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REVIEW

Non-neuronal cell outgrowth in *C. elegans*

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ABSTRACT

Cell outgrowth is a hallmark of some non-migratory developing cells during morphogenesis. Understanding the mechanisms that control cell outgrowth not only increases our knowledge of tissue and organ development, but can also shed light on disease pathologies that exhibit outgrowth-like behavior. *C. elegans* is a highly useful model for the analysis of genes and the function of their respective proteins. In addition, *C. elegans* also has several cells and tissues that undergo outgrowth during development. Here we discuss the outgrowth mechanisms of nine different *C. elegans* cells and tissues. We specifically focus on how these cells and tissues grow outward and the interactions they make with their environment. Through our own identification, and a meta-analysis, we also identify gene families involved in multiple cell outgrowth processes, which defined potential *C. elegans* core components of cell outgrowth, as well as identify a potential stepwise cell behavioral cascade used by cells undergoing outgrowth.

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Introduction

During cell morphogenesis, when cells change their shape during development, the process of cell outgrowth, during which a non-migratory cell expands outward in a projection-like path as it changes shape, is also a common event. Examples of normal cell outgrowth during morphogenesis include outgrowth in the mouse facial primodia, outgrowth of regenerating fins in Zebrafish, dramatic outgrowth in *Drosophila* cardioblast cells, and the development of human neurons.[1–4] Cell outgrowth-like behavior is also characteristic of certain diseases such as endometriosis, the outgrowth of endometrium cells outside of the uterus, and in metastatic cancer during the spreading of tumors from one tissue to another.[5,6] Therefore, understanding the mechanisms that control cell outgrowth not only sheds light on the genetic inputs that control development, but also provides information on the pathologies of certain diseases.

C. elegans is a small free-living nematode whose cell lineage is stereotypic and well-characterized.[7–11] This model organism is also transparent, making it a powerful tool to study cell morphology. Though much of *C. elegans* development consists of cells dividing and taking on different fates, certain cell types undergo outgrowth during morphogenesis. In this review, we describe the mechanisms used during outgrowth for nine non-neuronal cell types. We specifically focus on non-neuronal cells as numerous neuronal cells that undergo outgrowth have already been well discussed,[12–18] and addressing each neuronal cell type would result in content that would encompass its own review.

We will describe and compare the cell outgrowth processes in the uterine seam cell (utse), the anchor cell (AC), the vulval sex muscles, the muscle arms, the male tail, the excretory cell, the head mesodermal cells, and during two events of embryonic development, dorsal intercalation and ventral enclosure.

The utse and vulval sex muscles

The *C. elegans* utse attaches the uterus to the lateral epithelial seam cells of the body wall.[18] The utse forms from the fusion of eight cells, that require the expression of the Notch family receptor LIN-12 and its Delta

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family ligand LAG-2, as well as the fusogen AFF-1.[19–27] After fusion, the outgrowth process of the utse occurs over an eight hour period as the utse cell body grows bi-directionally along the anterior-posterior axis, and the utse nuclei segregate into two groups migrating along the anterior-posterior axis and settling at the anterior/posterior edges of the utse cell body (Fig. 1).[19,26] The utse cell body extends ahead of its nuclei during outgrowth, indicating that separate mechanisms control the movement of the cell body and the nuclei.[26]

During outgrowth, the utse requires the presence of several cells within the *C. elegans* uterus. Four uterine toroids line the lumen of the uterus, denoted uterine toroid 1 to uterine toroid 4, with numbers increasing for cells that are more distal to the vulva.[19] The presence of uterine toroid 1 and uterine toroid 2 is essential for proper utse outgrowth as ablation of these cells leads to defects in utse outgrowth.[26] In addition, the vulval sex muscles, which lie proximal and distal to the utse on either side of the body wall, are also necessary for utse outgrowth; ablation of the vulval sex muscle precursors, and knockdown of genes expressed in the vulval sex muscles, lead to defects in utse outgrowth development. During egg-laying, vulval sex muscles contract to open the vulva and allow eggs to be laid. Vulval sex muscles develop from the M cell that divides to create precursors for body wall muscles, coelomocytes, and two sex myoblasts. Through the function of *egl-15*, the sex myoblasts migrate, divide, and arrange into four sections within the uterine/vulval area, during the L3 stage. The two sections that lie proximal to the vulva become vulval sex muscles, vm1 and vm2, and the two sections distal to the vulva become uterine muscles. The vulval sex muscles undergo outgrowth when they extend processes ventrally to attach the vulva to the hypodermis, as well as longitudinally towards the seam cells (Fig. 2).[9,17] The function of *unc-53* has been shown to be necessary for generating these longitudinal processes,[17] as *unc-53* mutants do not form these processes and result in the muscles attaching to myofilaments and developing a rounded shape.

Similar to the sex muscles, cells and tissues surrounding the utse express several gene families that have also been shown to be involved in utse development. The uterine toroids express genes encoding a guanine nucleotide exchange factor (*unc-73*) and its interacting factors (*let-502, rho-1, unc-13*, and *unc-64*), and genes encoding Rab-like and RabGTPases (*rab-1, rab-5, rab-6.1, rab-10, rab-11.1*, and *rsef-1*). The vulval sex muscles express genes that encode migration-signaling proteins (*unc-53* and *egl-15*). In addition, genes expressed by the anchor cell that mediate its invasion (*aff-1, cdh-3, egl-43, fos-1, him-4, ina-1, lag-2, mig-10, pat-3*, and *zif-1*) also act on the utse.

Furthermore, through an RNAi screen (S. Ghosh et al., in preparation) we identified 52 additional genes that influenced utse cell outgrowth. These genes encode proteins involved in gene expression and regulation (DAF-16, EGL-13, F11A10.5, IMA-1, IMA2, IMA-3, IMB-2, IMB-3, LIN-11, LIN-31, LIN-39, PQN-85, RAN-2, RAN-3, TTX-3, VAB-3, ZAG-1, ZFH-2, ZFP-1), signal transduction (CWN-1, GIPC-1, GIPC-2, GLB-12, GLP-1, ITR-1, MIG-15, RCC-1, SAX-1), cellular vesicle transport (AMPH-1, DH11.5, SEC-15), cytoskeleton dynamics and binding (ANC-1, ARX-2, ARX-3, DEB-1, FRM-2, GEX-1/WVE-1, GEX-2, LIM-9, NUD-2, TOCA-1, UNC-70, UNC-83, UNC-84, UNC-97), extracellular matrix proteins (FBL-1, LMN-1), and

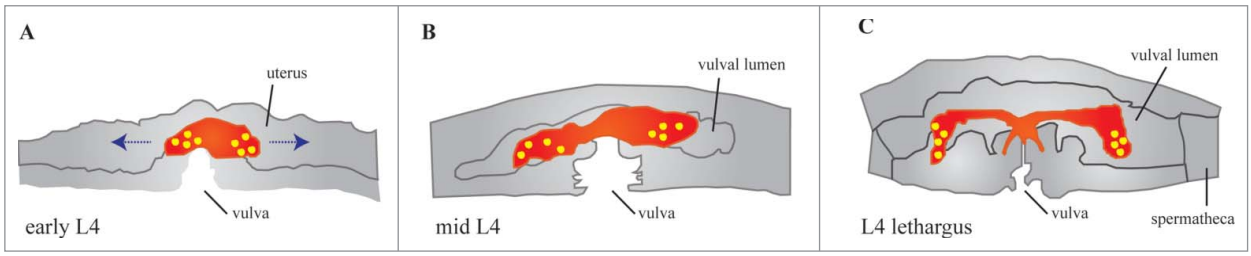

Figure 1. utse outgrowth over time. Schematic of utse outgrowth over time (A) Early L4 vulva and uterus. The utse has just formed at this stage after the fusion of eight ρ cells and the anchor cell. The cell has an ellipsoidal shape. Outlines indicate positions of vulva and uterus. utse is indicated in red. Blue dashed arrows indicate direction of outgrowth. (B) Mid L4 vulva and uterus. utse has begun elongating along the anterior-posterior axis. Outlines indicate positions of vulva and uterus. utse is indicated in red. (C) L4 lethargus vulva and uterus. utse has completed its outgrowth, and has taken on an elongated shape with the edges of its arms extending along the dorsal/ventral axis. Outlines indicate positions of vulva and uterus. utse is indicated in red.

