

Review

Gut development in *C. elegans*

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ABSTRACT

The midgut (intestine) of the nematode, *C. elegans*, is a tube consisting of 20 cells that arises from a single embryonic precursor. Owing to its comparatively simple anatomy and the advantages inherent to the *C. elegans* system, the gut has been used as a model for organogenesis for more than 25 years. In this review, the salient features of *C. elegans* gut development are described from the E progenitor through to the 20-cell intestine. The core gene regulatory network that drives specification of the gut, and other genes with roles in organogenesis, lumen morphogenesis and the cell cycle, are also described. Questions for future work are posed.

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1. Introduction

The major functions of the *C. elegans* gut are to import dietary macromolecules, process them metabolically, and store chemical energy. The gut also serves as an entry point for pathogens and activation of immune responses. In hermaphrodites, the gut supplies chemical energy to oocytes for embryonic development after fertilization. The gut is a simple tube consisting of 20 cells that arises from a single cell called E that proceeds through a stereotyped set of

cell divisions and movements. Its simple anatomy and importance in many biological functions, coupled with the many tools available in *C. elegans*, have made the gut the focus of studies for over 25 years. The least-studied aspect of the gut has been its cellular development, both in terms of the genes that drive it downstream of specification, and how the gut primordium dynamically generates the intestine. In this review, the salient features of gut morphogenesis will be described based on recent studies. As listed in Table 1, genes involved in gut development, including those important for specification, differentiation and morphogenesis, will be described. Finally, future work to fully elucidate the intestinal gene network, and address aspects of the robustness of intestinal development, will be proposed.

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Table 1
Examples of genes important for *C. elegans* gut development.

Gene/Product	Mutant Phenotype	Reference(s)
Specification and Maintenance of Differentiation		
<i>skn-1</i> /Nrf2	loss of gut specification in ~80% of embryos	[44]
<i>pop-1</i> /TCF	ectopic specification of gut fate from MS; synergy for loss of gut with <i>skn-1</i>	[50,88]
<i>med-1,2</i> /GATA	loss of gut specification in 15–50% of embryos	[46,56,89]
<i>end-1,3</i> /GATA	complete loss of gut specification (double mutant)	[33]
<i>elt-2</i> /GATA	loss of gut integrity	[54]
<i>elt-7</i> /GATA	no phenotype alone; enhances <i>elt-2</i> phenotype	[55]
Morphogenesis and Cell Polarization		
<i>vang-1</i> /Van Gogh	misalignment of E4 primordium cells in ~40% of embryos	[13]
<i>mab-20</i> /Semaphorin	defects in int2 intercalation	[13]
<i>eph-4</i> /Ephrin	defects in int2 intercalation	[13]
<i>lin-12</i> /Notch	failure of pre-rotation of int2L	[13]
<i>unc-6</i> /Netrin	failure of int3 and int4 rotation; partial failure of int2 rotation	[13]
<i>madd-2</i> /E3 ubiquitin ligase	asymmetric expression of <i>madd-2</i> in left side cells of int2, int3 and int4 important for rotation of int2-4	[13]
<i>erm-1</i> /Ezrin	disorganization of lumen	[36]
<i>chc-1</i> /Clathrin	loss of apicobasal polarity, failure of lumen organization	[37]
<i>dlg-1</i> /Discs Large	loss of adherens junction integrity	[35]
<i>sptl-1</i> /lipid enzyme	loss of apical targeting of vesicles, formation of ectopic lumen	[41]
<i>let-413</i> /Scribble	loss of maintenance of apical localization of terminal web	[17]
<i>ifo-1</i> /Novel	mislocalization of intermediate filaments; synergy with <i>dlg-1</i> and <i>erm-1</i>	[34]
Cell Division		
<i>cdc-25.1</i> /Cdc25	supernumerary gut nuclei (gain-of-function mutation)	[75,76]
<i>cdc-25.2</i> /Cdc25	no nuclear or cell divisions after E16 stage	[77]

