the degradation of cyclin E apprehended. *Genes Dev* 1999, 13:2751–2757.
47. Kipreos ET, Lander LE, Wing JP, He WW, Hedgecock EM. *cul-1* is required for cell cycle exit in *C. elegans* and identifies a novel gene family. *Cell* 1996, 85:829–839.
48. Boxem M, van den Heuvel S. *C. elegans* class B synthetic multivulva genes act in G(1) regulation. *Curr Biol* 2002, 12:906–911.
49. Buttitta LA, Edgar BA. Mechanisms controlling cell cycle exit upon terminal differentiation. *Curr Opin Cell Biol* 2007, 19:697–704.
50. Kaldis P, Richardson HE. When cell cycle meets development. *Development* 2012, 139:225–230.
51. Shemer G, Podbilewicz B. LIN-39/Hox triggers cell division and represses EFF-1/fusogen-dependent vulval cell fusion. *Genes Dev* 2002, 16:3136–3141.
52. Palmer RE, Inoue T, Sherwood DR, Jiang LI, Sternberg PW. *Caenorhabditis elegans* *cog-1* locus encodes GTX/Nkx6.1 homeodomain proteins and regulates multiple aspects of reproductive system development. *Dev Biol* 2002, 252:202–213.
53. Inoue T, Sternberg PW. *C. elegans* BED domain transcription factor BED-3 controls lineage-specific cell proliferation during organogenesis. *Dev Biol* 2010, 338:226–236.
54. Freyd G, Kim SK, Horvitz HR. Novel cysteine-rich motif and homeodomain in the product of the *Caenorhabditis elegans* cell lineage gene *lin-11*. *Nature* 1990, 344:876–879.
55. Gillies TE, Cabernard C. Cell division orientation in animals. *Curr Biol* 2011, 21:R599–R609.
56. Kishore RS, Sundaram MV. *ced-10* Rac and *mig-2* function redundantly and act with *unc-73* trio to control the orientation of vulval cell divisions and migrations in *Caenorhabditis elegans*. *Dev Biol* 2002, 241:339–348.
57. Chen Z, Han M. Role of *C. elegans* *lin-40* MTA in vulval fate specification and morphogenesis. *Development* 2001, 128:4911–4921.
58. Kiontke K, Barriere A, Kolotuev I, Podbilewicz B, Sommer R, Fitch DH, Felix MA. Trends, stasis, and drift in the evolution of nematode vulva development. *Curr Biol* 2007, 17:1925–1937.
59. Kolotuev I, Podbilewicz B. *Pristionchus pacificus* vulva formation: polarized division, cell migration, cell fusion, and evolution of invagination. *Dev Biol* 2004, 266:322–333.
60. Kolotuev I, Podbilewicz B. Changing of the cell division axes drives vulva evolution in nematodes. *Dev Biol* 2008, 313:142–154.
61. Wang M, Sternberg PW. Patterning of the *C. elegans* 1 degrees vulval lineage by RAS and Wnt pathways. *Development* 2000, 127:5047–5058.
62. Green JL, Inoue T, Sternberg PW. The *C. elegans* ROR receptor tyrosine kinase, CAM-1, non-autonomously inhibits the Wnt pathway. *Development* 2007, 134:4053–4062.
63. Green JL, Inoue T, Sternberg PW. Opposing Wnt pathways orient cell polarity during organogenesis. *Cell* 2008, 134:646–656.
64. Inoue T, Oz HS, Wiland D, Gharib S, Deshpande R, Hill RJ, Katz WS, Sternberg PW. *C. elegans* LIN-18 is a Ryk ortholog and functions in parallel to LIN-17/Frizzled in Wnt signaling. *Cell* 2004, 118:795–806.
65. Gleason JE, Szylyko EA, Eisenmann DM. Multiple redundant Wnt signaling components function in two processes during *C. elegans* vulval development. *Dev Biol* 2006, 298:442–457.