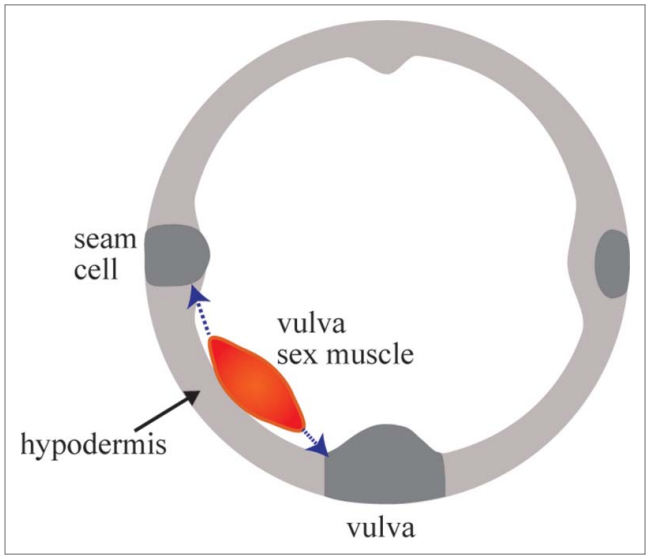

Figure 2. Vulval sex muscle outgrowth. Schematic of a sex myoblast at the L3 larval stage. A cross section of an L3 vulva shows that a sex myoblast daughter cell (in red) is in between the vulval epithelium and the seam cells of the hypodermis. Sex myoblasts extend protrusions ventrally towards the vulva and laterally towards the seam cells.

cellular adhesion and migration (NCAM-1, PXL-1, SAX-7, SRSX-18, UNC-33). Taken together, it appears that the utse is highly sensitive to perturbations of a broad range of cell biological functions.

The anchor cell

The AC undergoes cell outgrowth when it invades the underlying basement membrane to interact with vulval epithelial cells and establish vulval cell fates.[28] During the L3 larval stage, the AC extends a process ventrally to mediate a connection with the descendants of the 1° vulval precursor cell P6.p (Fig. 3).[28,30] Once P6.p reaches the two-cell stage (mid L3) (Fig. 3A), the basement membrane underneath the AC is interrupted, and the basolateral portion of the AC crosses the membrane (Fig. 3C). When P6.p reaches the four-cell stage (mid to late L3), the AC extends a fine cellular process that reaches ventrally between the P6.pap and P6.ppa cells (Fig. 3D). This invasive structure remains in place as the P6.p granddaughter cells continue to divide and the vulva invaginates. By the L3 lethargus/early L4 stage, the AC positions itself in the dorsal apex of the vulva and has completed its outgrowth process. The AC then induces the surrounding ventral uterine cells to generate the utse cell body.[24,26,27]

The presence of the 1° vulval cells are required for the initiation of AC outgrowth.[28] The 1° vulval cells are specified via LIN-3 signaling from the AC in late L2 to early L3 larval stage.[29] Without the AC, all vulval precursor cells take on the 3° fate and become external epithelial cells.[30–33] *lin-3* mutants, which have 3° vulval cells instead of 1° vulval cells, do not exhibit AC outgrowth.[28] Also, when the P8.p is ectopically induced by ablating all other vulval precursor cells in the L2 stage, the AC directs its projections to the distal P8.p cells which indicates that a long-range cue from the 1° vulval cells induces outgrowth.

Several genes are involved in inducing AC outgrowth and removing the basement membrane underlying the AC. A key pathway necessary for AC outgrowth involves the expression of the gene *fos-1* (C-FOS transcription factor), and five genes that encode the downstream effectors of FOS-1: *zmp-1* (zinc metalloprotease), *cdh-3* (protocadherin), *egl-43* (zinc finger protein), *him-4* (hemicentin), and *mig-10* (lamellopodin).[34–37] Other genes encoding proteins involved in genetic interactions that promote outgrowth include: *zif-1* and *cdc-42*, which promote the interaction between E3 ubiquitin ligase substrate-recognition subunit; *unc-6* and *unc-40*, which promotes outgrowth through enrichment of the actin regulators, F-actin, and phosphatidylinositol 4, 5-bisphosphate (PtdIns (4, 5) P2) with netrin (UNC-6) in the basement membrane and corresponding netrin receptor (UNC-40) in the AC plasma membrane; *ina-1* and *pat*-3, which targets the netrin receptor to the plasma membrane of the AC; *hlh-2*, which regulates levels of protocadherin and hemicentin in a separate pathway from *fos-1*; *vrk-1*, which regulates polarity of protrusions; *aff-1*, which encodes a fusogen that allows the AC to fuse with the utse syncytium, and *madd-2*, which prevents ectopic invasive structures from emanating from the AC.[38–47]

Muscle arms

The plasma membrane of body wall muscles extend protrusions to motor neurons, called muscle arms, in order to make neuromuscular junctions (Fig. 4).[48–50] Muscle arms contain a thin stalk from the body wall muscle and have bifurcated ends.[49] Actin is required to generate the outgrowth