2. Nomenclature, function and anatomy

The *C. elegans* digestive tract runs most of the length of the animal (Fig. 1). The fully formed intestine serves the animal for its life span, as cells are not replaced. It is subdivided into the pharynx, intestine, and hindgut; between each pair of regions are a small number of valve cells. In the wild, animals generally consume a moist diet of microbes found primarily on rotting plants and their fruits [1]. The pharynx, analogous to the vertebrate esophagus, is a muscular organ that pumps food [2]. Within the posterior half of the pharynx is the grinder, a cuticle-lined structure that mechanically breaks down food into smaller particles that travel through the pharynx-intestine valve and into the anterior lumen of the intestine. Within the intestinal lumen, the contents slosh around along its length, while macromolecules are broken down by enzymes and the products are absorbed. Defecation through the hindgut occurs by a set of body contractions approximately every 45 s [3]. Bacteria transit the intestine rapidly: With each defecation cycle, 43 +/- 10% of the intestinal volume is expelled, corresponding to a residence time of 1–2 min [4].

The *C. elegans* intestine has additional roles. Animals can store dietary fat in lipid droplets throughout lysosomal compartments in the gut cytoplasm [5]. In hermaphrodites, the intestine is the location of synthesis of vitellogenins, yolk protein precursors that will be delivered to oocytes [6]. It is also a primary site of innate immune responses [7]. Larger macromolecules and some microorganisms can also enter gut cells intact. Consumption of bacteria expressing double-stranded RNA (dsRNA) can stimulate RNA-mediated interference (RNAi) [8]. Particles of the Orsay virus can enter intestinal cells and replicate if the host strain is deficient in RNAi [9,10] and the eukaryotic intracellular pathogen, microsporidia, infects animals through the gut lumen [11].

The entire gut is descended from a single embryonic precursor called E (Fig. 1A). A single-cell origin for gut occurs among distantly related nematodes, suggesting that this trait is ancestral [12]. The E cell undergoes a stereotyped pattern of cell divisions to produce 20 cells (and on occasion, one or two more) that will form the intestine [13–15]. Cells in the gut primordium are identified by their pattern of descent from E and the pattern of division axes, i.e. anterior-posterior, left-right or dorsal-ventral. For example, the four grand-daughters of E are Eal, Ear, Epl and Epr, where 'Eal' indi-

cates the cell that is the left daughter of the anterior daughter of E. A particular stage is referred to by the total number of E descendants present, hence the aforementioned stage is called E4. It becomes more convenient to identify later cells according to their positions at the E16 stage [13].

The gut lumen is found on the apical surface of intestinal cells, lining the digestive tract. A transverse transmission electron microscope image through a larval intestine is shown in Fig. 2. The lumen appears as a flattened oval, lined with a brush border containing microvilli. A flattened shape may better support the body bends that occur during locomotion [13]. The microvillar membrane projections into the lumen are supported by an actin-rich network that emerges from the terminal web, a cytoplasmic layer of intermediate filament proteins (sometimes called the "endotube") that surrounds the lumen [16–18]. Along two sides of the lumen are adherens junction complexes that connect the intestinal cells [17]. The exterior surface of the microvilli, i.e. the apical surface within the lumen, is associated with a glycocalyx. This region likely consists of glycoproteins and enzymes that function in breakdown of macromolecules and protection of the microvilli [19]. The lumen can be modulated dynamically over time to suit developmental stage and food availability [20].