66. Deshpande R, Inoue T, Priess JR, Hill RJ. *lin-17*/Frizzled and *lin-18* regulate POP-1/TCF-1 localization and cell type specification during *C. elegans* vulval development. *Dev Biol* 2005, 278:118–129.
67. Chisholm AD, Hardin J. Epidermal morphogenesis. *WormBook* 2005:1–22.
68. Dalpe G, Brown L, Culotti JG. Vulva morphogenesis involves attraction of plexin 1-expressing primordial vulva cells to semaphorin 1a sequentially expressed at the vulva midline. *Development* 2005, 132:1387–1400.
69. Liu Z, Fujii T, Nukazuka A, Kurokawa R, Suzuki M, Fujisawa H, Takagi S. *C. elegans* PlexinA PLX-1 mediates a cell contact-dependent stop signal in vulval precursor cells. *Dev Biol* 2005, 282:138–151.
70. Roy PJ, Zheng H, Warren CE, Culotti JG. *mab-20* encodes Semaphorin-2a and is required to prevent ectopic cell contacts during epidermal morphogenesis in *Caenorhabditis elegans*. *Development* 2000, 127:755–767.
71. Wu Z, Sweeney LB, Ayoob JC, Chak K, Andreone BJ, Ohyama T, Kerr R, Luo L, Zlatic M, Kolodkin AL. A combinatorial semaphorin code instructs the initial steps of sensory circuit assembly in the *Drosophila* CNS. *Neuron* 2011, 70:281–298.
72. Chauvet S, Cohen S, Yoshida Y, Fekrane L, Livet J, Gayet O, Segu L, Buhot MC, Jessell TM, Henderson CE, et al. Gating of *Sema3E*/PlexinD1 signaling by neuropilin-1 switches axonal repulsion to attraction during brain development. *Neuron* 2007, 56:807–822.
73. Pellegrino MW, Farooqui S, Frohli E, Rehrauer H, Kaeser-Pebarnard S, Muller F, Gasser RB, Hajnal A. LIN-39 and the EGFR/RAS/MAPK pathway regulate *C. elegans* vulval morphogenesis via the VAB-23 zinc finger protein. *Development* 2011, 138:4649–4660.

74. Lynch AM, Hardin J. The assembly and maintenance of epithelial junctions in *C. elegans*. *Front Biosci* 2009, 14:1414–1432.
75. Hurd DD, Kemphues KJ. PAR-1 is required for morphogenesis of the *Caenorhabditis elegans* vulva. *Dev Biol* 2003, 253:54–65.
76. Williams-Masson EM, Malik AN, Hardin J. An actin-mediated two-step mechanism is required for ventral enclosure of the *C. elegans* hypodermis. *Development* 1997, 124:2889–2901.
77. Millard TH, Martin P. Dynamic analysis of filopodial interactions during the zipper phase of *Drosophila* dorsal closure. *Development* 2008, 135:621–626.
78. Podbilewicz B. Cell fusion. *WormBook* 2006:1–32.
79. Avinoam O, Fridman K, Valansi C, Abutbul I, Zeev-Ben-Mordehai T, Maurer UE, Sapir A, Danino D, Grunewald K, White JM, et al. Conserved eukaryotic fusogens can fuse viral envelopes to cells. *Science* 2011, 332:589–592.
80. Mohler WA, Shemer G, del Campo JJ, Valansi C, Opoku-Serebuoh E, Scranton V, Assaf N, White JG, Podbilewicz B. The type I membrane protein EFF-1 is essential for developmental cell fusion. *Dev Cell* 2002, 2:355–362.
81. Sapir A, Avinoam O, Podbilewicz B, Chernomordik LV. Viral and developmental cell fusion mechanisms: conservation and divergence. *Dev Cell* 2008, 14:11–21.
82. Sapir A, Choi J, Leikina E, Avinoam O, Valansi C, Chernomordik LV, Newman AP, Podbilewicz B. AFF-1, a FOS-1-regulated fusogen, mediates fusion of the anchor cell in *C. elegans*. *Dev Cell* 2007, 12:683–698.