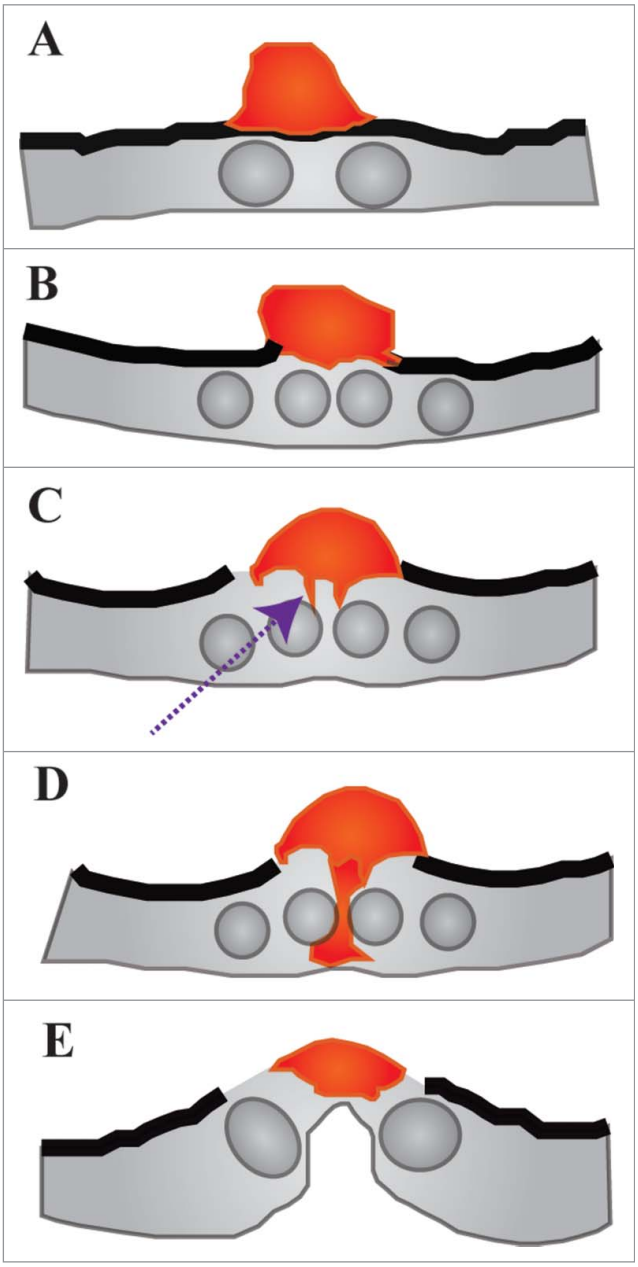

Figure 3. Anchor cell invasion. Schematic anchor cell invasion. Anchor cell shown in red, vulva shown in gray. Basement membrane shown in thick line. Adapted from Ihara et al., 2011 and Hagedorn and Sherwood, 2011. (A) Anchor cell at P6.p two cell stage during mid L3. Basement membrane (shown in thick line) is intact. Anchor cell is dorsal to the P6.p 1° VPC daughters (two circles below). (B) Anchor cell at P6.p four cell stage during mid to late L3. Anchor cell has generated a gap in the basement membrane (see separation between thick lines). Edges of the anchor cell are still in contact with the basement membrane. (C) Anchor cell at P6.p late four-cell stage (late L3). The anchor cell has begun forming protrusions that will invade the vulva (dashed purple arrow). (D) Anchor cell at P6.p late four-cell stage (late L3). The anchor cell has completely invaded the vulva, specifically invading between the 1° VPC granddaughters. (E) Anchor cell at early L4 stage. The 1□ VPCs have divided and proximal cells are shown. Vulval invagination has occurred and anchor cell will soon fuse with the ρ cells to form the utse.

extensions of the muscle arms, as knockdown of actin genes, *act-1,act-2, act-3*, reduces their formation. Of the 95 body wall muscles, 79 create muscle arms, of which 16 outgrow from the neck to the nerve ring, and the remaining 63 outgrow towards the nearest motor neuron on the nerve cord. When motor neurons take on aberrant paths, muscle arm outgrowth follows the path of those aberrant neurons, indicating that muscle arms respond to chemoattractant cues from motor neurons.[51] Muscle arms are also thought to direct their outgrowth in relation to areas containing dense core vesicles, as seen in *unc-104* mutants, in which muscle arms extend towards areas with increased localization of dense core vesicles.[50,52]

A screen performed by Alexander et al.[53] identified ten genes necessary for muscle arm outgrowth: *gex-2, madd-2, unc-33, unc-40, unc-51, unc-54, unc-60, unc-73, unc-93*, and *unc-95*. Knockdown of these genes cause a muscle arm extension defect, known as a MAD defect. The cofilin UNC-60 specifically affects muscle arm extension by regulating actin-severing activity.[49] Tropomyosin is known to antagonize cofilin activity, and *C. elegans* tropomyosin, LEV-11, acts to stabilize actin in muscle arms.[49,54–57] The ADAM ortholog MADD-4 and netrin signaling (*eva-1, unc-6, unc-40*,) work together to form and guide muscle arm outgrowth.[58,59] Netrin signaling also mediates the gene expression of *lin-12* and *madd-2* to affect muscle arm development.[60,61]

Other genes involved in muscle arm outgrowth include *pat-2*, *pat-4*, *pat-6*, *unc-52*, *lam-1*, *lam-2*, and *epi-1*.[62] The dense body components (*unc-97* and *unc-98*), as well as components of the WAVE complex (*gex-1*/*wve-1* and *wsp-1*) also regulate muscle arm extension. Myosin heavy chain B, UNC-54, is also necessary for muscle arm formation, as loss-of-function *unc-54* mutants contain fewer muscle arms and changes in arm width.[49,60] The fibroblast growth factor (FGF) pathway is also involved in regulating muscle arm formation; when *let-756* (FGF), *egl-15* (FGF receptor), or *sem-5* (adaptor protein GRB2) genes are knocked down, ectopic muscle membrane extensions form.[62] Loss-of-function of other FGF signaling components such as, *egl-17, let-60, ptp-2, soc-1, soc-2* and *sos-1* also result in ectopic muscle extensions. The body wall muscle-expressed tyrosine phosphatase

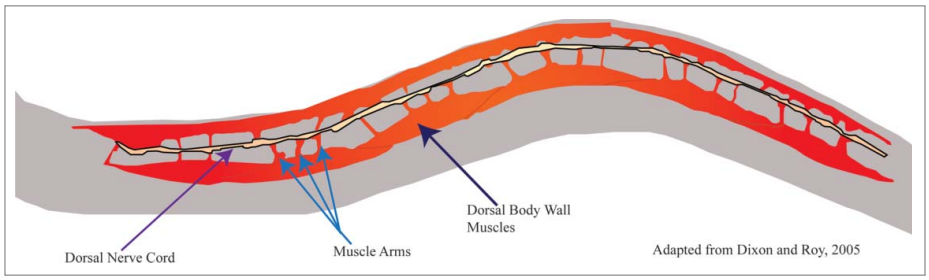

Figure 4. Muscle arm extension. Schematic of muscle arm extension. Adapted from Dixon and Roy, 2005. Example of muscle arms throughout the *C. elegans* body (light blue arrows). Muscle arms have formed from the dorsal body wall muscles (end of dark blue arrow) towards the dorsal nerve cord.

receptor CLR-1 inhibits EGL-15 activity, suppresses the EME phenotype from *egl-15* knockdown, and causes the MAD phenotype when knocked down on its own.[62,63]

The male tail

The male tail consists of the fan, the rays, the spicules, the proctodeum, the gubernaculum, and the hook (Fig. 5).[64,65] Cell outgrowth occurs during fan and ray formation, therefore we characterized the development and molecular inputs for these two processes.[66]

The male rays and fan originate from the lateral epidermal seam cells, of which the three most posterior give rise to the ray precursor cells, or Rn.p cells.[64] These Rn.p cells eventually generate nine pairs of sensory rays.[67] During the late L4 stage, the tip of the tail becomes rounded and retracts anteriorly by losing adhesion with the cuticle, leaving behind a clear fluid in the extracellular space (Fig. 5B).[64,68] The ray cells form papillae on the edge of the tail cell body (Fig. 5C and D), and these papillae eventually become the distal edges of the rays, which attach to the fan cuticle through an adherens junction (Fig. 5E).[67,69] As the fan extends from the cell body, it pulls the papillae with it, allowing the rays to extend outward.

