Developmental genetics of the *Caenorhabditis elegans* pharynx

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The *Caenorhabditis elegans* pharynx is a rhythmically pumping organ composed initially of 80 cells that, through fusions, amount to 62 cells in the adult worm. During the first 100 min of development, most future pharyngeal cells are born and gather into a double-plate primordium surrounded by a basal lamina. All pharyngeal cells express the transcription factor PHA-4, of which the concentration increases throughout development, triggering a sequential activation of genes with promoters responding differentially to PHA-4 protein levels. The oblong-shaped pharyngeal primordium becomes polarized, many cells taking on wedge shapes with their narrow ends toward the center, hence forming an epithelial cyst. The primordium then elongates, and reorientations of the cells at the anterior and posterior ends form the mouth and pharyngeal-intestinal openings, respectively. The 20 pharyngeal neurons establish complex but reproducible trajectories using 'fishing line' and growth cone-driven mechanisms, and the gland cells also similarly develop their processes. The genetics behind many fate decisions and morphogenetic processes are being elucidated, and reveal the pharynx to be a fruitful model for developmental biologists. © 2014 The Authors. *WIREs Developmental Biology* published by Wiley Periodicals, Inc.

INTRODUCTION

The *Caenorhabditis elegans* pharynx, or foregut, is a feeding organ: rhythmic contractions of this innervated muscular tube fill and empty its triangular lumen while retaining food particles that are crushed at its posterior end then passed to the intestine. The anatomy of the pharynx has been painstakingly described at the ultrastructural level by Albertson and Thomson,[1] its complete cell lineage was established in Sulston's classic studies,[2] and the electromechanical properties of its feeding motions have been studied in great detail by Avery and coworkers.[3,4] The isolation of informative pharyngeal-defective mutants has led to the elucidation of many genetic pathways that control

pharyngeal development, the most remarkable being the forkhead transcription factor PHA-4 that Gaudet and Mango have found regulates most and perhaps even all pharyngeal genes throughout development.[5] Several excellent and comprehensive reviews of pharyngeal function and developmental genetics have been written,[6–9] and the aim here is not to repeat what has already been thoroughly explained. Rather, I will provide an overview of pharyngeal function and development, then focus on aspects that have not previously been as thoroughly reviewed, namely the developmental genetics of the pharyngeal axons and gland cells, and post-developmental events such as growth and aging.

PHARYNX ANATOMY AND FUNCTION

WormAtlas is the definitive source of anatomical information.[10] The main features of the pharynx are,

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from anterior to posterior, the procorpus, the metacorpus, the isthmus, and the posterior bulb in which the grinder is located (Figure 1(a)). The mature pharynx is composed of 62 cells containing 80 nuclei; many cells are multinucleate as a result of cell fusions. There are five types of pharyngeal cells: neurons (20), muscles (20 cells; 37 nuclei), marginal cells (9), epithelial cells (9), and gland cells (4 cells; 5 nuclei). The muscle cells and marginal cells form a single-cell-thick tube with trifold symmetry (two bilateral symmetrical left and right subventral sides, and one dorsal side), continuous at its anterior end with the hypodermis that encloses the worm (Figure 1(b)). These cells are joined by tight junctions that divide the membrane into apical and basal surfaces, with the apical surfaces facing a lumen that is triangular when open, i.e., when the muscle cells contract along the triangle's sides, with the marginal cells located at the vertices. The pharyngeal lumen is lined with a cuticle that connects with that of the epidermis and includes specialized structures that serve as sieves and, in the posterior bulb, as a grinder; chitosan, a deacetylated form of chitin, may be a component of the pharyngeal cuticle.[12] The basal surfaces of the marginal and muscle cells face a basal lamina continuous with that covering the hypodermis and intestine. Components of this basal lamina are likely produced by body-wall muscles.[13] The nine epithelial cells form a narrow ring at the anterior end of the pharynx that connects with the mouth opening. There is otherwise no epithelial sheet covering the pharynx. The gland cell bodies are embedded within muscle cell membrane folds in the posterior bulb and extend processes that connect anteriorly to the lumen and secrete vesicles just before hatching, at each larval molt and during feeding; secretions include mucins that may provide a lubricated lining of the digestive tract.[14] Pharyngeal neurons also lie deep within folds of the basal membrane of pharyngeal muscle cells (the part of the muscle cell membranes that is on the 'basal' side and is not to be confused with the basal lamina itself).

Normal feeding consists of two primary motions: pumping and isthmus peristalsis.[3] A pump is a near-simultaneous contraction of the muscles of the corpus, anterior isthmus, and terminal bulb, followed by a near-simultaneous relaxation. Muscle contraction pulls the lumen open from its resting closed Y-shape. The second motion, isthmus peristalsis, typically occurs every fourth pump and after the main relaxation is complete: it is a peristaltic wave of contraction in the posterior isthmus that carries bacteria trapped in the anterior isthmus back to the grinder. The nervous system is not essential for pumping, which continues even when the entire pharyngeal
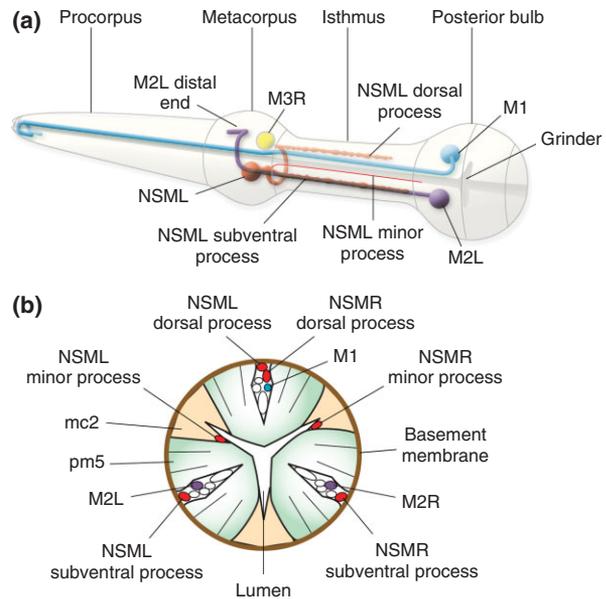

FIGURE 1 | Pharyngeal anatomy. The figure outlines the main features of the *Caenorhabditis elegans* pharynx of relevance for this review. (a) Outline of the pharynx in which the trajectories of the M1, M2L, and NSML neurons are drawn, together with M3R of which only the cell body is shown for clarity. Except for the unique M1, each of these cells has a similar contralateral cell with a mirror shape. The pale gray lines outline the boundaries between muscle cell layers: for example, the pm4 and pm5 muscle cells make up the bulk of the metacorpus and isthmus, respectively (two of each per sector). (b) A cross section in the isthmus, revealing the threefold symmetry with two sublateral sectors, and a dorsal sector, as well as the relative positions of the M1, M2, and NSM processes. Unlabelled ovals in the muscle folds of each sector represent other axons or gland cell ducts. The anatomy is from Albertson et al.[1] and Axäng et al.[86]

nervous system is killed.[15] However, many neurons are important: efficient pumping and trapping of bacteria by the pharynx requires the presence of the neurons I5, MC, M3, M4, and NSM.[15,16]

If one uses the criteria of *pha-4* positivity as a marker of pharyngeal cells, as suggested by Susan Mango,[7] then an additional 15 cells could be included as part of the pharynx: 9 arcade cells and 6 pharyngeal-intestinal valve cells. The arcade cells form two separate epithelial syncytia: the anterior arcade, made of three fused cells, and the posterior arcade, made of six fused cells.[17] Together, these arcades and the pharyngeal epithelium rings form the buccal cavity of the worm. At the posterior end of the pharynx, six equivalent cells form three rings of one, two and three cells that connect the posterior bulb to the first intestinal ring; this narrow passage for food macerate is the pharyngeal-intestinal valve. A short list of publications that together provide an introduction to the *C. elegans* pharynx is provided in Box 1.

A STARTER'S PUBLICATION LIST

A quite subjective selection of articles that ought to be recommended to any budding pharynx afficionado.

Anatomy: Albertson and Thomsom.[1] This article provides a description of pharynx anatomy at the ultrastructural level, including maps of neuronal connectivity.

Neuron ablation: Avery and Horvitz.[15] Shows that worms are still viable and fertile even when all pharyngeal neurons, except for M4, are ablated. This is good news for geneticists interested in isolating mutants with neuronal defects.

Mutant screen: Avery.[18] Describes the first and probably most successful screen for mutants with pharyngeal defects. The methodology is powerful and many mutants are even finely mapped, though some remain to be molecularly defined even to this day.

Electropharyngeograms: Avery et al.[19] Presents an elegant method to monitor the electrical activity of the pharynx using extracellular recordings, termed electropharyngeograms (EPGs).

The master gene *pha-4*: Gaudet and Mango.[5] A spectacular article identifying PHA-4 binding sequence motifs in hundreds of pharyngeal genes and showing that increasing levels of PHA-4 during development explains the timing of activation of most, and perhaps all, pharyngeal genes.

Food transport: Avery and Shtonda.[3] A study of the feeding mechanics in *C. elegans*, complete with an analysis of the effect of food particle sizes.

Neuronal development: Mörck et al.[20] Genetic analysis of axon guidance in the *C. elegans* pharynx demonstrating developmental robustness and the utilization of growth cone-dependent and -independent mechanisms.

Pharynx morphogenesis: Rasmussen et al.[21] Beautiful study explaining how the pharyngeal precursors organized themselves into two plates on either side of the midline to later form an epithelial tube by constriction along the apical surfaces in a laminin-dependent fashion.

PHARYNX DEVELOPMENT

The pharynx develops through the morphogenesis of a primordium composed of 80 undifferentiated cells,

as well as 19 apoptotic sisters of pharyngeal cells that die within 350–420 min of development.[2] Morphogenesis is accompanied by differentiation without cell division. The mature pharynx contains 80 nuclei but only 62 cells; this is due to cell fusion among muscle and gland cells that occur around the time of hatching and seem irrelevant to the developmental process.[2,22]

0–100 min: Early Cell Divisions and Establishment of Main Lineages

The *C. elegans* zygote, a cell called P_0, establishes its embryonic axes as a consequence of sperm entry that triggers cytoplasmic streaming, actin and microtubule-based processes, and differential segregation of maternally inherited cytoplasmic determinants. This produces, upon completion of the first division, two distinct cells, namely the posterior P_1 cell and the anterior AB cell (Figure 2(a)).[25,26] P_1 then produces EMS and P_2 while AB produces ABa and ABp. EMS then divides into MS and E. The ABa and MS cells will produce all future pharyngeal cells, as well as many other cells. Note that the cells AB, C, D, MS, E, and P_4 are called founder cells, or blastomeres, because they divide approximately equally and with characteristic periods.[2] Also, in *C. elegans*, descendents of blastomeres are named by adding the a, p, l, or r letter to the name of their mother cell depending on whether they were the anterior, posterior, left, or right daughter, respectively.[2] That all pharyngeal cells originate from ABa and MS is quite remarkable: these are two distinct lineages separated at the first division and inheriting completely different sets of maternal determinants. Strikingly, cells with these two very different ancestries may end up adopting nearly identical fates. For example, the muscle cell m4L has the ancestry MSaaapaap, whereas the contralateral identical cell m4R has the ancestry ABaraaapapp.