83. Podbilewicz B, Leikina E, Sapir A, Valansi C, Suissa M, Shemer G, Chernomordik LV. The *C. elegans* developmental fusogen EFF-1 mediates homotypic fusion in heterologous cells and in vivo. *Dev Cell* 2006, 11:471–481.
84. Fernandes JS, Sternberg PW. The tailless ortholog nhr-67 regulates patterning of gene expression and morphogenesis in the *C. elegans* vulva. *PLoS Genet* 2007, 3:e69.
85. Bulik DA, Wei G, Toyoda H, Kinoshita-Toyoda A, Waldrip WR, Esko JD, Robbins PW, Selleck SB. sqv-3, -7, and -8, a set of genes affecting morphogenesis in *Caenorhabditis elegans*, encode enzymes required for glycosaminoglycan biosynthesis. *Proc Natl Acad Sci U S A* 2000, 97:10838–10843.
86. Hwang HY, Horvitz HR. The *Caenorhabditis elegans* vulval morphogenesis gene sqv-4 encodes a UDP-glucose dehydrogenase that is temporally and spatially regulated. *Proc Natl Acad Sci U S A* 2002, 99:14224–14229.
87. Hwang HY, Horvitz HR. The SQV-1 UDP-glucuronic acid decarboxylase and the SQV-7 nucleotide-sugar transporter may act in the Golgi apparatus to affect *Caenorhabditis elegans* vulval morphogenesis and embryonic development. *Proc Natl Acad Sci U S A* 2002, 99:14218–14223.
88. Hwang HY, Olson SK, Brown JR, Esko JD, Horvitz HR. The *Caenorhabditis elegans* genes sqv-2 and sqv-6, which are required for vulval morphogenesis, encode glycosaminoglycan galactosyltransferase II and xylosyltransferase. *J Biol Chem* 2003, 278:11735–11738.
89. Hwang HY, Olson SK, Esko JD, Horvitz HR. *Caenorhabditis elegans* early embryogenesis and vulval morphogenesis require chondroitin biosynthesis. *Nature* 2003, 423:439–443.
90. Bulik DA, Robbins PW. The *Caenorhabditis elegans* sqv genes and functions of proteoglycans in development. *Biochim Biophys Acta* 2002, 1573:247–257.
91. Bender AM, Kirienko NV, Olson SK, Esko JD, Fay DS. lin-35/Rb and the CoREST ortholog spr-1 coordinately regulate vulval morphogenesis and gonad development in *C. elegans*. *Dev Biol* 2007, 302:448–462.
92. Lane MC, Koehl MA, Wilt F, Keller R. A role for regulated secretion of apical extracellular matrix during epithelial invagination in the sea urchin. *Development* 1993, 117:1049–1060.
93. Herman T, Horvitz HR. Mutations that perturb vulval invagination in *C. elegans*. *Cold Spring Harb Symp Quant Biol* 1997, 62:353–359.
94. Newman AP, White JG, Sternberg PW. The *Caenorhabditis elegans* lin-12 gene mediates induction of ventral uterine specialization by the anchor cell. *Development* 1995, 121:263–271.
95. Kimble J, Hirsh D. The postembryonic cell lineages of the hermaphrodite and male gonads in *Caenorhabditis elegans*. *Dev Biol* 1979, 70:396–417.
96. Ziel JW, Hagedorn EJ, Audhya A, Sherwood DR. UNC-6 (netrin) orients the invasive membrane of the anchor cell in *C. elegans*. *Nat Cell Biol* 2009, 11:183–189.
97. Kao G, Huang CC, Hedgecock EM, Hall DH, Wadsworth WG. The role of the laminin β subunit in laminin heterotrimer assembly and basement membrane function and development in *C. elegans*. *Dev Biol* 2006, 290:211–219.
98. Matus DQ, Li XY, Durbin S, Agarwal D, Chi Q, Weiss SJ, Sherwood DR. In vivo identification of regulators of cell invasion across basement membranes. *Sci Signal* 2010, 3:ra35.