The gut is classically described as consisting of nine "rings" numbered int1 through int9 from anterior to posterior (Fig. 3) [14]. Rings 2 through 9 consist of a pair of cells each, while the first ring consists of four cells. The rings are conveniently referred to by their relative locations in the E16 primordium, using the naming system of Asan et al. (2016). In this notation, int2 through int9 have a left (L) and right (R) cell (e.g. int2L and int2R form int2), while the four cells in int1 are also marked dorsal (D) and ventral (V) (i.e. int1RD, int1LD, int1LV, int1RV). In the later stages of gut morphogenesis, a rotation of several rings occurs to cause most of the left-right pairs to adopt a nearly dorsal/ventral orientation [13,21]. The pairs of cells that form rings 3–9 are often diagrammed as being in anterior-posterior register with respect to one another as shown in Fig. 3. However, the right-side cells are displaced anteriorly from the left side by some 10–30% of their lengths [13]. The outside of the gut is surrounded by a basement membrane [21], and aside from attachment to the foregut and hindgut, is not rigidly attached to the body. The gut is not associated with neurons and is connected to a single muscle at its posterior [18].

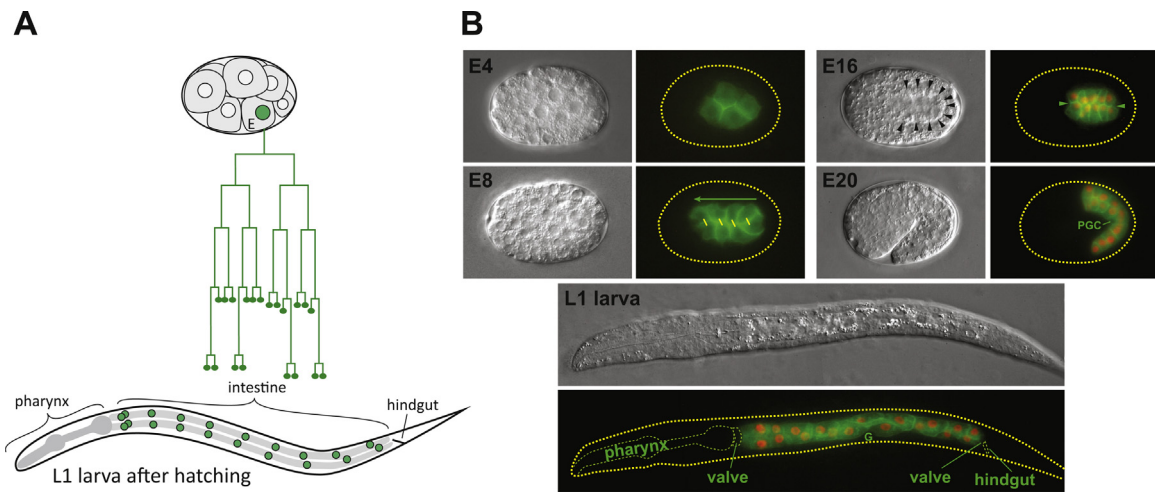


Fig. 1. Lineal origin of the *C. elegans* gut and its appearance in embryos and larvae. (A) The gut forms through a stereotyped pattern of cell divisions that generates 20 descendants of the E blastomere [14]. The intestine is the largest organ in the L1 larva, occupying two thirds of the body length and a substantial fraction in cross section. (B) The appearance of the developing gut by differential interference contrast (DIC) and fluorescence microscopy. Gut cells are marked by a membrane-targeted *end-1::CAAX::GFP* reporter [86] while the nuclei are marked by expression of *elt-2::mCherry* after the E8 stage. At E8, the anterior displacement of the right-hand cells is shown by a green arrow and yellow marks indicating the left/right cell pairs. At E16, the polarization of gut granules away from the midline is shown on the DIC image by black arrowheads, and the midline is shown by green arrowheads in the fluorescence image. At E20, the Primordial Germ Cell lobes (PGC) can be seen inserted into the gut by a reduction in cytoplasmic GFP. In the larva, the gonad (G) remains associated with int5. Methods: Images were acquired on a Canon DSLR and Olympus BX51 microscope, then merged and enhanced in Adobe Photoshop. Anterior is to the left. The E4, E8 and E16 stages are shown in a dorsal view with right upwards, while the E20 (1.5-fold) and larva stages are shown in a lateral aspect with dorsal upwards. A *C. elegans* embryo is $\sim 50 \mu\text{m}$ long, while the larva is $\sim 180 \mu\text{m}$ long.