The spatial contributions of the ABa and MS lineages more or less reflect their initial positions within the 8-cell embryo. Specifically, ABa contributes cells of the anterior pharynx, whereas the more posterior MS cell contributes mostly posterior pharyngeal cells. This holds true for later descendents and narrower scopes of spatial contributions. Figure 3 shows the contributions of the pharyngeal precursors at the 100-cell stage embryo to the adult pharynx, and emphasizes the preservation of spatial relationships during development. Thus, ABalpa contributes mostly to the anterior left subventral area, etc.

100–250 min: Gastrulation

Gastrulation begins at approximately 100 min after first cleavage, at the 28-cell stage.[2] During
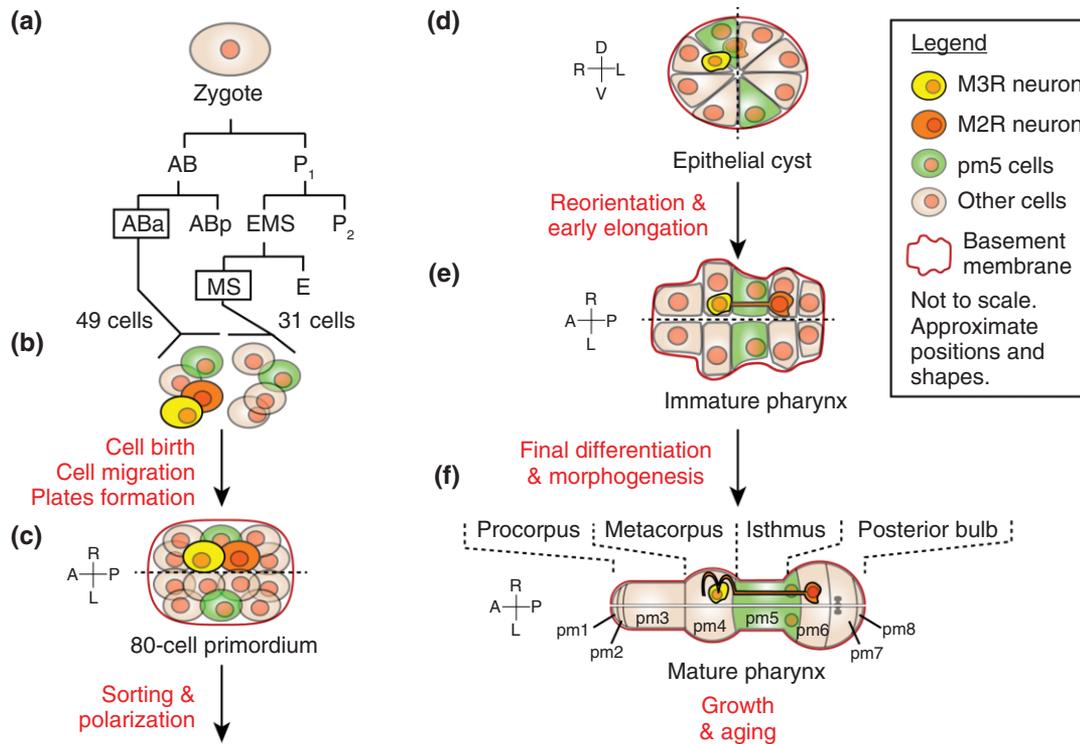
FIGURE 2 | Overview of pharyngeal development. All pharyngeal cells are descendants of the ABa and MS cells which are born after 2 and 3 cell divisions of the zygote, respectively (a). ABa will produce 49 pharyngeal cells while MS will produce 39. These cells are born and migrate during gastrulation (b) to form two plates of cells 6–8 cells deep along the dorsal–ventral axis (c). Many cells then constrict along the midline facing apical surfaces to create an elongated epithelial cyst depicted as a cross section in (d). Reorientation of the cells at the anterior and posterior ends of the primordium create openings toward the mouth and intestine, and retraction of the apical tips of marginal cells create a lumen that runs through the entire pharynx as it elongates; thin cellular processes, such as the axons of the M2 and other neurons, are drawn by cellular elongations such as that of the pm5 muscle cells in the isthmus (e). Pharyngeal cells then complete their differentiation and morphogenesis, including completing growth cone-driven axon trajectories, to produce a mature pharynx that will grow in size during larval development and of which the muscle cells will gradually become disorganized during aging (f). See text for details. The lineage is from Sulston et al.,[2] and the developmental processes shown are mostly from Portereiko and Mango,[23] Rasmussen et al.[21] and Rauthan et al.[24]

gastrulation, several cells enter deep into the embryo through a ventral cleft. The first cells to enter are the gut precursor cells Ea and Ep, followed by the P_4 and MS progeny at 120–200 min of development, and the AB-derived pharyngeal precursors that enter more anteriorly at 210–250 min. The ventral cleft closes from posterior (230 min) to anterior (290 min). As gastrulation proceeds, the E cell descendents and the pharyngeal precursors form a central cylinder. Note that cell divisions continue during gastrulation: pre-pharyngeal cell divisions continue until approximately 350 min of development, and some late divisions occur until approximately 400 min (Figure 2(b)).

250–400 min: Compaction of the Pharyngeal Primordium

Between 250 and 400 min the pharyngeal primordium becomes clearly defined. The nonpharyngeal precursor cells are somehow excluded from the pharyngeal

primordium. Perhaps the pharyngeal cells have more adhesive affinity to each other than to any other cell, which would in line with the theory of Malcolm Steinberg; e.g., see Duguay et al.[27] By 290 min, the pharyngeal precursor cells become organized into two bilaterally symmetrical 6–8 cell deep plates (Figure 2(c)).[21] These rectangular plates, of which the cellular polarity depends on laminin, will transform into a cyst by apical constriction: midplane-facing surfaces of pharyngeal precursor cells shrink, whereas the peripheral surfaces show relatively little change in size (Figure 2(d)).

400–430 min: Extension of Pharyngeal Primordium

The pharyngeal primordium is surrounded by a basement membrane that is visible at 400 min[2]; there is no evidence of basement membrane within the pharynx itself.[13] The presence of a surrounding basement membrane effectively means that the pharyngeal
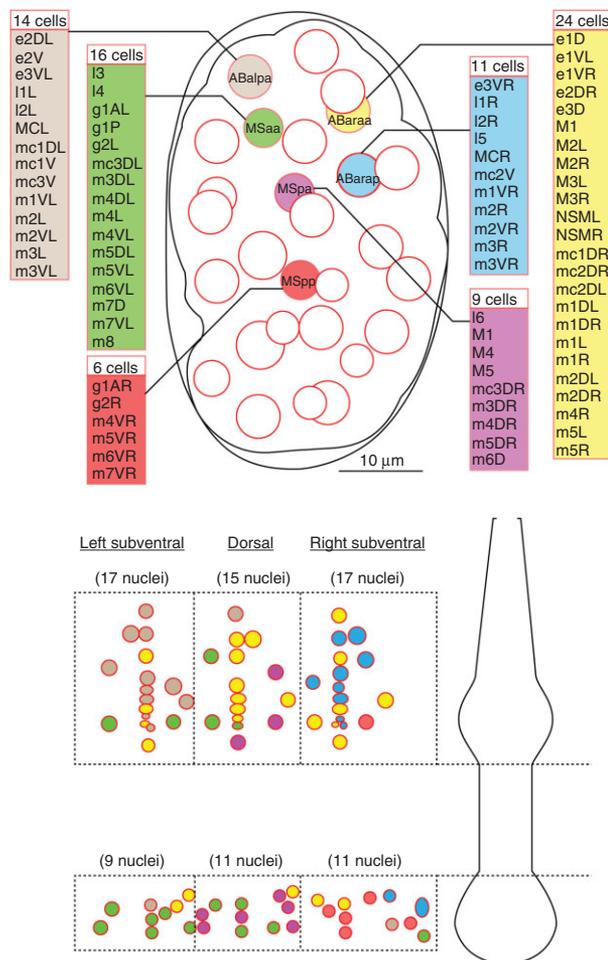
FIGURE 3 | Cellular origins and positions of pharyngeal cells. Color-coded fates of the pharyngeal cells in the 28-cell embryo (a) and location of their nuclei in the mature pharynx (b). Note the approximate preservation of spatial relationships in the mature pharynx, and absence of nuclei in the isthmus. The lineage is from Sulston et al.,[2] and the anatomy is from Albertson et al.[1]

primordium develops in isolation from the rest of the worm. Such autonomous development seems also true of the 20-cell intestine that, together with the pharynx, makes up the *C. elegans* digestive tract.[28] At approximately 400 min, the pharyngeal primordium is more or less oblong, and most of the cells are in spatial relationships that will be preserved in the mature pharynx, although their relative distances change greatly. For example, at approximately 430 min, the sister cells M2 and M3 are neighbors, whereas in the final pharynx M2 has its cell body in the posterior bulb and M3 in the metacorpus. Another example is the g1 gland cells that migrate into the posterior bulb, with their movements tracing the course of their secretory processes, which are probably laid down during the migration.[2]

At approximately 400 min, the primordium begins to elongate anteriorly then posteriorly, and

the adherens junctions that connect many pharyngeal cells with each other form. Portereiko and Mango have divided this period of pharyngeal morphogenesis into three stages (Figure 2(e)): (1) lengthening of the nascent pharyngeal lumen by reorientation of the apicobasal polarity of anterior pharyngeal cells ('Reorientation'); (2) formation of an epithelium by the buccal cavity cells, which mechanically couples the buccal cavity to the pharynx and anterior epidermis ('Epithelialization'); and (3) a coordinated movement of the pharynx anteriorly and the epidermis of the mouth posteriorly to bring the pharynx, buccal cavity, and mouth into close apposition ('Contraction').[23] The lumen is thought to form during the 400–430 min period by the retraction of the tips of marginal cells from the midline of the primordium when the pharyngeal cells begin to differentiate and contract.[28] Maintenance of the attachment between the pharynx and the arcade cells is dependent on the microRNA miR-51 that regulates the Fat cadherin ortholog CDH-23 in the arcade cells.[29]

430–800 min: Completion of Functional Pharynx

The pharyngeal bulbs and isthmus become apparent between 430 and 490 min, as the pharyngeal cells interpret their final differentiation programs and adopt their final shapes (Figure 2(f)). The pharyngeal cuticle is produced and the lumen becomes distinct between 600 and 650 min. The pharyngeal glands are active by 720 min and the pharynx is pumping by 750 min. Hatching occurs at approximately 800 min following first cleavage.