99. Sherwood DR. Cell invasion through basement membranes: an anchor of understanding. *Trends Cell Biol* 2006, 16:250–256.
100. Hagedorn EJ, Yashiro H, Ziel JW, Ihara S, Wang Z, Sherwood DR. Integrin acts upstream of netrin signaling to regulate formation of the anchor cell's invasive membrane in *C. elegans*. *Dev Cell* 2009, 17:187–198.
101. Ozanne BW, Spence HJ, McGarry LC, Hennigan RF. Transcription factors control invasion: AP-1 the first among equals. *Oncogene* 2007, 26:1–10.

102. Sherwood DR, Butler JA, Kramer JM, Sternberg PW. FOS-1 promotes basement-membrane removal during anchor-cell invasion in *C. elegans*. *Cell* 2005, 121:951–962.
103. Adiseshaiah P, Vaz M, Machireddy N, Kalvakolanu DV, Reddy SP. A Fra-1-dependent, matrix metalloproteinase driven EGFR activation promotes human lung epithelial cell motility and invasion. *J Cell Physiol* 2008, 216:405–412.
104. Perez SE, Cano DA, Dao-Pick T, Rougier JP, Werb Z, Hebrok M. Matrix metalloproteinases 2 and 9 are dispensable for pancreatic islet formation and function in vivo. *Diabetes* 2005, 54:694–701.
105. Estes KA, Hanna-Rose W. The anchor cell initiates dorsal lumen formation during *C. elegans* vulval tubulogenesis. *Dev Biol* 2009, 328:297–304.
106. Estes KA, Kalamegham R, Hanna-Rose W. Membrane localization of the NlpC/P60 family protein EGL-26 correlates with regulation of vulval cell morphogenesis in *Caenorhabditis elegans*. *Dev Biol* 2007, 308:196–205.
107. Hanna-Rose W, Han M. The *Caenorhabditis elegans* EGL-26 protein mediates vulval cell morphogenesis. *Dev Biol* 2002, 241:247–258.
108. Newman AP, Inoue T, Wang M, Sternberg PW. The *Caenorhabditis elegans* heterochronic gene *lin-29* coordinates the vulval-uterine-epidermal connections. *Curr Biol* 2000, 10:1479–1488.
109. Cinar HN, Richards KL, Oommen KS, Newman AP. The EGL-13 SOX domain transcription factor affects the uterine pi cell lineages in *Caenorhabditis elegans*. *Genetics* 2003, 165:1623–1628.
110. Hanna-Rose W, Han M. COG-2, a sox domain protein necessary for establishing a functional vulval-uterine connection in *Caenorhabditis elegans*. *Development* 1999, 126:169–179.
111. Chang C, Newman AP, Sternberg PW. Reciprocal EGF signaling back to the uterus from the induced *C. elegans* vulva coordinates morphogenesis of epithelia. *Curr Biol* 1999, 9:237–246.
112. Rajakumar V, Chamberlin HM. The Pax2/5/8 gene *egl-38* coordinates organogenesis of the *C. elegans* egg-laying system. *Dev Biol* 2007, 301:240–253.
113. Chamberlin HM, Palmer RE, Newman AP, Sternberg PW, Baillie DL, Thomas JH. The PAX gene *egl-38* mediates developmental patterning in *Caenorhabditis elegans*. *Development* 1997, 124:3919–3928.
114. Ihara S, Hagedorn EJ, Morrissey MA, Chi Q, Motegi F, Kramer JM, Sherwood DR. Basement membrane sliding and targeted adhesion remodels tissue boundaries during uterine-vulval attachment in *Caenorhabditis elegans*. *Nat Cell Biol* 2011, 13:641–651.
115. Gurskaya NG, Verkhusha VV, Shcheglov AS, Staroverov DB, Chepurnykh TV, Fradkov AF, Lukyanov S, Lukyanov KA. Engineering of a monomeric green-to-red photoactivatable fluorescent protein induced by blue light. *Nat Biotechnol* 2006, 24:461–465.