The expression of several genes is required for the formation of the rays. *mab-21* is necessary for maintaining cell shape in ray 6. In wild-type males, ray 6 is thicker and more conical than other rays, whereas in *mab-21* mutants, ray 6 takes on the morphology of other rays, and also, exhibit an ectopic tenth papillae between rays 5 and 7. The Smad genes *sma-2, sma-3* and *smad-4* are necessary for preventing rays 5 and 7 from taking on ray 6 morphology. Similarly, mutants for *daf-4, mab-20, mab-21, mab-26, sma-2, sma-3*, and *sma-4* can have rays that are fused together, caused by displacement of papillae prior to retraction.[66–71] The *ram* genes *ram-1, ram-2* and *ram-4*, affect collagen within the male tail, as mutants of these genes exhibit rays with an expanded, lumpy shape.[65–72] Other genes that affect ray shape include *dpy-11*, which encodes the thioredoxin-like protein, and *dpy-18*, which encodes a collagen hydroxylase.[73–75] In addition, *mab-7* and *ram-5* transcription gene expression mediates communication between the ray cells and the hypodermis.[65–76]

A variety of molecular inputs have also been characterized for male tail retraction. The expression of *tlp-1*, which encodes a C2H2 Zn-finger presumptive transcription factor, promotes hyp8–11 anterior retraction.[77] The *doublesex*-related DM gene *dmd-3* is necessary to trigger retraction during the L4 stage.[78] When defects in retraction are present, ray formation is often also affected. The RBCC (Ring finger-B box-Coiled coil) protein LIN-41 functions with LET-7 to regulate male tail retraction.[79] Reduction-of-function *lin-41* mutants begin retraction in the L3 stage, and either form disrupted fans or rays, or no fans or rays at all. The hox gene *egl-5* mediates the retraction of cells other than hyp8–11, and in *egl-5* mutants, rays and the fan do not form.[80] The gene *let-765*, encoding the strawberry notch 1 receptor is also necessary for retraction and ray formation as *let-765* mutants with reduced function do not form rays or fans.[81] RME-8, which is necessary for receptor-mediated endocytosis, also plays a role in both retraction and ray and fan morphogenesis. RNAi against *rme-8* causes defects in the disruption of these behaviors.[82] Furthermore, Nelson et al. identified 25 other genes that are involved in male tail retraction.[82] These genes encode proteins involved in cellular transport (*abcx-1, arl-1, wht-5*),

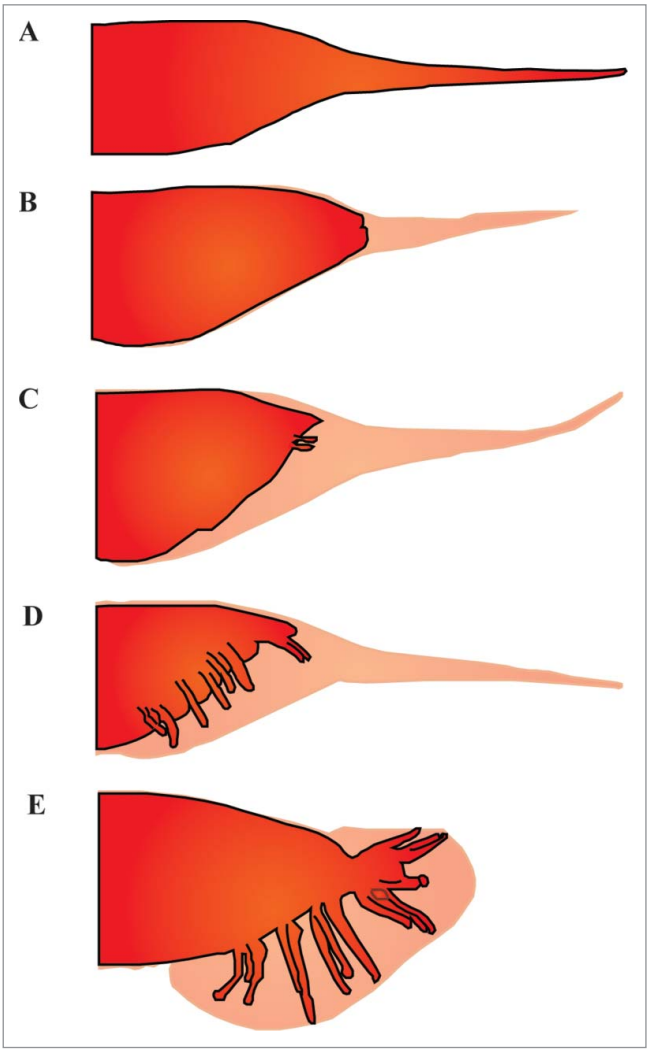

Figure 5. Male tail cell shape change and outgrowth. Schematic of male tail retraction and ray formation from L3 to adulthood. Adapted from Nguyen et al., 1999. (A) Male tail at L3 stage, retraction and ray formation have not occurred and entire cell is composed of tail epithelium. (B) Beginning of tail retraction in L4. Light red indicates fluid-filled extracellular space previously inhabited by tail epithelium. (C) L4 stage. Continuation of retraction in male tail, start of ray formation. (D) L4 stage, male tail has finished retracting and rays have all formed. (E) Adult male tail. Rays have reached their final shape. Fluid-filled extracellular space has taken peloderan shape.

cellular organization (*inx-12, inx-13, nmy-2, ptl-1*), nuclear transport (*expo-2, nnp-3, nnp-6*), signal transduction (*bub-1, cdc-42, nhr-25, pkl-1, rcn-1*) and general gene expression and regulation (*blmp-1, cdt-1, egl-18, mix-1, nob-1, php-3, pri-2, ran-3, rpa-1, smc-4*).

Excretory cell

The excretory cell is the largest mono-nuclear cell in *C. elegans*.[83] As this review focuses on cell outgrowth, the lumen formation of the excretory cell will not be discussed. The excretory cell originates from the AB blastomere within the embryo, specifically AB plpappaap.[11] During the three-fold embryonic stage, this cell grows outward dorsolaterally toward the lateral midline (Fig. 6A–C).[83,84] The proximal and distal edges of the cell, known as canals, initially grow outward dorsally (Fig. 5C), then branch out and extend anteriorly and posteriorly (Fig. 6D). By the time the worm hatches, the posterior canal has extended outward measuring half the length of the organism. Extension is completed within the L1 stage 12–14 hours after hatch, when the canal spans the entire worm body from the anterior tip to the tip of the tail (Fig. 6E).[85] The canal connects to the hypodermis through gap junctions, and once the canal extension is completed, the canal grows as the body of the worm grows, expanding from approximately 300 μm at the L1 stage to 1 mm as an adult size.[84]

The tail hypodermis acts in mediating excretory cell outgrowth.[83,86] Mutations in genes that control tail hypodermis integrity, *lin-17* and *bli-6*, result in exaggerated posterior canal growth.[87] Several basement membrane proteins are also necessary for proper excretory canal outgrowth. These include both α integrins INA-1 and PAT-2 and their corresponding β integrin PAT-3; perlecan UNC-52, and laminins EPI-1 and LAM-1.[88–92] In *pat-3* mutants, canals grow approximately 30% slower than in wild type animals during the L1 stage and do not reach the ends of the animal. Canals continue to grow with the rest of the animal throughout the later larval stages, but they do not extend further along the hypodermis, indicating that separate mechanisms control initial canal outgrowth, and later, passive canal growth as the worm develops. The seam cells may also affect excretory cell outgrowth, since knockdown of *cdh-3*, which is expressed in the seam, leads to outgrowth defects.[92]