The postembryonic gut is functional at hatching (the first larval stage, L1). The gut grows with the animal through the L2, L3 and L4 and adult stages. The gut undergoes no further cell divisions but instead increases in size. During L1, the 14 nuclei among the int3 through int9 rings typically undergo one nuclear division to become binucleate, bringing the number of gut nuclei to 30–34 per animal [15,22]. At each larval molt the nuclei undergo an endoreduplication, such that by the adult stage, each nucleus carries a DNA content of 32C [22].

While gut cells are generally similar in overall appearance, some differences occur. The anterior-most gut cells are rich in rough endoplasmic reticulum and secretory vesicles, and have fewer storage granules, while the rest of the intestine has more storage organelles and fewer secretory vesicles [16]. This suggests that most enzyme secretion into the lumen occurs where food enters the gut, while nutrient absorption and storage of chemical energy occurs more posteriorly. This is consistent with the observation that the lumen is larger in int1, and that it is the only ring comprised of four cells. Transcriptional differences also support regional specialization of the gut: Expression of the cysteine protease gene *cpr-1* occurs primarily in the anterior [23], while the phosphatase gene *pho-1* is expressed in the rings posterior to int2 [24].

3. Morphogenesis of the gut

3.1. Cellular events

C. elegans embryos develop from the zygote to 558-cell stage in approximately 14 h at 25 °C [14]. The embryo is transparent and exhibits a nearly invariant pattern of cell divisions, features that have made it a useful system for the study of embryonic development, as changes in cell division or positioning, as a result of experimental manipulation, can be easily detected.

Studies over many years have built up a detailed description of how the gut forms from the E progenitor. These are summarized diagrammatically in Fig. 3. Gut is autonomously specified when E is born, as an E cell that has been removed from the embryo will produce gut-like descendants, although these do not form a tube [21]. After E undergoes mitosis, its daughters Ea and Ep move

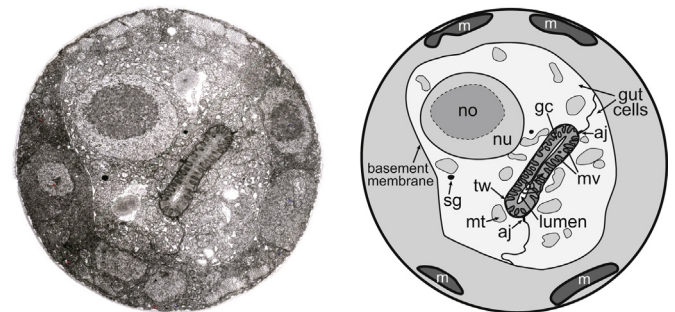


Fig. 2. Midbody cross-section of an intestinal ring in a first larval stage hermaphrodite. The section is located approximately 60% along the anterior-posterior length of the animal and likely shows the int6 ring. Abbreviations: aj, adherens junction; gc, glycocalyx; m, body muscle; mt, mitochondrion; mv, microvilli; no, nucleolus; nu, nucleus. The original micrograph was obtained from wormimage.org (animal L1Q, image print #102, negative #3152-03 taken by Nichol Thomson, Medical Research College, Cambridge, England, and annotated by David H. Hall). The image was adjusted for contrast and stretched to restore circularity to the section in Adobe Photoshop. A similar tracing of a section through the int2 ring in an embryo appears in another work [13].

toward the interior of the embryo, marking the onset of gastrulation [25]. The migration of Ea and Ep into the interior has been studied extensively, and requires both cell-intrinsic and cell-extrinsic mechanisms [25].

Following internalization, the E daughter cells divide along the left-right axis of the embryo, and then divide again along the anterior-posterior to form E8, in which the cells are arranged in a roughly planar configuration. The four E4 cells must be in correct alignment for the E8 cells to adopt this arrangement. However, variation of the E4 cell positions occurs in 27% of wild-type embryos, even though embryos typically show normal positioning of the E8 cells [13]. E4 variation is suppressed by a VANG-1/Van Gogh-dependent mechanism, as *vang-1* mutants exhibit abnormal positioning among the E8 and E16 cells, causing abnormal gut development in 32% of embryos [13,26]. Therefore, variation in positions of cells in the gut primordium can be suppressed by interactions with the surrounding cells.