116. Choi J, Richards KL, Cinar HN, Newman AP. N-ethylmaleimide sensitive factor is required for fusion of the *C. elegans* uterine anchor cell. *Dev Biol* 2006, 297:87–102.
117. Newman AP, Acton GZ, Hartwig E, Horvitz HR, Sternberg PW. The *lin-11* LIM domain transcription factor is necessary for morphogenesis of *C. elegans* uterine cells. *Development* 1999, 126:5319–5326.
118. Broday L, Kolotuev I, Didier C, Bhoumik A, Gupta BP, Sternberg PW, Podbilewicz B, Ronai Z. The small ubiquitin-like modifier (SUMO) is required for gonadal and uterine-vulval morphogenesis in *Caenorhabditis elegans*. *Genes Dev* 2004, 18:2380–2391.
119. Bettinger JC, Euling S, Rougvie AE. The terminal differentiation factor LIN-29 is required for proper vulval morphogenesis and egg laying in *Caenorhabditis elegans*. *Development* 1997, 124:4333–4342.
120. White JG, Southgate E, Thomson JN, Brenner S. The structure of the nervous system of the nematode *Caenorhabditis elegans*. *Philos Trans R Soc Lond B Biol Sci* 1986, 314.
121. Li C, Chalfie M. Organogenesis in *C. elegans*: positioning of neurons and muscles in the egg-laying system. *Neuron* 1990, 4:681–695.
122. Garriga G, Desai C, Horvitz HR. Cell interactions control the direction of outgrowth, branching and fasciculation of the HSN axons of *Caenorhabditis elegans*. *Development* 1993, 117:1071–1087.
123. Shen K, Bargmann CI. The immunoglobulin superfamily protein SYG-1 determines the location of specific synapses in *C. elegans*. *Cell* 2003, 112:619–630.
124. Colavita A, Tessier-Lavigne M. A Neurexin-related protein, BAM-2, terminates axonal branches in *C. elegans*. *Science* 2003, 302:293–296.
125. Asakura T, Ogura K, Goshima Y. UNC-6 expression by the vulval precursor cells of *Caenorhabditis elegans* is required for the complex axon guidance of the HSN neurons. *Dev Biol* 2007, 304:800–810.
126. Shen K, Fetter RD, Bargmann CI. Synaptic specificity is generated by the synaptic guidepost protein SYG-2 and its receptor, SYG-1. *Cell* 2004, 116:869–881.
127. Christiaen L, Davidson B, Kawashima T, Powell W, Nolla H, Vranizan K, Levine M. The transcription/migration interface in heart precursors of *Ciona intestinalis*. *Science* 2008, 320:1349–1352.
128. Maloof JN, Kenyon C. The Hox gene *lin-39* is required during *C. elegans* vulval induction to select the outcome of Ras signaling. *Development* 1998, 125:181–190.
129. Yang L, Sym M, Kenyon C. The roles of two *C. elegans* HOX co-factor orthologs in cell migration

- and vulva development. *Development* 2005, 132: 1413–1428.
130. Wang Z, Sherwood DR. Dissection of genetic pathways in *C. elegans*. *Methods Cell Biol* 2011, 106: 113–157.
131. Datta A, Bryant DM, Mostov KE. Molecular regulation of lumen morphogenesis. *Curr Biol* 2011, 21: R126–136.
132. Avinoam O, Poddilewicz B. Eukaryotic cell-cell fusion families. *Curr Top Membr* 2011, 68:209–234.
133. Schindler AJ, Sherwood DR. The transcription factor HLH-2/E/Daughterless regulates anchor cell invasion across basement membrane in *C. elegans*. *Dev Biol* 2011, 357:380–391.
134. Ziel JW, Matus DQ, Sherwood DR. An expression screen for RhoGEF genes involved in *C. elegans* gonadogenesis. *Gene Expr Patterns* 2009, 9:397–403.
135. Levine M, Davidson EH. Gene regulatory networks for development. *Proc Natl Acad Sci U S A* 2005, 102: 4936–4942.