Other genes that are involved in promoting excretory cell outgrowth are *mig-10, mig-15, unc-34, unc-53, unc-71, unc-73, unc-104, unc-116*, and *vab-8*.[12,14,17,93–100] The kinesin motor proteins UNC-104 and VAB-8 act with ROBO signaling proteins, SAX-3, SLT-1, and EVA-1, in the excretory cell to mediate cell migration and transport.[95] Another gene that acts in multiple pathways in excretory cell outgrowth is *abi-1*.[84] ABI-1 acts with both MIG-10 and UNC-53 to promote outgrowth of the excretory cell by inducing branched actin accumulation through activation of

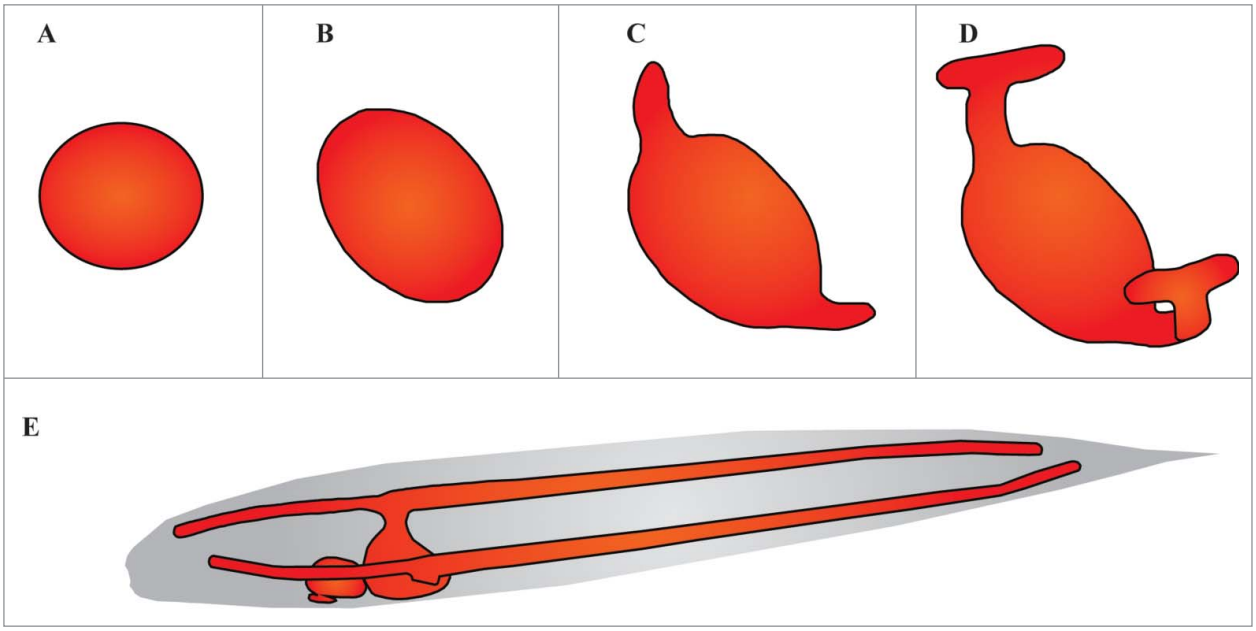

Figure 6. Excretory cell outgrowth. Schematic of excretory cell outgrowth. Excretory cell in red, outline of worm in black. Adapted from Buechner et al., 2002. (A-D) Excretory cell outgrowth during three-fold embryonic stage. (A) Excretory cell at birth, cell has a spherical shape. (B) Cell takes on an ellipsoidal shape as it begins to grow laterally from left to right over the ventral muscle quadrants. (C) Apical and basal edges of the excretory cell begin migrating dorsally. (D) Edges of dorsal protrusions bifurcate and begin to grow outward laterally along the anterior posterior axis. (E) Completed excretory cell outgrowth (L1). Cell has extended laterally along the anterior-posterior axis to span the entire length of the worm.

the Arp2/3 complex.[84,99] Lastly, expression of *unc-5* and *unc-6* provides netrin signaling that mediates dorsal guidance cues for the canals.[51,101,102] Many of the genes discussed, as well as *lin-17* and *bli-6*, also affect guidance of neurons, indicating that they could be serving as diffusible cues from the basal surface to affect outgrowth of multiple tissues.[83]

Head mesodermal cells

Head mesodermal cells are branched cells that lie dorsal to the terminal bulb of the pharynx (Fig. 7).[11,103] These cells, which originate from hmcR and hmcL (head mesodermal cell right and head mesodermal cell left), migrate circumferentially to the dorsal midline.[103] Once they reach the dorsal midline, the hmcR undergoes programmed cell death and the hmcL extends processes anteriorly and posteriorly along the dorsal and ventral margins of the body wall.[103] These two branches split at the pharynx and grow adjacent to the terminal bulb of the pharynx. The ventral process grows along the anterior loop of the right excretory gland and adjacent to the ventral hypodermal ridge. This process also runs adjacent to the body wall muscle and makes gap junctions with the body wall muscle.[104] The dorsal process grows adjacent to the dorsal hypodermal ridge and also makes gap junctions with dorsal muscle arms.

UNC-39, the homolog of human myotonic dystrophy-associated homeodomain protein SIX5, is involved in regulating the outgrowth of the processes formed by head mesodermal cells.[105] When *unc-39* is knocked down, ectopic processes form around the nerve ring, and posteriorly directed processes become shorter. In addition, netrin and its receptors (*unc-5, unc-6*, and *unc-40*) affect hmcL cell body positioning but not arm projections.[51]

Outgrowth processes during embryonic development

The epidermal cells of *C. elegans* are generated during the 9th round of embryonic cell divisions, at which point the embryo comprises 365 cells.[106–108] Epidermal cells originate from four lineages, ABarp, ABpla, ABpra, and C. These cells undergo several rounds of division, with the majority of epidermal cells localizing to the dorsal region of the embryo. Once terminal divisions are complete, three groups of major

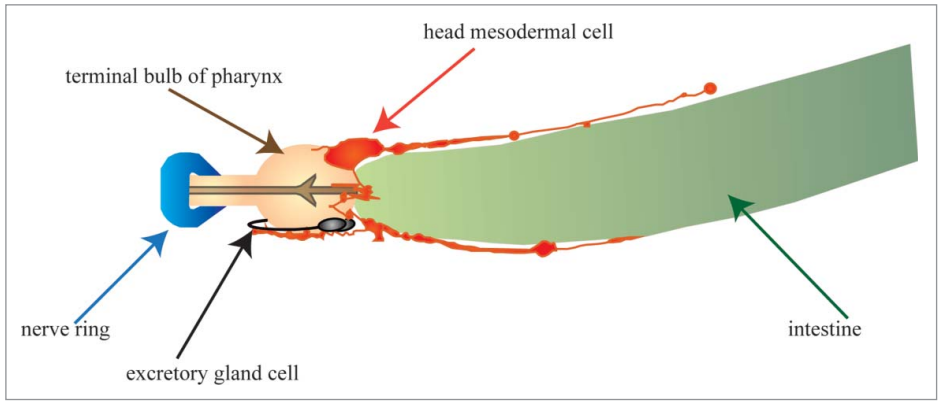

Figure 7. Head mesodermal cell. Schematic of head mesodermal cell. Positioning of head mesodermal cell in adult worm. Head mesodermal cell lies dorsomedial to the terminal bulb of the pharynx. It extends processes that split at the pharynx and extend anteriorly and posteriorly along the dorsal and ventral margins of the body wall. These processes also lie adjacent to the intestine as well as the excretory gland cell. Adapted from Altun and Hall, 2009.

epidermal cells are generated: the dorsal epidermal cells, seam epidermal cells, and ventral epidermal cells. The dorsal and ventral epidermal cells take on a sheet-like shape and undergo morphogenetic movements to encase the remaining cells of the embryo. Before the embryo can undergo elongation, two morphogenetic movements must occur, dorsal intercalation and ventral enclosure.[109] Both dorsal intercalation and ventral enclosure require changes in cell shape and outgrowth, and we discuss them here.