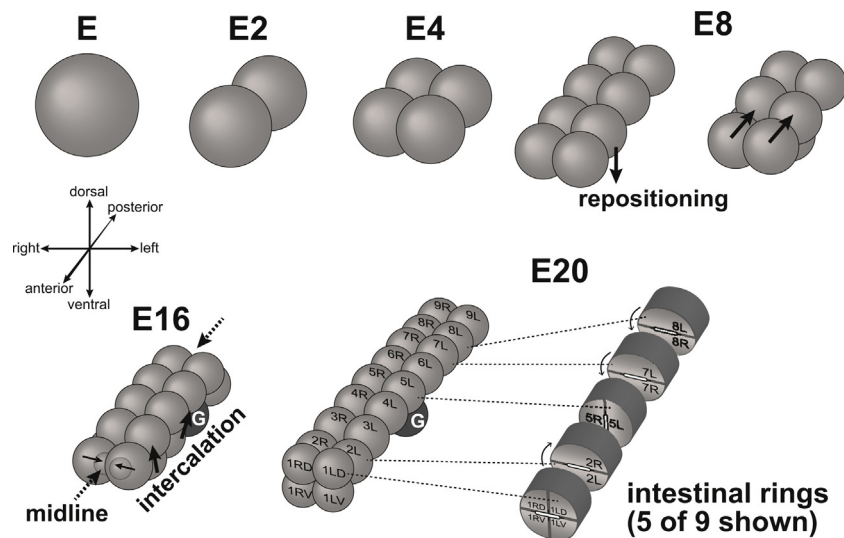


Fig. 3. Features of development of the *C. elegans* intestine. For clarity, E and its descendants are shown by themselves; in later stages, the intestine is depicted as being straight as it would be in the larva. From E to the E16 stage, most cell divisions are roughly synchronous and occur along the antero-posterior axis; only the E2 cells divide in a left-right manner. Hallmarks of gut development are indicated as follows. (i) At E8 stage, two cells reposition ventrally and the space is closed by movement of the most anterior cells. (ii) At E16, the daughters of the repositioned cells intercalate back into the upper tier. (iii) At E16, the nuclei (shown for the anterior-most pair) migrate toward the midline that runs along the anterior-posterior axis. (iv) A cell body of the gonad primordium (G) is shown in close association with the int5 cells. (v) At E20, division of the anterior and posterior pairs of cells results in the four cells of int1, and the posterior-most int8 and int9 pairs. (vi) At E16, a 'rotation' of the int2, int3 and int4 pairs of cells begins. (For simplicity this rotation is not shown in the leftmost E20 diagram.) A later rotation occurs in the posterior rings; int5 does not rotate. The rotations are shown by arrows on the appropriate subset of rings depicted in the final diagram, which also shows the lumen and adherens junctions. Diagrams were drawn in Adobe Illustrator, modified from prior work [13,21].

Although the E8 primordium consists of four pairs of cells in a planar array, the right-hand cells are displaced anteriorly (shown in Fig. 1B, E8 fluorescence panel). This displacement, which persists through to the adult intestine, requires Notch signaling in the primordium [13]. Following formation of the E8 primordium, the second left-right pair of E8 cells (just behind the anterior-most pair) becomes repositioned underneath the remaining six cells prior to the next round of cell division, a movement driven by dorsal constriction [13]. The E8 cells then divide along the anterior-posterior axis with the posterior-most pair of daughter cells displaced slightly ventrally. The four cells, descended from the two that were repositioned earlier, undergo an intercalation back into the top plane of cells to align the E16 cells to a common A/P axis.