GENETICS OF PHARYNGEAL DEVELOPMENT

What follows is an overview of particularly illustrative genes for pharyngeal development. Many of the pharyngeal phenotypes discussed are also summarized in Box 2.

pha-4 Specifies Pharyngeal Cell Identity and Regulates Pharyngeal Genes

pha-4 encodes the *C. elegans* homolog of FoxA, a forkhead transcription factor.[37] Expression of PHA-4 is detected in all pharyngeal precursor cells beginning from at least 200 min of development (and perhaps even earlier). By the comma stage (~430 min), all the pharyngeal cells are present and continue to express PHA-4. The *pha-4* mutants completely lack all pharyngeal cells, even though the AB and MS lineages

PHARYNGEAL MUTANTS AND PHENOTYPES

eat **(EATing mutants).** These mutants have feeding defects due to abnormal pharyngeal pumping includes at least 20 members and was introduced by Leon Avery with the publication of a screen for feeding mutants.[18] Several of the *eat* mutants are molecularly defined and affect various aspects of pharyngeal function. For example, *eat-4* encodes vesicular glutamate transporter in glutamatergic pharyngeal neurons[30,31] and *eat-5* encodes a transmembrane protein important for electrical coupling of pharyngeal muscle cells.[32]

mnm **(M Neuron Morphology abnormal).** These mutants have abnormal morphologies of the pharyngeal motorneurons.[20] Currently this class has eight members, three of which are molecularly defined: *mnm-2* encodes a C2H2-type zinc finger protein important for the differentiation of the M2 and M3 neurons,[24] *mnm-4* corresponds to *mig-6* and encodes an extracellular matrix protein,[33] and *mnm-5* corresponds to *ten-1* which encodes the only *C. elegans* teneurin gene.[34]

pha **(defective PHArynx development).** These mutants have pharynges that fail to develop or develop abnormally.[18,35] There are four members in this class: *pha-1* mutants form a pharynx that is unattached fto the buccal opening, *pha-2* mutants fail to form a thin isthmus, *pha-3* mutants have an abnormal anteriorly narrow isthmus and *pha-4* mutants fail to form pharyngeal cells. *pha-3* is not molecularly defined; *pha-1, -2,* and *-4* are described in the main text.

phm **(PHaryngeal Muscle).** There are three *phm* mutants, characterized by abnormal pharyngeal muscle morphologies.[18] *phm-1* is now known as *unc-89* and is required for proper organization of A bands in the striated pharyngeal muscles.[36] *phm-2* and *phm-3* are not molecularly defined.

pun **(Pharynx UNattached).** These mutants have pharynges that are unattached or detached from the buccal cavity. Only one *pun* mutant exists, *pun-1*, and it is molecularly undefined. However, several other mutants display a Pun phenotype, including *lin-35, pha-1, ubc-18* (see main text on *pha-1*).

twp **(TWisted pharynx).** A phenotype where the pharynx is twisted along its anterior-posterior axis. This is often a result of ECM attachment or remodelling defects.[18,119]

are otherwise completely normal.[38] PHA-4 therefore acts as an organ identity factor. Consistently, ectopic expression of PHA-4 causes ectopic expression of *myo-2* (encodes a pharyngeal-specific muscle myosin), *ceh-22* (encodes a homeodomain protein that is also a coactivator of the *myo-2* gene), *pha-2* (encodes another homeodomain protein important for pharynx development, see below), and most likely other pharyngeal-specific genes.[37,39] Indeed, Gaudet and Mango have proposed that the PHA-4 protein may directly activate most or all pharyngeal genes, via the consensus binding site TRTTKRY (R = A/G, K = T/G, Y = T/C), and the expression timing being regulated by the presence of binding sites of varying affinity: poor binding sites will have delayed expression since they require higher levels of PHA-4.[5] Furthermore, specific sequence motifs of 8–11 base pairs in length have been identified that likely recruit other transcription factors that, in conjunction with PHA-4, regulate specific differentiation programs for pharyngeal muscle, marginal, and epithelial cells,[40] and yet other motifs work in combination with PHA-4 to achieve the temporal regulation of gene expression for pharyngeal genes.[41,42] PHA-4 also mediates dynamic chromatin organization during pharynx development, which likely has important consequences for the expression of developmentally important genes.[43] All in all, PHA-4 together with the tissue-specific motifs and the temporal regulatory motifs account for most of the gene regulation that occurs in the pharynx during development. Specific patterning within the relatively homogeneous primordium likely also integrates positional information established during early embryogenesis.[44]

Taking a step back to the very beginning, we may ask how *pha-4* itself is regulated in the ABa and MS lineages? The answer involves segregation of cytoplasmic determinants and induction of zygotically encoded T-Box transcription factors. Specifically, expression of *pha-4* in descendents of ABa is regulated by the zygotically expressed LAG-1 (the *C. elegans* CSL; CBF, Suppressor of Hairless, LAG-1 family member[45]), and the T-Box transcription factors TBX-37 and TBX-38.[46,47] Upstream, expression of TBX-37 and TBX-38 is *inhibited* in the ABp lineage by the maternally supplied Notch receptor GLP-1, with signaling coming from P$_2$ at the 4-cell stage,[46,48] while LAG-1 activation in ABa descendents is *activated* by a second wave of GLP-1 signaling coming this time from MS at the 12-cell stage.[45,49,50] In the MS lineage, *pha-4* expression is also regulated by a T-Box transcription factor, TBX-35, that is itself regulated by the GATA factors MED-1 and MED-2 which are the first zygotic genes downstream of the maternally encoded SKN-1, a bZIP

transcription factor that determines the fate of MS.[51] Thus, pharyngeal development comes under zygotic control once the TBX-37/TBX-38 and TBX-35 factors become activated in the ABa and MS lineages, respectively.

pha-4 is also expressed in several rectal cells, including the two rectal valve cells and the three rectal epithelial cells,[37] and regulates gene expression in these cells during development.[52] The expression pattern and function of PHA-4 during development therefore echoes that of the *Drosophila forkhead* gene: high levels in the foregut/pharynx and hindgut/rectum. Finally, *pha-4* also plays a key role in metabolic adaptation to environmental variables, such as food availability. In particular, PHA-4 chromatin immunoprecipitation studies identified thousands of genes that may be regulated by *pha-4* during starvation.[53]

pha-1 Mutants and the Pun Phenotype

In *pha-1* mutants, the pharyngeal primordium forms normally and elongation also appears normal up to at least 420 min of development. However, after elongating and contacting the mouth opening, the pharynx detaches and retracts away from the buccal cavity, causing a 'Pun' (pharynx unattached) phenotype. The end result is a worm in which the incompletely formed pharynx is slightly elongated, surrounded by the visible basement membrane and unattached to the mouth. It is difficult to determine if all pharyngeal cell differentiation events take place in the *pha-1* mutant, but expression of MYO-2::GFP is detected and a pharyngeal lumen forms.[54] Thus *pha-1* affects pharynx development after pharynx cells are committed to a specific cell fate, but before terminal differentiation/morphogenesis of the different pharyngeal cell types occurs.[54,55] *pha-1* encodes a cytosolic protein that is widely expressed (essentially all cells at 100-cell stage)[54] but of which the biochemical function is unknown. Genetic interaction experiments have shown that *pha-1*, *lin-35* (the *C. elegans* Retinoblastoma protein homolog), and *ubc-18* (a ubiquitin-conjugating enzyme) play partially redundant functions to control pharyngeal morphogenesis.[54,56,57] For example the double mutant *lin-35*/Rb; *ubc-18* shows a synthetic pharyngeal phenotype: failure to undergo pharyngeal primordium elongation, typically failing already at the reorientation step during which the anterior epithelial cells of the primordium should align their long axis with the dorsoventral axis of the embryo.[58] Similarly, the *ubc-18* and *pha-1* also both show strong synthetic pharyngeal phenotypes when combined with class B synthetic multivulval (SynMuv) genes. The SynMuv genes form two molecularly heterogeneous classes (classes A and B) of genes that contribute redundantly to vulva development; class B SynMuv genes obviously also play a hitherto unknown role in pharyngeal development that is redundant with both *ubc-18* and *pha-1*.[54,58]

pha-2 and Isthmus Formation

pha-2 mutant worms exhibit a late defect in pharyngeal morphogenesis such that the narrow isthmus does not form, causing the two bulbs to remain next to each other. PHA-2 is a homeodomain-containing protein homologous to the vertebrate Hex.[39,59] A rescue-capable PHA-2::GFP translational fusion reporter is expressed in the pm5 muscle cells that form the isthmus, the pm4 cells that make up the bulk of the metacorpus, and pharyngeal epithelial cells. We suspect that PHA-2 confers an isthmus cell identity to the pm5 cells, which have an elongated shape with their cell bodies in the posterior bulb. This shape likely results from directional growth after the comma stage (~430 min) because it is only during the 430–490 min interval that the bulbs become apparent and the nucleus-free isthmus forms.[2] The late effects of the *pha-2* mutation suggest that it regulates late effectors of the differentiation program, such as cytoskeletal genes. Indeed, experiments using optical tweezers showed that the pm5 cells of the *pha-2* mutant have a structurally weak cytoskeleton, which correlates with their inability to elongate anteriorly and their inability to prevent nuclei from being misplaced into the isthmus (Figure 4).[59] Also, *pha-2* likely acts as a repressor of *ceh-22* in the pm5 cells: in *pha-2* mutants, expression of a CEH-22::GFP reporter persists in the isthmus during late embryogenesis and post-embryonically, while it is silenced in the isthmus of wild-type worms during late embryogenesis.[39]

ceh-22 and Pharyngeal-Specific Myosins

Like cardiomyocytes, the pharyngeal muscles exhibit autonomous rhythmic contractions and do not depend on members of the MyoD myogenic factor family for their development. Two myosin heavy chain genes, *myo-1* and *myo-2*, are specifically expressed in pharyngeal muscles where they are regulated by the homeodomain protein CHE-22.[60] This transcription factor is homologous to the vertebrate Nkx2.5 and the *Drosophila tinman*, which regulate heart development vertebrates and flies, respectively. Furthermore, expression of the zebrafish nkx2.5 gene in *C. elegans* can activate *myo-2* and rescue the *ceh-22* mutant, suggesting that *ceh-22* and nkx2.5 share a conserved molecular function.[61] The *ceh-22* mutant

WT   *pha-2*

pm5/isthmus cells   pm5 nuclei stay in isthmus
elongate anteriorly.   as cells elongate

The pm5 cells fuse and   Weak isthmus deforms
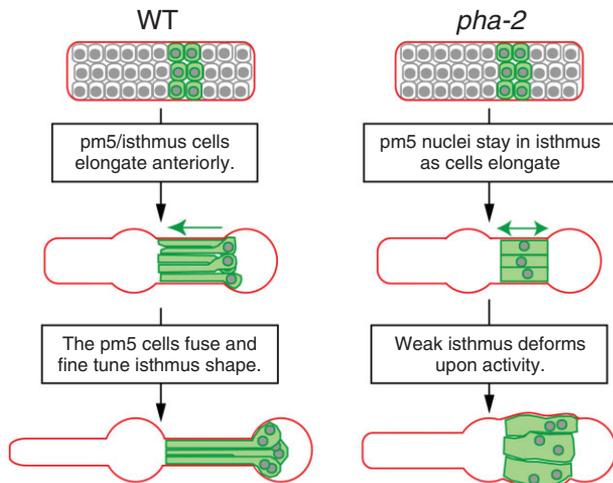fine tune isthmus shape.   upon activity.