Dorsal intercalation

Dorsal intercalation is the process by which dorsal epidermal cells form a single row across the dorsal midline.[11,109,110] During this process, dorsal epidermal cells arrange themselves into six rows and change their morphology from a round shape to a wedge shape (Fig. 8A).[109] These cells then begin intercalating, with the anterior cells interdigitating first, followed by the remaining cells along the anterior-posterior axis (Fig. 8A–B). The interdigitating cells intercalate by forming basolateral protrusions (Fig. 8B), which touch neighboring cells and help them move towards one another.[106,109–112]

Molecular inputs controlling the change in cell morphology, from round to wedge shape, have been characterized. The T-box transcription factors TBX-8 and TBX-9 are inputs for this process, as animals with RNAi knockdown of *tbx-8* and *tbx-9* do not form wedge shaped cells and intercalation arrests prematurely.[113] Another gene that affects cell shape of dorsal epidermal cells is *sax-3*, which encodes a ROBO

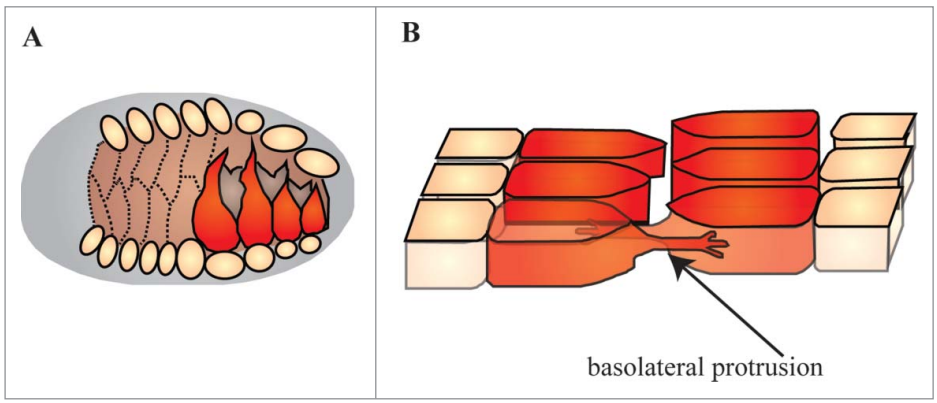

Figure 8. Dorsal intercalation. Schematic of dorsal intercalation. Adapted from Chisholm and Hardin, 2005. (A) Dorsal view of embryo undergoing intercalation. Intercalating epidermal cells shown in red. Dashed lines indicate cells that have already intercalated. Cells that are intercalating are changing from a rounded shape to a more wedge/protrusion-like shape. (B) Expanded view of intercalating cells. Basolateral protrusions that also touch neighboring cells, and help the intercalating cells move towards one another.

receptor protein that functions cell autonomously within the dorsal epidermal cells.[114]

Additional genes that promote dorsal intercalation include genes that encode cytoskeleton structure proteins (arx-2, gex-1/*wve-1*), GTPases (*gex-2, gex-3, ced-10*), and signaling proteins (*apr-1, frk-1, mig-5, rib-1*, and *ten-1*).[115–123] Though all of these genes are necessary for induction of dorsal intercalation, none have been characterized to promote protrusion formation. Dorsal intercalation and protrusion formation may be uncoupled behaviors, for in *die-1* mutants protrusions form normally, but intercalation does not occur.[112] Therefore, it may be useful to further analyze genes that have been implicated in dorsal intercalation for roles in dorsal protrusion formation.

Ventral enclosure

During ventral enclosure, ventral epidermal cells move towards the ventral midline to encase underlying cells in an epithelial sheath.[106] Specifically, the epidermal sheet migrates laterally and ventrally to encase the embryo (Fig. 9A–B).[124] This process commences when two anterior leading cells, known as ventral marginal cells (Fig. 9A), extend large protrusions towards the ventral midline and form epithelial junctions.[106,124] Once these cells reach the midline, the remaining cells, known as ventral pocket cells (Fig. 9A), move towards the midline by extending protrusions and encase the embryo. The presence of ventral marginal cells is necessary to mediate the rest of the ventral enclosure, for if the marginal cells are ablated, ventral enclosure cannot occur.[124] Marginal and pocket cell protrusions consist of actin filaments at the apical domain, which constrict as the ventral pocket closes.

The formation of marginal and pocket cell protrusions is modulated by several molecular cues. The WAVE complex (GEX-2, GEX-3, WSP-1), WASP (WIP-1), and Ena/VASP (UNC-34) activate the Arp2/3 complex (ARX-1, ARX-2, ARX-3, ARX-4, ARX-5, ARX-6. ARX-7), which enables actin polymerization at the leading edge of ventral marginal cells.[116,119,125] Knock down of components of each of these complexes results in defects in ventral enclosure. The 1,4,5-inositol trisphosphate (IP_3) receptor ITR-1 is also necessary for generating filopodia and organizing actin at the leading edge of marginal cells.[126] The cadherin-catenin complex (CCC), consisting of cadherin (HMR-1), α-catenin (HMP-1) and β-catenin (HMP-2), is also necessary for enabling protrusions to make adhesive contacts with the ventral midline.[127–129]

Other proteins that affect protrusion formation include the receptor tyrosine kinase VAB-1 and its ligands VAB-2, EFN-2 and EFN-3, which properly direct protrusions in both marginal and pocket cells.[130–133] The semaphorin MAB-20 is necessary for preventing the formation of ectopic protrusions in the ventral pocket cells.[134,135] The Plexin PLX-2 binds with MAB-20, but acts redundantly with MAB-20 and VAB-1 to generate protrusions in the pocket cells.[133,136] DPY-18, the catalytic subunit of collagen prolyl 4-hydroxylase, acts with TEN-1 (tenurin) to prevent ectopic protrusion formation *in dpy-18:ten-1* double mutant animals, in which ventral protrusions were present after ventral enclosure had completed.[137] Dishevelled (DSH-2) is necessary for mediating the length of the protrusions formed by marginal cells, for *dsh-2* mutants have marginal cells with longer