The movement of these cells back into the dorsal plane occurs in two steps [13]. First, the posterior pair of cells intercalates between the dorsal third and fourth pair of cells to become the future L/R cells of int5. This is driven by ventral constriction of the dorsal cells, permitting the int5 cells to migrate fully into the dorsal plane of cells, and takes ~65 min at 20–22 °C. Second, the anterior pair intercalates between the first and second pair of cells to become the L/R cells of int2; this is shown in the first part of Fig. 4. The intercalation of the int2 cells is more variable than int5, taking between 24 and 48 min, and generally occurring through one of two modes. The first, which occurs in 27% of wild-type embryos, involves intercalation of int2L/R together in a manner similar to int5L/R: Both cells extend dorsal processes and that meet up at the dorsal midline. In the second mode, int2L extends dorsally but stalls, while int2R rotates to position the int2 ring dorsally. Intercalation requires the Ephrin EPH-4, and the semaphorin-2a ortholog MAB-20 [13]. Ephrins and semaphorins act in short-range cell signaling and morphogenesis in many developmental contexts [27,28]. A failure of int2 intercalation is not a lethal condition in the laboratory, as mutations in *eph-4* or *mab-20* are generally viable; the gut is intact and functional, although abnormal patterning of the adherens junctions becomes apparent [13].

The E16 stage serves as the reference point for the nomenclature used by Asan et al. (2016), as the arrangement of the rings is now established. A rotation, called intestinal twist, occurs around the future lumen [21] (Figs. 3 and 4). The int2, 3 and 4 rings rotate in their respective pairs, in a way that eventually positions their right-hand cells dorsally. Viewed from the front toward the posterior, the rotation occurs in a clockwise direction. In generating the final E20 gut, the anterior-most cells undergo a D/V pair of divisions, resulting in the four cells of int1, while the posterior-most cells undergo mitosis to form the int8L/R and int9L/R cells (Fig. 4). The E20 diagram in Fig. 3 shows the final orientation of the int2 cells as an example; this can be compared with the int5 cells that do not rotate. The posterior rings (e.g. int7 and int8 in Fig. 3) are also rotated in the newly hatched larva, implying that they also undergo a rotation. These are apparently counterclockwise, as it is their left cells that lie dorsally in the E20 gut [13].

Cellular and genetic requirements for the int2–4 rotations have been investigated [13,29]. Laser ablation of the surrounding cells, or their precursors, established that rotation requires contact with overlying dorsal ectoderm cells and underlying neural cells. The int2L cell begins rotation 30 min earlier than the remaining cells; this 'pre-rotation' requires LIN-12/Notch. Components of the UNC-6/Netrin pathway are also required, as mutants in *unc-6* show a complete failure of int3 and int4 rotation. Rotation of int2 can occur in *unc-6* mutants, however its rotational direction is often counterclockwise instead of clockwise, and fails to show the int2L pre-rotation. Similar phenotypes are observed with loss of the Netrin ligand UNC-40/DCC and MADD-2, an E3 ubiquitin ligase that regulates UNC-40 signaling [13]. A MADD-2:GFP reporter is expressed specifically in the left-hand int2–4 cells, and this asymmetric expression is correlated with correct rotation [13]. The failure of int2–4 rotation is also not a lethal condition, as animals survive but exhibit abrupt changes in the orientation of the lumen between int1 and the int2–4 region. This shows that intestinal twist likely occurs to maintain the alignment of the flattened shape of the lumen between rings.