FIGURE 4 | Isthmus defect in *pha-2* mutants. In wild-type worms (left), pm5 (green) and other isthmus cells elongate anteriorly while their nuclei remain in the nascent posterior bulb. This drives the formation of the narrowed, nucleus-free isthmus. Near the time of hatching, two cells fuse in each muscle sector to create the multinucleated pm5 cells that make up the mature isthmus. In *pha-2* mutants (right), the pm5 cells have a weak cytoskeleton that does not drive anterior elongation nor prevent movement of nuclei into the isthmus, which therefore does not form. The result is that the metacorpus and posterior bulb remain near each other, connected by a short abnormally thick isthmus containing many nuclei. The state of cellular fusion in the *pha-2* mutant is not known.

has only a mild pharyngeal phenotype: slight thickening of the isthmus, and no defect in the expression of *myo-2*. This suggests that other regulatory pathways act in parallel with *ceh-22* to regulate *myo-2*.[62] Indeed, PHA-1 also directly regulates the *myo-2* gene, and a *pha-1; ceh-22* double mutant is more severe than either mutant alone.[62] Finally, it is important to note that *ceh-22* has roles outside the pharynx, notably during gonad development.[63] Indeed, it is likely that most, if not all, genes important for pharyngeal development also have other roles.

MORPHOGENESIS OF INDIVIDUAL CELLS

Introduction to Pharyngeal Neurons

The pharyngeal cells that are best understood from a developmental genetics point of view are the neurons, which establish intricate but reproducible trajectories and synapses.[1] More than 50 axon guidance decisions are made within the metacorpus, a near-spherical structure with a diameter of about 20 μm. The pharyngeal neurons send axons along three pharyngeal nerve cords, one in each muscle sector, and exhibit some of the most complicated cell morphologies found

within *C. elegans*: they are often branched, have many turns, and several form actual closed circles with their axons. The fact that they mostly lie within folds of the pharyngeal muscle membranes also distinguish them from the body neurons that are typically sandwiched between a basement membrane lining the hypodermis and the surface of the body muscles.[1] As previously mentioned, most of the pharyngeal neurons can be ablated in adults with little or no consequences for pharyngeal pumping or viability in the laboratory, which is an ideal situation for genetic studies since mutations affecting these neurons are usually viable.[15] Such genetic studies have revealed three features that explain how individual *C. elegans* pharyngeal neurons can develop their specific trajectories in spite of the fact that all are exposed to a similar environment: (1) they seem to utilize most of the known guidance pathways, with the exception of the ephrins; (2) they can establish long portions of their trajectories without growth cones; and (3) their growth cones are reprogrammed during axon growth. What follows is an overview of the developmental genetics of the three best-studied pharyngeal neuron types.

The M2L/R Neurons
Morphology and Function
M2L and M2R are bilaterally symmetrical motor neurons with cell bodies in the subventral tiers of the terminal bulb (Figure 1(a)).[1,10] Each sends a single axon with a proximal trajectory that travels through the isthmus, within deep folds of the pm5 muscle cells, and a distal end that forms two arcs within the metacorpus: the first arc is on the same horizontal plane as the proximal trajectory and follows the shape of the pm4 cells while the second arc first extends through the pm4 muscle cell dorsally and anteriorly, beyond the pharyngeal nerve ring that lies at the boundary between the pm4 and pm5 cells, then project toward the midline to connect at its end in a gap junction with the contralateral M2 neuron. While the M2 neurons form *en-passant* synapses with the pm5 and pm4 muscle cells, their function is unknown. Indeed, they can be laser ablated without detectable effect on pharynx function in *C. elegans*,[15] although they are essential for anterior isthmus peristalsis in some other species.[64]

Growth Cone-Dependent Distal Ends
Axons typically establish their trajectories via a motile structure at their distal ends, the growth cone, that bears receptors on its surface and reacts to the presence of molecular attractants or repellants by locally stabilizing or destabilizing its internal cytoskeleton.[65–67]

The pharyngeal neurons M2L/R were studied in mutant backgrounds to determine the roles of growth cones during their development, and to identify the guidance pathways that may guide them.[20] Rather unexpectedly, most tested guidance pathways do contribute to M2 neuron development, but only for the distal end. Mutations that impair basic growth cone functions, such as *unc-51*[68,69] and *unc-73*,[70,71] usually caused the highest incidence of defects and truncations in the distal end of the M2 neurons. The *unc-6*/netrin pathway is important for circumferential and antero-posterior guidance decisions in *C. elegans*.[72–76] This pathway helps the axon of M2 to move dorsally within the metacorpus and to form the two arcs at the distal end. The *unc-6* pathway therefore guides the circumferential projections of pharyngeal neurons, just as it guides circumferential trajectories in body neurons, and may also influence the length of the processes. Similarly, mutations in the *sax-3*/roundabout pathway, which controls neuron polarity and also regulates midline crossing in *C. elegans* body neurons,[74,76–78] often cause the axons of the M2 neurons to migrate erroneously across the midline, or in the wrong direction along the anterior–posterior axis, as well as frequently causing the cell bodies to be misplaced. *sax-3* therefore provides a midline crossing barrier in the pharynx and also conveys anterior–posterior information to the M2 neurons. A mutation in *unc-119*, which encodes a protein acting as a regulator of axon branching,[79,80] caused abnormally long axons and excessive branching in the M2 neurons. Other mutations that affected the M2 neurons include *fax-1*,[81] *unc-69*,[82,83] *unc-115*,[84,85] and *mnm-5*.[24,86] The ephrin pathway does not seem to play an important role during the development of the M2 neurons: a mutation in the only known ephrin receptor in *C. elegans*, *vab-1*,[87,88] has no effect on M2 morphology.

Growth Cone-Independent Proximal Trajectory: Fishing Line Paradigm

The proximal trajectory of the M2 axons projects straight from the cell body through the isthmus, and is impervious to mutations in growth cones or guidance cues; it must therefore be established in a growth-cone independent manner.[20,86,89] Consistently, microscopic monitoring of the M2 neuron shows that a growth cone appears only after the axon has elongated to span the nascent isthmus during early embryogenesis. This growth cone emerges within the developing metacorpus and therefore cannot participate in the elongation of the axon through the isthmus.[24] The simplest hypothesis for the development of the M2 proximal trajectory without using a growth cone is that the M2 cell initially makes an attachment to

a cell that moves to the other side of the isthmus during morphogenesis, dragging along the elongating M2 axon. This process is not unlike a fishing line (the axon) being pulled from the reel (the M2 cell body) by a fish swimming away (the other cell to which M2 is attached). An excellent candidate for the 'fish' is M2's sister cell M3, which has its cell body in the metacorpus (Figure 5). Being the sister of M2, M3 is necessarily in contact with M2 after their birth. The two cells are then pushed away from each other as the isthmus forms by the elongation of the pm5 muscle cells that make up its bulk.[39,59] The elongating pm5 cells provide some of the force that separates M2 and M3 as the isthmus forms, as evidenced from the fact that in *pha-2* mutants, which have a weak pm5 cytoskeleton, the M2 cell body can become drawn into the isthmus.[59]

The fishing line paradigm, first proposed by Bray in 1984,[90] is a robust way of establishing axon projections and could be important whenever axons develop from migrating neurons or from neurons that are neighbors to migrating cells. This process is likely related to retrograde extension of the sensilla neurons in *C. elegans*[2,91] and possibly even to that of the radial migration of cerebellar granule cells in mammals,[92] both of which are cases where the cell bodies lay down an axon as they move away from an initial attachment point. A related example is also found in *Drosophila* where the larval optic nerve undergoes a period of elongation by intercalation of membrane as the neuron cell body and a distant guidepost cell move away from each other; later, a growth cone-dependent process completes the establishment of the distal trajectory.[93]

mnm-2, and M3 as a Time-Delayed Guidepost Cell for M2

The *mnm-2* gene encodes a Krüppel-like transcription factor that in the pharynx is initially expressed by the mother cell to M2 and M3, but persists only in the M3 cell.[24] In the *mnm-2* mutant, the M3 cell is still present but functionally defective, as assessed using EPGs that can detect M3-specific spikes in the electrical activity of the pharynx. The *mnm-2* gene is essential for the ability of M3 to deliver an instructive signal to the tip of the M2 axon at the time when it should begin to migrate along the dorsoventral axis to generate the second arc of the distal end (Figure 5). The term 'guidepost cell' is generally applied to cells that act as stepping stones along the path of an elongating axon, often resulting in a change in direction after the contact point between the guide post cell and the growth cone.[94,95] It therefore seems appropriate to say that M3 acts as a 'time-delayed guidepost cell'
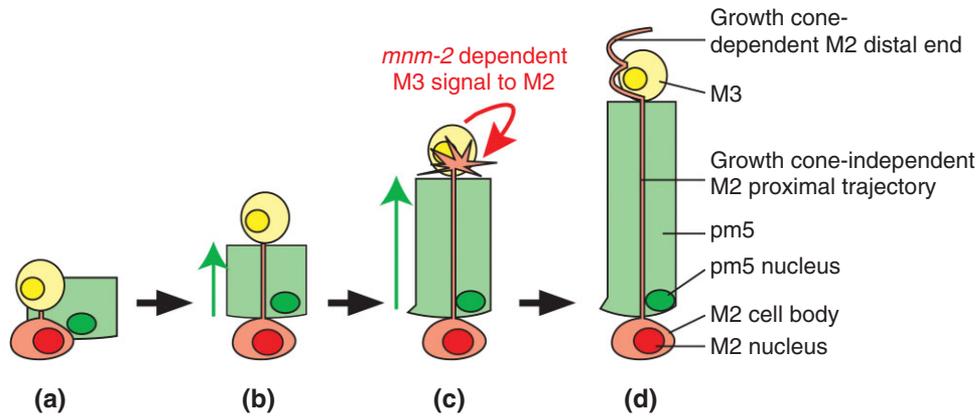
FIGURE 5 | Model for the development of the M2 axon. Elongation of the pm5 cells likely separates the cell bodies of the sister cells M2 and M3. However, M2 remains attached to M3, such that their separation causes lengthening of the M2 axon proximal trajectory through the isthmus (a, b). Later, the distal end of the M2 cell forms a growth cone that requires a signal from M3 to be specified correctly. M3 depends on its expression of *mnm-2* to produce this signal (c). The M2 growth cone then interprets local cues and establishes the two arcs of the distal end (d), and meets its contralateral cellular homolog at the midline where they become connected by a gap junction. Anterior is up. (Reprinted with permission from Ref 24. Copyright 2007 Elsevier Ltd.)