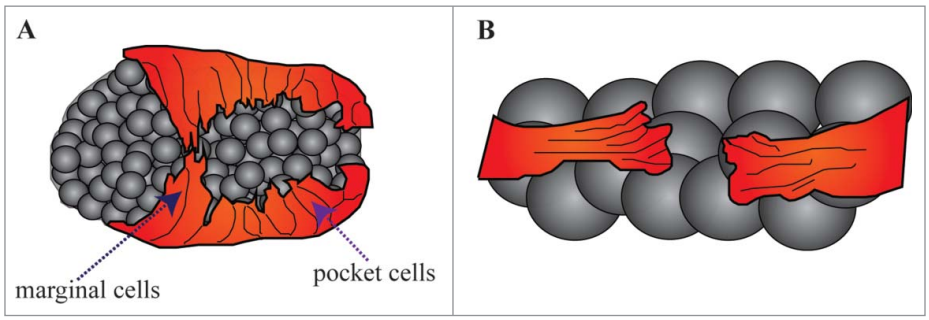

Figure 9. Ventral Enclosure. Schematic of ventral enclosure. Adapted from Chisholm and Hardin, 2005. Ventral epidermal cells shown in red, neuroblasts shown in gray spheres. (A) Outgrowth of epidermal cells during ventral enclosure. Ventral marginal cells are indicated with blue arrow and pocket cells are indicated with green arrow. (B) Expanded view of ventral epidermal cells moving along the neuroblast substratum.

protrusions than normal.[138] Likewise, *dsh-2* mutant marginal cell protrusive activity also lags behind pocket cell protrusion formation, which prevents ventral enclosure from occurring.

Genes that affect ventral enclosure but not protrusion formation have also been characterized. The expression of these genes affect migration of epidermal cells and include the APC-related gene *apr-1*, as well as *rib-1* and *rib-2*, which are involved in heparan sulfate biosynthesis.[115,139] Expression of *kal-1* (kallikrein) with *efn-4* (ephrin-B2) mediate ventral epidermal cell migration.[140] The expression of *wip-1* activates WASP and affects ventral epidermal cell migration, but since WSP-1 affects protrusion formation, WIP-1 may also act in the protrusion formation pathway.[141] The Fer-related kinase-1 FRK-1 also affects enclosure.[121] Actin distribution is normal in mutants of the polymerase-associated factor 1 complex (PAF1C) member *ctr-9*; however, defects in closure are present.[142] The anilin ANI-1 is necessary for aligning cells as they come together during enclosure.[143] Lastly, the GTPase Arl2 homolog EVL-20 is necessary for mediating integrity of the hypodermis during ventral enclosure.[144]

Analysis of pathways

In this review we have discussed 205 genes involved in nine different examples of cell outgrowth (Table 1). To determine which genes might act as key regulators of non-neuronal cell outgrowth in *C. elegans*, we identified genes that control cell outgrowth in multiple tissues, as well as examined those genes affecting multiple different tissues, and found that 44 genes were involved in affecting outgrowth in more than one tissue (Table 1). This analysis demonstrated that two genes, *gex-2* and *unc-6*, controlled outgrowth in four different tissues. Interestingly, these two genes belong to families that are also involved in neuronal cell outgrowth.

GEX-2 is a member of the WAVE/SCAR complex necessary for actin initiation, and many components of the WAVE/SCAR complex are involved in multiple tissues characterized in this work (as described below). Expression of *gex-1/wve-1* is involved in the utse, dorsal intercalation and muscle arms.[26,53,116] Expression of *gex-3* is involved in dorsal intercalation, ventral enclosure, and muscle arm extension.[49,116,117] Expression of netrin (*unc-6*) is well-characterized to provide signaling guidance cues. The netrin receptor *unc-40* is involved in regulating outgrowth in the anchor cell, ventral enclosure, muscle arm extension, and head mesodermal cell positioning.[40,53,58,59,99] Taken together we believe that these two master cell outgrowth regulators function in multiple tissues and are differentially activated through interaction with other pathways.

Based on all available information, ten genes are shared by at least three cell types or tissues. These genes encoded proteins involved in cytoskeletal dynamics (ARX-2, PAT-3), signal transduction (EGL-15, GEX-1/WVE-1, UNC-40, UNC-53, UNC-73), and adhesion (CDH-3, INA-1, MIG-10). Interestingly, of these genes, *cdh-3, ina-1, pat-3*, and *mig-10*, were all commonly expressed in the utse, AC, and excretory cell, suggesting that connections to the cytoskeleton and ECM networks are vital for outgrowth.

Behaviors undertaken by outgrowing cells

The core components *gex-2* and *unc-6* affect different aspects of cell behavior. *gex-2* is involved in branched actin initiation and modulating cell outgrowth in systems that generate actin-like protrusions during outgrowth,[116] while UNC-6 serves as a guidance cue.[51,96] In addition, attachment and connections to the surrounding cytoskeletal and ECM structures were equally important for outgrowth processes, therefore we wanted to see if these broader mechanisms are used for cell outgrowth by other genes in the nine tissues we described (Table 2). Specifically, we determined if each of these tissues had protrusions that contained actin/branched actin, used guidance cues or made direct contact with their environment, and modulated levels of basement membrane proteins and adhesion complexes. Of the nine examples of non-neuronal cell outgrowth, we observed that aside from early elongation and the vulval sex muscles (whose protrusive mechanisms have not been well characterized), each cell type uses two or more of these mechanisms for outgrowth. Strikingly, the excretory cell uses all five of these mechanisms as determined from our initial core component gene set.

Our meta-analysis indicates that non-neuronal cells in *C. elegans* rely on more than one mechanism for cell outgrowth. We show that not only is outgrowth not confined to one pathway or family of genes, but

Table 1. Genes involved in cell outhgrowth.

	utse	anchor cell	vulval sex muscles	muscle arms	male tale	exretory cell	head mesodermal cells	dorsal intercalation	ventral enclosure
abcx-1					X				
abi-1						X			
act-1				X					
act-2				X					
act-3				X					
aff-1	X	X							
amph-1	X								
anc-1	X								
ani-1									X
ap-1/							C42C1.11		
apr-1								X	X
arl-1					X				
arx-1									X
arx-2	X							X	X
arx-3 *	X								X
arx-4									X
arx-5									X
arx-6									X
arx-7									X
bli-6						X			
blmp-1					X				
bub-1					X				
cdc-42		X			X				
cdh-3 *	X	X				X			
cdt-1					X				
ced-10								X	
clr-1				X					
ctr-9									X
cwn-1	X								
daf-4					X				
daf-16	X								
deb-1	X								
dh11.5	X								
die-1								X	
dmd-3					X				
dpy-11					X				
dpy-18					X				X
dsh-2									X
efn-2									X
efn-3									X
efn-4									X
egl-5					X				
egl-13	X								
egl-15 *	X		X	X					

	utse	anchor cell	vulval sex muscles	muscle arms	male tale	exretory cell	head mesodermal cells	dorsal intercalation	ventral enclosure
fos-1	X	X							
frk-1								X	X
frm-2	X								
gex-1 *	X			X				X	
gex-2 **	X			X				X	X
gex-3								X	X
gipc-1	X								
gipc-2	X								
glb-12	X								
							glp-1	X	
him-4	X	X							
hlh-2		X							
hmp-1									X
hmp-2									X
hmr-1									X
ima-1	X								
ima-2	X								
ima-3	X								
imb-2	X								
imb-3	X								
ina-1 *	X	X				X			
inx-12					X				
inx-13					X				
itr-1	X								X
kal-1									X
lag-2	X	X							
lam-1				X		X			
lam-2				X					
let-7					X				
let-60				X					
let-502	X								
let-756				X					
let-765					X				
let-805									
lev-11				X					
lim-9	X								
lin-3		X							
lin-11	X								
lin-12	X			X					
lin-17						X			
lin-31	X								
lin-39	X								
lin-41					X				
lmn-1	X								