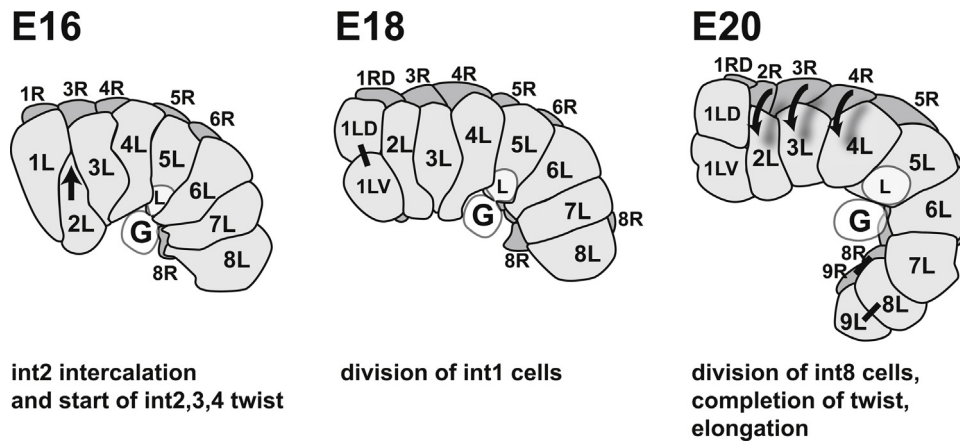


Fig. 4. Features of gut development from mid-E16 to early E20, a period of approximately 70 min. The diagrams outline the cells of the gut primordium; see Fig. 1, E20 fluorescence image for comparison. Following the intercalation of the int5L/R cells, int2L/R undergo intercalation (arrow) as shown in the E16 diagram. Intestinal twist, the rotation of the int2, int3 and int4 rings, commences at this stage. The int1 cells divide in the dorsal/ventral direction to generate the four cells of int1 at the E18 stage. At the E20 stage, the int8 cells divide along the anterior/posterior axis to generate the full complement of 20 intestinal cells. Intestinal twist is apparent, shown by the three arrows. In all three diagrams, the approximate location of one of the Primordial Germ Cell bodies (G) and lobe (L), inserted into int5, is shown. Diagrams were traced from 3D reconstructions in Asan et al. (2016) [13]. Appearance of the gonad primordium is interpreted from fluorescence images in Asan et al. (2016) [13] and Abdu et al. (2016) [30]. Anterior is to the left and dorsal is up.

From the E16–E20 stages, the two Primordial Germ Cells (PGCs) each have a process, or lobe, inserted into the int5 pair of cells [14] (Fig. 4). Over a period of approximately two hours, the lobes are actively removed by scission of the ‘neck’ joining to lobe to the cell body outside the gut, in an event described as cell cannibalism [30]. This event remodels the contents of the gonad, removing most of the mitochondria and other cell debris from the PGCs.

Throughout morphogenesis, the remaining somatic tissues develop in parallel. The embryo becomes enclosed in the epidermis (classically called the hypodermis) and elongates, ultimately reaching some 3.5x the length of the egg. When body muscles become functional at the two-fold stage of elongation, the animal begins to writhe about within the egg. Intestine elongation follows embryo elongation and is likely driven by the same muscular contractions [31], as mutations that affect embryo elongation also prevent elongation of the intestine [21,32]. Morphogenesis of the rest of the embryo can occur without the gut, at least some of the time, although the resulting larvae undergo developmental arrest [33].

3.2. Formation of the gut lumen

The earliest signs of lumen formation occur at E16. Microtubule-dependent cytoplasmic polarization becomes apparent as nuclei migrate toward the midline that runs along the length of the primordium (Figs. 1B and 3, E16 stage), while gut granules and other organelles migrate away from the midline [21]. The lumen forms ‘piecemeal’ within each of the rings, beginning as small regions of cell separation between pairs of cells. Appearance of these gaps coincides with intracellular accumulation of small apical vesicles near where the lumen will form, and localization of adherens junction (AJ) components near the midline [21]. The AJs are enriched in actin, the novel coiled-coil protein AJM-1, and DLG-1/discs large [34,35]. As the lumen forms, the AJs become visible as distinct pairs of belts around the lumen (Figs. 2 and 3), and at the junction between rings, resembling a ladder running the length of the intestine. The integrity of the AJs requires LET-413/Scribble and DLG-1 [17,35].

Proper lumen structure requires proteins that support the microvilli and establish or maintain apicobasal polarity. One is the Ezrin/Radixin/Moesin membrane-cytoskeleton linker protein ERM-1, which localizes just inside the forming luminal surface [36].