for the M2 axon that it likely pulled through the isthmus. The timing of the M3 signal to the M2 axon is apparently determined by the *mnm-2*-driven differentiation of M3: without *mnm-2* the M2 growth cone errs without moving dorsally, precisely as it does when M3 is genetically ablated.[24]

Growth cone reprogramming is well documented for many neurons. For example, it occurs when *Drosophila* commissural neurons cross the midline: these growth cones begin expressing ROBO on their surface only after contacting COMM-expressing cells.[96,97] *C. elegans* growth cones are also reprogrammed when the hermaphrodite specific neurons HSNL encounter guidepost cells in the vulval epithelium, a process that requires the guidepost protein SYG-2 and its receptor SYG-1.[98] What is unusual about the M3 reprogramming of the M2 growth cone is that M3 seems to functions as a time-delayed guidepost cell. M2 and M3 are sister cells that contact each other throughout development, but it is only when M3 differentiates in a manner dependent on *mnm-2* that it provides a reprogramming signal to the M2 growth cone. This allows the timely redirecting of the M2 growth cone within the narrow confines of the metacorpus and presumably coordinates this process with that of the other pharyngeal neurons that also establish trajectories in the neighboring space.

The M1 Neuron
Morphology and Function
The developmental genetics of the M1 neuron has also been investigated in detail.[89] M1 is a unique neuron with a very long rather straight trajectory: its cell body is located within the posterior bulb and its axon extends a proximal trajectory through the isthmus, and a distal portion that projects from the metacorpus and all the way to the anterior end of the pharynx where it terminates into two short posteriorly directed branches (Figure 1(a)). M1 innervates the procorpus by forming synapses with the pm1, pm2, and pm3 muscle cells, while receiving input from interneurons in each of the three pharyngeal sectors. While the M1 connectivity suggests a role in regulating procorpus behavior, no function has been experimentally documented.

Growth Cone-Dependent and -Independent Axon Guidance
M1 uses growth cone-dependent mechanisms only for the development of its distal end: the growth cone function genes *unc-34*, *unc-51*, *unc-115*, and *unc-119* were all required for the distal end but not the proximal trajectory to form normally. Intriguingly, no mutation in the tested guidance pathways (netrin, semaphorins, ephrins, TGF-*β*) had strong effects on the M1 distal end, indicating either that novel guidance pathways are involved or that multiple redundant pathways are at work to insure robust development. The M1 straight proximal trajectory, just like that of the M2 neurons, is impervious to mutations in growth cones or guidance cues and must therefore be established in a growth-cone independent manner.[89] Also like the M2 neurons, the developing M1 axon uses an instructive interaction with a specific cell, the gland cell g1P, for the guidance of its growth cone-dependent distal end.[89] Thus,

growth cone-independent development of axon trajectories through the isthmus followed by instructive cell–cell interactions in the metacorpus may be a general strategy for the development of many pharyngeal neurons.

The NSML/R Neurons
Morphology and Function

The NSML and NSMR neurosecretory motorneurons have their cell bodies located within the subventral tiers of the metacorpus, embedded inside the pm4 muscle cells (Figure 1(a)).[1,10] Each produces a main trunk at its posterior side, from which three processes emerge. The major NSM subventral branch projects within the ipsilateral nerve cord all the way through the isthmus and ending near the boundary to the terminal bulb. The major NSM dorsal branch first loops under the pharyngeal lumen, within the pharyngeal nerve ring at the boundary between the pm4 and pm5 cells, then rises into the dorsal tier to project into the isthmus, within the dorsal pharyngeal nerve cord. Both major processes run along the outer edge of the nerve cords, in close contact with the basement membrane, and both are periodically swelled or even extend short projections filled with synaptic vesicles that they secrete through the basement membrane, into the pseudocoelomic fluid. Each major branch also forms multiple *en passant* electron-dense synapses with the pm5 muscle cells in the isthmus and with the pharyngeal basement membrane. Finally, the NSM minor process is thin, synapse free and projects ipsilaterally along the boundary between a pm5 muscle cell and a mc2 marginal cell, in close contact with the cuticle that lines the pharyngeal lumen.[86] The thin process may serve a sensory function to monitor the state of the pharyngeal lumen. The NSM neurons can secrete serotonin,[99,100] glutamate[31] and the neuropeptide-like proteins NLP-13, NLP-18, and NLP-19.[101] Given their trajectories and connectivity, the NSM neurons are likely able to convey information about the presence of food in the pharynx to the rest of the worm, thus affecting foraging behavior. Consistently, ablation of the NSMs impairs a slowing of locomotion response that starved worms usually exhibit when encountering food.[102]

Growth Cone-Dependent Development of the NSM Major Branches

The three NSM processes are differently sensitive to mutations.[86] The major dorsal branch is most sensitive to mutations that affect growth cone guidance and function (e.g., *unc-6*, *unc-34*, *unc-73*), while the major subventral branch is more sensitive to

mutations that affect components of the extracellular matrix (e.g., *sdn-1*). In *sax-3* mutants, the NSM neurons often project one axon branch anteriorly (37% of cases) or exhibit a very abnormal morphology with cell bodies frequently misplaced (19% of cases). This indicates that *sax-3* is important to establish the anterior–posterior polarity of the NSM neurons and to specify their correct position within the primordium. It is interesting to note that while *sax-3* provides anterior–posterior information to both the M2 and NSM neurons, these two neuron types interpret the signal to guide axons in opposite directions (M1 is not strongly affected by *sax-3* mutation[89]). Another difference between the M2 and NSM neurons concerns the effect of *unc-119*: for M2 this mutation causes abnormally long axons and extra branching but for the NSMs it causes short axons and no excessive branching.

Growth Cone-Independent Development of the NSM Minor Branch

The minor NSM process (shown as a thin red line in Figure 1(a)) is mostly resistant to mutations that affect growth cones, although low penetrance defects such as occasional truncations are observed in these mutants, suggesting that some growth cone function is required for its elongation.[86] However, the development of the minor NSM process is unusual in one respect: a mutation in *unc-101*, which encodes a mu1 subunit of the AP-1 clathrin adaptor complex, results in its complete absence. This suggests that the NSM minor process may initiate and elongate via the polarized transport of proteins using a *unc-101*-dependent pathway. This is consistent with the observation that, in *C. elegans*, clathrin-associated proteins are known to determine the polarity of axons and dendrites, a process to which *unc-101* contributes. *unc-101* could, e.g., help establish a gradient of adhesion at the leading edge of the growing NSM minor process, in analogy with the role of clathrin adaptor molecules in cell adhesion molecular (CAM)-dependent growth cone migration.[103] Given that the NSM minor process is most likely a sensory dendrite,[86] it is interesting that secretory pathways play a specific role during dendrite elongation also in other organisms, such as *Drosophila*[104]: it suggests the evolutionary conservation of a dendrite-specific growth mechanism.

A Word on the Developmental Robustness of Pharyngeal Neurons

That many genes contribute to the development of pharyngeal neurons was put in evidence in a screen of 2500 mutagenized haploid genomes for

M2 neuron mutants, from which only one of six newly isolated mutants were alleles of the numerous previously known mutants that affect M2 development.[20] Similarly, a screen of 5000 haploid genomes for M1 guidance defects found nine mutants affecting five genes, three of which were novel.[89] Extrapolating from this dataset, it would seem that very many genes contribute to M1 and M2 guidance in a way that could be studied using a developmental genetics approach, i.e., produce viable worms with M1 or M2 defects when mutated. We have seen that growth cone-dependent processes are important for the development of the anterior-projecting distal ends of the M1 and M2 neurons, and for the two posterior-projecting major NSM branches. Similarly, the M2, M3, M4, I3, and NSM neurons all are affected by a mutation in *ten-1*, which encodes a transmembrane protein that likely regulates extracellular matrix composition, hence the substrate for growth cones.[34] However, each axon responds differently to mutations in specific guidance pathways, which helps explain why they develop different trajectories within the same environment. The multiplicity of pathways that cooperate to guide the pharyngeal neurons likely contributes to the robustness of their development. Even in *unc-73* mutants, more than 30% of M2 or NSM neurons develop normally, and the most severe guidance cue mutations, e.g., *unc-6* or *sax-3*, also produce 20% or more normal M2 and NSM neurons; the effects of other mutations were usually much weaker. Robustness through multiplicity of guidance processes was directly tested by scoring the M2 neurons in double mutants between *mnm-2* and other mutations: in all cases the double mutants were more severe than either single mutant. Indeed, only 3% of *unc-73;mnm-2*, 2% of *unc-40;mnm-2*, and 1.5% of *unc-5;mnm-2* double mutants produced normal M2 trajectories, compared to 35% or better for any of the single mutants.[24] Clearly, proper development of the M2 neurons can often withstand the loss of one guidance pathway, but not the loss of two. This conclusion is generally applicable to body neurons as well: the penetrance of guidance/growth cone mutations rarely exceeds 70% for most *C. elegans* neurons studied, including mechanosensory neurons,[105] canal-associated neurons (CANs),[106] hermaphrodite-specific neurons (HSNs)[106] and amphid neurons in the nerve ring.[107] Given the poor state in which naturally isolated *C. elegans* individuals are found,[108] with a range of developmental defects likely due to environmental stresses, it may be essential for many processes to rely on multiple pathways that buttress each other to achieve developmental robustness.