(*Continued on next page*)

Table 1. (*Continued*)

	utse	anchor cell	vulval sex muscles	muscle arms	male tale	exretory cell	head mesodermal cells	dorsal intercalation	ventral enclosure
egl-17				X					
egl-18					X				
egl-43	X	X							
epi-1				X		X			
eva-1				X		X			
evl-20									X
F11A10.5	X								
fbl-1	X								

	utse	anchor cell	vulval sex muscles	muscle arms	male tale	exretory cell	head mesodermal cells	dorsal intercalation	ventral enclosure
mab-7					X				
mab-20					X				X
mab-21					X				
mab-26					X				
madd-2	X			X					
madd-4				X					
mig-5								X	
mig-10*	X	X				X			

	utse	anchor cell	vulval sex muscles	muscle arms	male tale	exretory cell	head mesodermal cells	dorsal intercalation	ventral enclosure
mix-1				X					
ncam-1	X								
nhr-25				X					
nmy-2				X					
nob-1				X					
npp-3				X					
npp-6				X					
nud-2	X								
pat-2				X		X			
pat-3*	X	X				X			
pat-4				X					
pat-6				X					
php-3					X				
plk-1					X				
plx-2									X
pqn-85	X								
pri-2					X				
ptl-1					X				
ptp-2				X					
pxl-1	X								
rab-1	X								
rab-5	X								

	utse	anchor cell	vulval sex muscles	muscle arms	male tale	exretory cell	head mesodermal cells	dorsal intercalation	ventral enclosure
sos-1				X					
srsx-18	X								
tbx-8								X	
tbx-9								X	
ten-1								X	X
tlp-1					X				
toca-1	X								
ttx-3	X								
unc-5						X	X		
unc-6**		X		X		X	X		
unc-13	X								
unc-33	X			X					
unc-34						X			X
unc-39							X		
unc-40*		X		X			X		
unc-51				X					
unc-52				X		X			
unc-53*	X		X			X			
unc-54				X					
unc-60				X					
unc-64	X								
unc-70	X								

rab-6.1	X					
rab-10	X					
rab-11.1	X					
ram-1			X			
ram-2			X			
ram-4			X			
ram-5			X			
ran-2	X					
ran-3	X		X			
rcn-1			X			
rho-1	X					
rib-1					X	X
rib-2						X
rme-8			X			
rpa-1			X			
rrc-1	X					
rsef-1	X					
sax-1	X					
sax-3				X	X	
sax-7	X					
sec-15	X					
sem-5		X				
slt-1				X		
sma-2			X			
sma-3			X			
sma-4			X			
smc-4			X			
soc-1		X				

unc-71					X		
unc-73*	X		X		X		
unc-82							
unc-83	X						
unc-84	X						
unc-93			X				
unc-94							
unc-95			X				
unc-97	X		X				
unc-98			X				
unc-104			X		X		
unc-116					X		
vab-1							X
vab-2							X
vab-3	X						
vab-8					X		
vrk-1		X					
wip-1							X
wrt-5				X			
wsp-1			X				X
wve-1*	X		X			X	
xpo-2				X			
zag-1	X						
zfh-1	X						
zfp-1	X						
zif-1	X	X					
zmp-1		X					

**Gene expressed in four cells or tissues.
* Gene expressed in three cells or tissues.
**Gene expressed in four cells or tissues. * Gene expressed in three cells or tissues.

Table 2. Characteristics used by different outgrowth systems.

	Actin filament branching	Actin filament generation	Requirement of guidance cues	Involvement or adhesion to the extracellular membrane	Contacts to surrounding tissues
utse	X		X	X	X
anchor cell		X	X	X	X
vulval sex muscles			X		
muscle arms	X	X	X	X	X
male tail			X	X	
excretory cell	X	X	X	X	X
head mesodermal cells			X		X
dorsal intercalation	X		X		X
ventral enclosure	X	X	X		X

requires multiple players. When cells form a protrusion, they must guide the protrusion in the proper direction, while maintaining adhesive contacts with the extracellular matrix.

This stepwise set of behaviors is also characteristic of cell outgrowth in other organisms. For instance, branch formation in mammary tissues requires regulation of actin polymerization, manipulation of levels of ECM and adhesion molecules, and contact with surrounding cells and guidance cues.[145–149] Mouse mammary gland branch formation occurs during adolescence and involves a two-step process.[147] The duct cells first elongate through the fat pad (fatty tissue in the mammary gland) to form a primary duct, then branches form from the initial primary ducts throughout the mammary tissue through terminal end bud bifurcation and lateral side branching. The mammary duct cells are composed of many layers (from internal to external), which are two layers of epithelial cells, a sheath of basement membrane, an outer ring of fibrous interstitial ECM, and lastly, a layer of loose connective tissue. During branch formation, considerable change is observed in the composition of the basement membrane and the interstitial ECM, indicating that the proteins in these layers play roles in cell outgrowth. For instance, $\alpha 2$ integrin negatively controls branching, as $\alpha 2$ integrin knockouts exhibit diminished branching.[148] Other adhesion complexes required include P-cadherin, which is expressed in the monolayer of epithelial cap cells at the end buds and is necessary for the maintenance of mammary tissue integrity.[149] Mammary branch morphogenesis also requires proper arrangement of the actin cytoskeleton for disruption of genes that regulate actin dynamics and polymerization, such as gelsolin and ROR-2 which prevent branch formation.[145,146] As these ducts are branching out they make contacts with surrounding adipose tissues.[147] Hormonal guidance cues are also necessary for inducing branch development in adolescence.[150] In order for branching morphogenesis to take place estrogen hormone (GH), estrogen receptor α (ERα), progesterone, and its receptor (PR) need to be present.[151,152] The neural guidance cue netrin-1 is also necessary for mammary branch formation, for it maintains connections between the preluminal cells to the cap cells in the terminal end buds of the mammary branches.[153,154] Therefore, the four cell outgrowth behaviors we have identified due to the presence of common genes between *C. elegans* outgrowth systems, are also behaviors used by outgrowth in other organisms.

Conclusions

We have discussed both the process and mechanisms used during outgrowth by nine non-neuronal cell types in *C. elegans*. We discussed the lineages from which these tissues emerge, and characterized in detail how these non-migratory tissues and cells grow outward as they change their shape during morphogenesis, and also described the genes and proteins that are necessary for mediating this outgrowth (Table S1). While we have shown that several gene families/types regulate outgrowth in multiple tissues, such as WAVE/SCAR proteins, integrins, and netrin signaling proteins, we must remember that expression levels of these genes will vary amongst each cell type and tissue. Equally, although we have shown that the behaviors regulated by *C. elegans* core components are hallmarks of cell behavior in other tissues, the importance of each gene's function can easily be impacted by the expression or inhibition of others, and therefore we must be cautious when determining the exact role each gene plays within each cell or tissue.

We hope that this work can be used as a resource not only for better understanding cell outgrowth, but

also for presenting a broad set of models that can be used for studying cell outgrowth. In our previous work studying the mechanisms involved in utse development, we were able to glean more information about utse outgrowth by testing genes involved in outgrowth in other tissues. Cell outgrowth is a process that is present in a plethora of developing systems, and studying multiple cell outgrowth systems can shed light on the specific ways cells change their shape.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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