Gland Cells
Morphology and Function
The pharyngeal glands are five cells in the posterior bulb of the pharynx with cellular projections that open into the pharyngeal lumen at discrete points along the length of the pharynx. The glands are further divided into two subgroups: three g1 cells extending long projections that empty into the lumen in the isthmus or procorpus and have a lamellar cytoplasm and few vesicles, and two g2 cells that empty near the grinder and have a rather clear cytoplasm and more vesicles. Periodic episodes of secretion have been seen in the g1 ducts near the time of molting suggesting a role in digestion of cuticle at this time.[1,109] The gland cells also express a mucin-like gene, *phat-1*, that may help to lubricate the lumen.[14]

Development of the Gland Cells
A screen of 5000 mutagenized haploid genomes for mutants with abnormal expression or morphology of a gland specific reporter (*phat-1::YFP*) identified five recessive mutations, two of which were molecularly defined.[110] One was a new allele of *hlh-6*, a bHLH transcription factor that, together with several other factors, is required for the expression of many gland cell genes, including *pha-1*.[14,111,112] The other identified mutant was a new allele of *sma-1*, which encodes a β-spectrin important for pharyngeal muscle development.[113–115] A detailed analysis of several other mutants that also affect the properties of the pharyngeal muscles revealed that they impose mechanical constraints that shape the gland cells: mutations in such genes result in abnormal gland cell morphology, including formation of supernumerary branches and swellings suggestive of hypertrophy. This suggests that pharyngeal muscles act primarily to regulate expansion of the gland cells.

Development of the Grinder
The grinder is a cuticular structure on the luminal side of the pm6 and pm7 muscle cells in the posterior bulb. Its three serrated surfaces interlock in a grinding motion to macerate food and may act as a one-way valve for food. The molecular composition of the grinder is not well defined, but it is thought to form as a result of secretions from the pm6 and pm7 cells and to depend on vesicular trafficking requiring RAB-6.2, a Rab6 homolog in *C. elegans*, and EAT-16, its regulatory Rab GAP.[116]

POST-DEVELOPMENTAL EVENTS: PLASTICITY, GROWTH, AND AGING

Developmental Plasticity

Even though each cell is normally specified to adopt a developmental fate, there is some degree of developmental plasticity. For example, M4 has a sister cell that usually dies of apoptosis but that is able to take over the function of M4 if the latter is laser-ablated in a *ced-3* mutant (in which the sister cell of M4 does not die of apoptosis).[117] Even more spectacular are examples where fully differentiated pharyngeal muscle cells of larvae and adults can be made to transdifferentiate into fully differentiated intestinal cells. This is done by a brief ectopic expression of the ELT-7 GATA factor in the pharyngeal cells and does not require cell division; ELT-7 usually regulates terminal differentiation of intestinal cells.[118]

Larval Growth and the Twisted Pharynx Phenotype

The organs of animal embryos are typically covered with an ECM that must be carefully remodeled as these organs enlarge during post-embryonic growth, and the *C. elegans* pharynx is no exception. Mutants with defects in ECM component proteins (e.g., FBL-1, UNC-52, DPY-7, LAM-1), ECM attachment proteins (e.g., DIG-1 or SAX-7) or ECM metalloproteinases (e.g., MIG-6) can all cause or influence a distinctive post-developmental twisted pharynx phenotype (Twp) that occurs when the ECM or attachment to the ECM cannot be remodeled during pharyngeal growth.[33,119] The Twp phenotype is therefore a promising experimental system to study ECM remodeling during organ growth.

Aging

The pharynx undergoes changes in morphology and texture that correlates with its age and, therefore provide a quantifiable measurement of the aging process.[120,121] These changes include an increase in 'entropy', or disorder, in the pharynx muscles and show a stepwise increase between days 2 and 4, then again between days 8 and 10. This morphological deterioration is correlated with elevated levels of free radical production by pharyngeal mitochondria, the intensity of which is a predictor of lifespan.[122] Furthermore, the timing of the step-changes correlates also with the nonlinear changes in the expression of *elt-5* during aging; *elt-5* controls many age-regulated genes.[123] All in all, these results suggest that the *C. elegans* pharynx proceeds through distinct stages during aging and that it faithfully reflects organismal aging. Thus, aging in *C. elegans* may be regulated by genetic pathways and reflected in measurable pharyngeal changes.

CONCLUSION

There is clearly much more that can be done using forward genetics to understand pharyngeal development. For example, a screen of 10,000 mutagenized haploid genomes yielded 83 mutants with abnormal pharynges.[113] Specific aspects remain to be understood in detail, including cell fusions[124] and the secretion and deposition of many extracellular components, including glycans,[125] specialized cuticle[126] and chitin.[12] One of the most pressing issues toward a better understanding of axon guidance in the *C. elegans* pharynx concerns the source and distribution of the guidance cues. *In vivo* reporters suggest that some neurons may act as morphogen sources within the pharynx. For example, the neuron I5 expresses *unc-6*,[127] and I4 expresses *unc-129*.[128] However, no morphogen has been imaged in the developing pharynx.

REFERENCES

1. Albertson DG, Thomson JN. The pharynx of *Caenorhabditis elegans*. *Philos Trans R Soc Lond B Biol Sci* 1976, 275:299–325.
2. Sulston JE, Schierenberg E, White JG, Thomson JN. The embryonic cell lineage of the nematode *Caenorhabditis elegans*. *Dev Biol* 1983, 100:64–119.
3. Avery L, Shtonda BB. Food transport in the *C. elegans* pharynx. *J Exp Biol* 2003, 206:2441–2457.

4. Raizen DM, Avery L. Electrical activity and behavior in the pharynx of *Caenorhabditis elegans*. *Neuron* 1994, 12:483–495.

5. Gaudet J, Mango SE. Regulation of organogenesis by the *Caenorhabditis elegans* FoxA Protein PHA-4. *Science* 2002, 295:821–825.

6. Avery L, You YJ. *C. elegans* feeding. In: Worm-Book, ed. *The C. elegans Research Community, Worm-Book*. doi:10.1895/wormbook.1.150.1. Available at: http://www.wormbook.org. (Accessed May 21, 2012).

7. Mango SE. The molecular basis of organ formation: insights from the *C. elegans* foregut. *Annu Rev Cell Dev Biol* 2009, 25:597–628.

8. Song B-M, Avery L. The pharynx of the nematode *C. elegans*: a model system for the study of motor control. *Worm* 2013, 2:e21833.

9. Kormish JD, Gaudet J, McGhee JD. Development of the *C. elegans* digestive tract. *Curr Opin Genet Dev* 2010, 20:346–354.

10. Altun ZF, Herndon LA, Crocker C, Lints R, Hall DH. WormAtlas, 2014. Available at: http://www.wormatlas.org. (Accessed March 1, 2014).

11. Pilon M. Fishing lines, time-delayed guideposts, and other tricks used by developing pharyngeal neurons in *Caenorhabditis elegans*. *Dev Dyn* 2008, 237:2073–2080.

12. Heustis RJ, Ng HK, Brand KJ, Rogers MC, Le LT, Specht CA, Fuhrman JA. Pharyngeal polysaccharide deacetylases affect development in the nematode *C. elegans* and deacetylate chitin in vitro. *PLoS One* 2012, 7:e40426.

13. Graham PL, Johnson JJ, Wang S, Sibley MH, Gupta MC, Kramer JM. Type IV collagen is detectable in most, but not all, basement membranes of *Caenorhabditis elegans* and assembles on tissues that do not express it. *J Cell Biol* 1997, 137:1171–1183.

14. Smit RB, Schnabel R, Gaudet J. The HLH-6 transcription factor regulates *C. elegans* pharyngeal gland development and function. *PLoS Genet* 2008, 4:e1000222.

15. Avery L, Horvitzt HR. Pharyngeal pumping continues after laser killing of the pharyngeal nervous system of *C. elegans*. *Neuron* 1989, 3:473–485.

16. Avery L. Motor neuron M3 controls pharyngeal muscle relaxation timing in *Caenorhabditis elegans*. *J Exp Biol* 1993a, 175:283–297.

17. Wright KA, Thomson JN. The buccal capsule of *C. elegans* (Nematoda: Rhabditoidea): an ultrastructural study. *Can J Zool* 1981, 59:1952–1961.

18. Avery L. The genetics of feeding in *Caenorhabditis elegans*. *Genetics* 1993b, 133:897–917.

19. Avery L, Raizen D, Lockery S. Electrophysiological methods. *Methods Cell Biol* 1995, 48:251–269.

20. Mörck C, Axäng C, Pilon M. A genetic analysis of axon guidance in the *C. elegans* pharynx. *Dev Biol* 2003, 260:158–175.

21. Rasmussen JP, Reddy SS, Priess JR. Laminin is required to orient epithelial polarity in the *C. elegans* pharynx. *Development* 2012, 139: 2050–2060.

22. Podbilewicz B. Cell fusion. *WormBook* 2006:1–32.

23. Portereiko MF, Mango SE. Early morphogenesis of the *Caenorhabditis elegans* pharynx. *Dev Biol* 2001, 233:482–494.

24. Rauthan M, Morck C, Pilon M. The *C. elegans* M3 neuron guides the growth cone of its sister cell M2 via the Kruppel-like zinc finger protein MNM-2. *Dev Biol* 2007, 311:185–199.

25. Goldstein B, Hird SN. Specification of the anteroposterior axis in *Caenorhabditis elegans*. *Development* 1996, 122:1467–1474.

26. Cheeks RJ, Canman JC, Gabriel WN, Meyer N, Strome S, Goldstein B. *C. elegans* PAR proteins function by mobilizing and stabilizing asymmetrically localized protein complexes. *Curr Biol* 2004, 14:851–862.

27. Duguay D, Foty RA, Steinberg MS. Cadherin-mediated cell adhesion and tissue segregation: qualitative and quantitative determinants. *Dev Biol* 2003, 253:309–323.

28. Leung B, Hermann GJ, Priess JR. Organogenesis of the *Caenorhabditis elegans* intestine. *Dev Biol* 1999, 216:114–134.

29. Shaw WR, Armisen J, Lehrbach NJ, Miska EA. The conserved miR-51 microRNA family is redundantly required for embryonic development and pharynx attachment in *Caenorhabditis elegans*. *Genetics* 2010, 185:897–905.

30. Bellocchio EE, Reimer RJ, Fremeau RT Jr, Edwards RH. Uptake of glutamate into synaptic vesicles by an inorganic phosphate transporter. *Science* 2000, 289:957–960.

31. Lee RY, Sawin ER, Chalfie M, Horvitz HR, Avery L. EAT-4, a homolog of a mammalian sodium-dependent inorganic phosphate cotransporter, is necessary for glutamatergic neurotransmission in *Caenorhabditis elegans*. *J Neurosci* 1999, 19:159–167.

32. Starich TA, Lee RY, Panzarella C, Avery L, Shaw JE. *eat-5* and *unc-7* represent a multigene family in *Caenorhabditis elegans* involved in cell-cell coupling. *J Cell Biol* 1996, 134:537–548.

33. Jafari G, Burghoorn J, Kawano T, Mathew M, Mörck C, Axäng C, Ailion M, Thomas JH, Culotti JG, Swoboda P, et al. Genetics of extracellular matrix remodeling during organ growth using the *Caenorhabditis elegans* pharynx model. *Genetics* 2010, 186:969–982.

34. Mörck C, Vivekanand V, Jafari G, Pilon M. *C. elegans ten-1* is synthetic lethal with mutations in

cytoskeleton regulators, and enhances many axon guidance defective mutants. *BMC Dev Biol* 2010, 10:55.

35. Schnabel H, Schnabel R. An organ-specific differentiation Gene, *pha-1*, from *Caenorhabditis elegans*. *Science* 1990, 250:686–688.

36. Benian GM, Tinley TL, Tang X, Borodovsky M. The *Caenorhabditis elegans* gene *unc-89*, required fpr muscle M-line assembly, encodes a giant modular protein composed of Ig and signal transduction domains. *J Cell Biol* 1996, 132:835–848.

37. Kalb JM, Lau KK, Goszczynski B, Fukushige T, Moons D, Okkema PG, McGhee JD. *pha-4* is Ce-fkh-1, a fork head/HNF-3α,β,γ homolog that functions in organogenesis of the *C. elegans* pharynx. *Development* 1998, 125:2171–2180.

38. Mango SE, Lambie EJ, Kimble J. The *pha-4* gene is required to generate the pharyngeal primordium of *Caenorhabditis elegans*. *Development* 1994, 120:3019–3031.

39. Morck C, Rauthan M, Wagberg F, Pilon M. *pha-2* encodes the *C. elegans* ortholog of the homeodomain protein HEX and is required for the formation of the pharyngeal isthmus. *Dev Biol* 2004, 272:403–418.

40. Ao W, Gaudet J, Kent WJ, Muttumu S, Mango SE. Environmentally induced foregut remodeling by PHA-4/FoxA and DAF-12/NHR. *Science* 2004, 305:1743–1746.

41. Gaudet J, Muttumu S, Horner M, Mango SE. Whole-genome analysis of temporal gene expression during foregut development. *PLoS Biol* 2004, 2:e352.

42. Raharjo WH, Logan BC, Wen S, Kalb JM, Gaudet J. In vitro and in vivo characterization of *Caenorhabditis elegans* PHA-4/FoxA response elements. *Dev Dyn* 2010, 239:2219–2232.

43. Fakhouri THI, Stevenson J, Chisholm AD, Mango SE. Dynamic chromatin organization during foregut development mediated by the organ selector gene PHA-4/FoxA. *PLoS Genet* 2010, 6:e1001060.

44. Labouesse M, Mango SE. Patterning the *C. elegans* embryo: moving beyond the cell lineage. *Trends Genet* 1999, 15:307–313.

45. Christensen S, Kodoyianni V, Bosenberg M, Friedman L, Kimble J. *lag-1*, a gene required for *lin-12* and *glp-1* signaling in *Caenorhabditis elegans*, is homologous to human CBF1 and Drosophila Su(H). *Development* 1996, 122:1373–1383.

46. Good K, Ciosk R, Nance J, Neves A, Hill RJ, Priess JR. The T-box transcription factors TBX-37 and TBX-38 link GLP-1/Notch signaling to mesoderm induction in *C. elegans* embryos. *Development* 2004, 131:1967–1978.

47. Smith PA, Mango SE. Role of T-box gene *tbx-2* for anterior foregut muscle development in *C. elegans*. *Dev Biol* 2014, 196:211–223.

48. Neves A, Priess JR. The REF-1 family of bHLH transcription factors pattern *C. elegans* embryos through Notch-dependent and Notch-independent pathways. *Dev Cell* 2005, 8:867–879.

49. Moskowitz IP, Gendreau SB, Rothman JH. Combinatorial specification of blastomere identity by *glp-1*-dependent cellular interactions in the nematode *Caenorhabditis elegans*. *Development* 1994, 120:3325–3338.

50. Moskowitz IP, Rothman JH. *lin-12* and *glp-1* are required zygotically for early embryonic cellular interactions and are regulated by maternal GLP-1 signaling in *Caenorhabditis elegans*. *Development* 1996, 122:4105–4117.

51. Broitman-Maduro G, Lin KT-H, Hung WWK, Maduro MF. Specification of the *C. elegans* MS blastomere by the T-box factor TBX-35. *Development* 2006, 133:3097–3106.

52. Marshall SD, McGhee JD. Coordination of *ges-1* expression between the Caenorhabditis pharynx and intestine. *Dev Biol* 2001, 239:350–363.

53. Zhong M, Niu W, Lu ZJ, Sarov M, Murray JI, Janette J, Raha D, Sheaffer KL, Lam HYK, Preston E, et al. Genome-wide identification of binding sites defines distinct functions for *Caenorhabditis elegans* PHA-4/FOXA in development and environmental response. *PLoS Genet* 2010, 6:e1000848.

54. Fay DS, Qiu X, Large E, Smith CP, Mango S, Johanson BL. The coordinate regulation of pharyngeal development in *C. elegans* by *lin-35*/Rb, *pha-1*, and *ubc-18*. *Dev Biol* 2004, 271:11–25.

55. Granato M, Schnabel H, Schnabel R. Genesis of an organ: molecular analysis of the *pha-1* gene. *Development* 1994, 120:3005–3017.

56. Mani K, Fay DS. A mechanistic basis for the coordinated regulation of pharyngeal morphogenesis in *Caenorhabditis elegans* by LIN-35/Rb and UBC-18-ARI-1. *PLoS Genet* 2009, 5:e1000510.

57. Polley SRG, Kuzmanov A, Kuang J, Karpel J, Lazetic V, Karina EI, Veo BL, Fay DS. Implicating SCF complexes in organogenesis in *Caenorhabditis elegans*. *Genetics* 2014, 196:211–223.

58. Fay DS, Large E, Han M, Darland M. *lin-35*/Rb and *ubc-18*, an E2 ubiquitin-conjugating enzyme, function redundantly to control pharyngeal morphogenesis in *C. elegans*. *Development* 2003, 130:3319–3330.

59. Morck C, Axang C, Goksor M, Pilon M. Misexpression of acetylcholinesterases in the *C. elegans pha-2* mutant accompanies ultrastructural defects in pharyngeal muscle cells. *Dev Biol* 2006, 297:446–460.

60. Okkema PG, Fire A. The *Caenorhabditis elegans* NK-2 class homeoprotein CEH-22 is involved in combinatorial activation of gene expression in pharyngeal muscle. *Development* 1994, 120:2175–2186.

61. Haun C, Alexander J, Stainier DY, Okkema PG. Rescue of *Caenorhabditis elegans* pharyngeal development by a vertebrate heart specification gene. *Proc Natl Acad Sci USA* 1998, 95:5072–5075.

62. Okkema PG, Ha E, Haun C, Chen W, Fire A. The *Caenorhabditis elegans* NK-2 homeobox gene *ceh-22* activates pharyngeal muscle gene expression in combination with pha-1 and is required for normal pharyngeal development. *Development* 1997, 124:3965–3973.

63. Lam N, Chesney MA, Kimble J. Wnt signaling and CEH-22/tinman/Nkx2.5 specify a stem cell niche in *C. elegans*. *Curr Biol* 2006, 16:287–295.

64. Chiang JT, Steciuk M, Shtonda B, Avery L. Evolution of pharyngeal behaviors and neuronal functions in free-living soil nematodes. *J Exp Biol* 2006, 209:1859–1873.

65. Chilton JK. Molecular mechanisms of axon guidance. *Dev Biol* 2006, 292:13–24.

66. Dickson BJ. Molecular mechanisms of axon guidance. *Science* 2002, 298:1959–1964.

67. Tessier-Lavigne M, Goodman CS. The molecular biology of axon guidance. *Science* 1996, 274:1123–1133.

68. Ogura K, Wicky C, Magnenat L, Tobler H, Mori I, Muller F, Ohshima Y. *Caenorhabditis elegans unc-51* gene required for axonal elongation encodes a novel serine/threonine kinase. *Genes Dev* 1994, 8:2389–2400.

69. Okazaki N, Yan J, Yuasa S, Ueno T, Kominami E, Masuho Y, Koga H, Muramatsu M. Interaction of the Unc-51-like kinase and microtubule-associated protein light chain 3 related proteins in the brain: possible role of vesicular transport in axonal elongation. *Brain Res Mol Brain Res* 2000, 85:1–12.

70. Steven R, Kubiseski TJ, Zheng H, Kulkarni S, Mancillas J, Ruiz Morales A, Hogue CW, Pawson T, Culotti J. UNC-73 activates the Rac GTPase and is required for cell and growth cone migrations in *C. elegans*. *Cell* 1998, 92:785–795.

71. Steven R, Zhang L, Culotti J, Pawson T. The UNC-73/Trio RhoGEF-2 domain is required in separate isoforms for the regulation of pharynx pumping and normal neurotransmission in *C. elegans*. *Genes Dev* 2005, 19:2016–2029.

72. Hedgecock EM, Culotti JG, Hall DH. The *unc-5*, *unc-6*, and *unc-40* genes guide circumferential migrations of pioneer axons and mesodermal cells on the epidermis in *C. elegans*. *Neuron* 1990, 4:61–85.

73. Ishii N, Wadsworth WG, Stern BD, Culotti JG, Hedgecock EM. UNC-6, a laminin-related protein, guides cell and pioneer axon migrations in *C. elegans*. *Neuron* 1992, 9:873–881.

74. Levy-Strumpf N, Culotti JG. VAB-8, UNC-73 and MIG-2 regulate axon polarity and cell migration

functions of UNC-40 in *C. elegans*. *Nat Neurosci* 2007, 10:161–168.

75. Quinn CC, Pfeil DS, Chen E, Stovall EL, Harden MV, Gavin MK, Forrester WC, Ryder EF, Soto MC, Wadsworth WG. UNC-6/netrin and SLT-1/slit guidance cues orient axon outgrowth mediated by MIG-10/RIAM/lamellipodin. *Curr Biol* 2006, 16:845–853.

76. Watari-Goshima N, Ogura K, Wolf FW, Goshima Y, Garriga G. *C. elegans* VAB-8 and UNC-73 regulate the SAX-3 receptor to direct cell and growth-cone migrations. *Nat Neurosci* 2007, 10:169–176.

77. Hao JC, Yu TW, Fujisawa K, Culotti JG, Gengyo-Ando K, Mitani S, Moulder G, Barstead R, Tessier-Lavigne M, Bargmann CI. *C. elegans* slit acts in midline, dorsal-ventral, and anterior-posterior guidance via the SAX-3/Robo receptor. *Neuron* 2001, 32:25–38.

78. Zallen JA, Yi BA, Bargmann CI. The conserved immunoglobulin superfamily member SAX-3/Robo directs multiple aspects of axon guidance in *C. elegans*. *Cell* 1998, 92:217–227.

79. Knobel KM, Davis WS, Jorgensen EM, Bastiani MJ. UNC-119 suppresses axon branching in *C. elegans*. *Development* 2001, 128:4079–4092.

80. Maduro M, Pilgrim D. Identification and cloning of *unc-119*, a gene expressed in the *Caenorhabditis elegans* nervous system. *Genetics* 1995, 141:977–988.

81. Much JW, Slade DJ, Klampert K, Garriga G, Wightman B. The *fax-1* nuclear hormone receptor regulates axon pathfinding and neurotransmitter expression. *Development* 2000, 127:703–712.

82. Luo S, Nonet ML. Regulators of kinesin involved in polarized trafficking and axon outgrowth. *J Biol* 2006, 5:8.

83. Su CW, Tharin S, Jin Y, Wightman B, Spector M, Meili D, Tsung N, Rhiner C, Bourikas D, Stoeckli E, et al. The short coiled-coil domain-containing protein UNC-69 cooperates with UNC-76 to regulate axonal outgrowth and normal presynaptic organization in *Caenorhabditis elegans*. *J Biol* 2006, 5:9.

84. Gitai Z, Yu TW, Lundquist EA, Tessier-Lavigne M, Bargmann CI. The netrin receptor UNC-40/DCC stimulates axon attraction and outgrowth through enabled and, in parallel, Rac and UNC-115/AbLIM. *Neuron* 2003, 37:53–65.

85. Lundquist EA, Herman RK, Shaw JE, Bargmann CI. UNC-115, a conserved protein with predicted LIM and actin-binding domains, mediates axon guidance in *C. elegans*. *Neuron* 1998, 21:385–392.

86. Axäng C, Rauthan M, Hall DH, Pilon M. Developmental genetics of the *C. elegans* pharyngeal neurons NSML and NSMR. *BMC Dev Biol* 2008, 8:38.

87. George SE, Simokat K, Hardin J, Chisholm AD. The VAB-1 Eph receptor tyrosine kinase functions in neural

and epithelial morphogenesis in *C. elegans*. *Cell* 1998, 92:633–643.

88. Ghenea S, Boudreau JR, Lague NP, Chin-Sang ID. The VAB-1 Eph receptor tyrosine kinase and SAX-3/Robo neuronal receptors function together during *C. elegans* embryonic morphogenesis. *Development* 2005, 132:3679–3690.

89. Refai O, Rohs P, Mains PE, Gaudet J. Extension of the *Caenorhabditis elegans* Pharyngeal M1 neuron axon is regulated by multiple mechanisms. *G3 (Bethesda)* 2013, 3:2015–2029.

90. Bray D. Axonal growth in response to experimentally applied mechanical tension. *Dev Biol* 1984, 102:379–389.

91. Heiman MG, Shaham S. DEX-1 and DYF-7 establish sensory dendrite length by anchoring dendritic tips during cell migration. *Cell* 2009, 137:344–355.

92. Komuro H, Yacubova E. Recent advances in cerebellar granule cell migration. *Cell Mol Life Sci* 2003, 60:1084–1098.

93. Schmucker D, Jackle H, Gaul U. Genetic analysis of the larval optic nerve projection in Drosophila. *Development* 1997, 124:937–948.

94. Bentley D, Keshishian H. Pathfinding by peripheral pioneer neurons in grasshoppers. *Science* 1982, 218:1082–1088.

95. Palka J, Whitlock KE, Murray MA. Guidepost cells. *Curr Opin Neurobiol* 1992, 2:48–54.

96. Keleman K, Ribeiro C, Dickson BJ. Comm function in commissural axon guidance: cell-autonomous sorting of Robo in vivo. *Nat Neurosci* 2005, 8:156–163.

97. Kidd T, Bland KS, Goodman CS. Slit is the midline repellent for the robo receptor in Drosophila. *Cell* 1999, 96:785–794.

98. 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.

99. Horvitz HR, Chalfie M, Trent C, Sulston JE, Evans PD. Serotonin and octopamine in the nematode *Caenorhabditis elegans*. *Science* 1982, 216:1012–1014.

100. Sze JY, Victor M, Loer C, Shi Y, Ruvkun G. Food and metabolic signalling defects in a *Caenorhabditis elegans* serotonin-synthesis mutant. *Nature* 2000, 403:560–564.

101. Nathoo AN, Moeller RA, Westlund BA, Hart AC. Identification of neuropeptide-like protein gene families in *Caenorhabditis elegans* and other species. *Proc Natl Acad Sci USA* 2001, 98:14000–14005.

102. Sawin ER, Ranganathan R, Horvitz HR. *C. elegans* locomotory rate is modulated by the environment through a dopaminergic pathway and by experience through a serotonergic pathway. *Neuron* 2000, 26:619–631.

103. Kamiguchi H, Yoshihara F. The role of endocytic l1 trafficking in polarized adhesion and migration of nerve growth cones. *J Neurosci* 2001, 21:9194–9203.

104. Ye B, Zhang Y, Song W, Younger SH, Jan LY, Jan YN. Growing dendrites and axons differ in their reliance on the secretory pathway. *Cell* 2007, 130:717–729.

105. Colavita A, Culotti JG. Suppressors of ectopic UNC-5 growth cone steering identify eight genes involved in axon guidance in *Caenorhabditis elegans*. *Dev Biol* 1998, 194:72–85.

106. Forrester WC, Garriga G. Genes necessary for *C. elegans* cell and growth cone migrations. *Development* 1997, 124:1831–1843.

107. Zallen JA, Kirch SA, Bargmann CI. Genes required for axon pathfinding and extension in the *C. elegans* nerve ring. *Development* 1999, 126:3679–3692.

108. Barriere A, Felix MA. High local genetic diversity and low outcrossing rate in *Caenorhabditis elegans* natural populations. *Curr Biol* 2005, 15:1176–1184.

109. Hall DH, Hedgecock EM. Kinesin-related gene *unc-104* is required for axonal transport of synaptic vesicles in *C. elegans*. *Cell* 1991, 65:837–847.

110. Raharjo WH, Ghai V, Dineen A, Bastiani M, Gaudet J. Cell architecture: surrounding muscle cells shape gland cell morphology in the *Caenorhabditis elegans* pharynx. *Genetics* 2011, 189:885–897.

111. Ghai V, Gaudet J. The CSL transcription factor LAG-1 directly represses *hlh-6* expression in *C. elegans*. *Dev Biol* 2008, 322:334–344.

112. Ghai V, Smit RB, Gaudet J. Transcriptional regulation of HLH-6-independent and subtype-specific genes expressed in the *Caenorhabditis elegans* pharyngeal glands. *Mech Dev* 2012, 129:284–297.

113. Ferrier A, Charron A, Sadozai Y, Switaj L, Szutenbach A, Smith PA. Multiple phenotypes resulting from a mutagenesis screen for pharynx muscle mutations in *Caenorhabditis elegans*. *PLoS One* 2011, 6:e26594.

114. Norman KR, Moerman DG. α spectrin is essential for morphogenesis and body wall muscle formation in *Caenorhabditis elegans*. *J Cell Biol* 2002, 157:665–677.

115. McKeown C, Praitis V, Austin J. *sma-1* encodes a *β*H-spectrin homolog required for *Caenorhabditis elegans* morphogenesis. *Development* 1998, 125:2087–2098.

116. Straud S, Lee I, Song B, Avery L, You Y-j. The jaw of the worm: GTPase-activating protein EAT-17 regulates grinder formation in *Caenorhabditis elegans*. *Genetics* 2013, 195:115–125.

117. Avery L, Horvitz HR. A cell that dies during wild-type *C. elegans* development can function as a neuron in a *ced-3* mutant. *Cell* 1987, 51:1071–1078.

118. Riddle MR, Weintraub A, Nguyen KCQ, Hall DH, Rothman JH. Transdifferentiation and remodeling of

post-embryonic *C. elegans* cells by a single transcription factor. *Development* 2013, 140:4844–4849.

119. Axäng C, Rauthan M, Hall DH, Pilon M. The twisted pharynx phenotype in *C. elegans*. *BMC Dev Biol* 2007, 7:61.

120. Johnston J, Iser WB, Chow DK, Goldberg IG, Wolkow CA. Quantitative image analysis reveals distinct structural transitions during aging in *Caenorhabditis elegans* tissues. *PLoS One* 2008, 3:e2821.

121. Shamir L, Wolkow CA, Goldberg IG. Quantitative measurement of aging using image texture entropy. *Bioinformatics* 2009, 25:3060–3063.

122. Shen EZ, Song CQ, Lin Y, Zhang WH, Su PF, Liu WY, Zhang P, Xu J, Lin N, Zhan C, et al. Mitoflash frequency in early adulthood predicts lifespan in *Caenorhabditis elegans*. *Nature* 2014, 508:128–132.

123. Budovskaya YV, Wu K, Southworth LK, Jiang M, Tedesco P, Johnson TE, Kim SK. An *elt-3/elt-5/elt-6* GATA transcription circuit guides aging in *C. elegans*. *Cell* 2008, 134:291–303.

124. Hardin J. To thine own self be true: self-fusion in single-celled tubes. *Dev Cell* 2008, 14:465–466.

125. Laughlin ST, Bertozzi CR. In vivo imaging of *Caenorhabditis elegans* glycans. *ACS Chem Biol* 2009, 4:1068–1072.

126. Keskiaho K, Kukkola L, Page AP, Winter AD, Vuoristo J, Sormunen R, Nissi R, Riihimaa P, Myllyharju J. Characterization of a novel *Caenorhabditis elegans* prolyl 4-hydroxylase with a unique substrate specificity and restricted expression in the pharynx and excretory duct. *J Biol Chem* 2008, 283:10679–10689.

127. Wadsworth WG, Bhatt H, Hedgecock EM. Neuroglia and pioneer neurons express UNC-6 to provide global and local netrin cues for guiding migrations in *C. elegans*. *Neuron* 1996, 16:35–46.

128. Colavita A, Krishna S, Zheng H, Padgett RW, Culotti JG. Pioneer axon guidance by UNC-129, a *C. elegans* TGF-*β*. *Science* 1998, 281:706–709.