ERM-1 is first detected at the E16 stage when polarization occurs [37]. Loss of *erm-1* results in a distorted lumen with regions of varying width, although the AJs and polarity remain intact. ERM-1 function is mediated in part by the actin gene *act-5* and the β -H-Spectrin gene *sma-1*, consistent with a role for ERM-1 in stabilization of the apical membrane onto the underlying cytoskeleton.

Trafficking of endosomal vesicles plays an essential role in formation and maintenance of the lumen. When the Clathrin ortholog *chc-1* is depleted, ERM-1:GFP becomes distributed throughout the membranes of the gut primordium while the AJs remain intact [37]. Partial loss of *chc-1* results in surviving larvae and adults with ectopic lumen structures that contain an underlying terminal web. Restoration of *chc-1* function to larvae can decrease the severity of this phenotype, consistent with an ongoing role for CHC-1 during growth of the intestine when new luminal membrane must be deposited apically. As in other systems, Clathrin and AP-1 likely function in proper apical trafficking of post-Golgi vesicles. The AP-1 adaptor genes *aps-1* and *apb-1* show similar loss-of-function phenotypes as loss of *chc-1*, consistent with the known dependence of Clathrin on adapter proteins [37,38]. Loss of Clathrin and AP1 also cause a decrease in apical enrichment of vesicles labeled with GFP fusions to endosomal proteins such as RAB-11 [37,39].

Recent work revealed that lipid biogenesis plays an important role in lumen formation, likely due to the role of specialized lipids in sorting of lumen components to the apical membrane. Apical trafficking is dependent on synthesis of glycosphingolipids (GSLs), as disruption of enzymes in lipid biosynthesis results in ectopic lumen formation [40]. Loss of function of *let-767*, which encodes an enzyme in sphingolipid biosynthesis, results in defects similar to loss of *chc-1* [37]. Maintenance of apicobasal polarity also requires branched-chain fatty acid synthesis, as knockdown of either the fatty acid elongase gene *elo-5*, or the serine palmitoyltransferase gene *sptl-1*, dramatically affects trafficking of apically localized lumen components [41].

Additional factors restrict lumen to the apical surface. When LET-413/Scribble is absent, ERM-1, cortical actin and the terminal web become progressively localized on the lateral and basal surfaces of the gut cell membranes [17,40]. LET-413 is normally distributed basolaterally, where it likely restricts expansion of the terminal web beyond the apical surface. The novel protein IFO-1 is required for proper organization of the intermediate filaments and actin in the terminal web [34]. LET-413 also restricts IFO-1

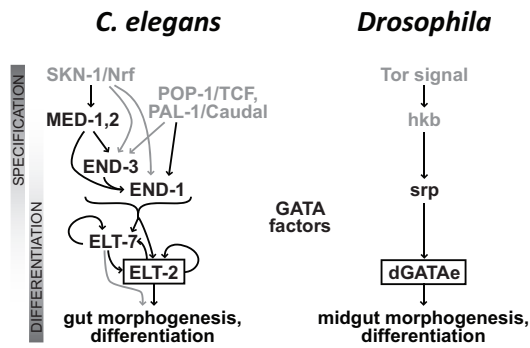


Fig. 5. A GATA factor network controls gut specification in *C. elegans* and *Drosophila*. In *C. elegans*, specification results from the combined maternal inputs of SKN-1/Nrf2, POP-1/TCF and PAL-1/Caudal, which activate expression of zygotic END-1 and END-3. SKN-1 acts on the *end* genes in part through direct activation of *med-1,2* [46]. In turn, END-1,3 activate the self-maintaining expression of *elt-2* and *elt-7*, with *elt-2* being the main gut fate maintenance factor [55,69]. The endogenous expression of the upstream specification factors is transient. In *Drosophila*, a similar cascade of GATA transcription factors (dark text) controls gut development, even though the upstream activators are different and downstream gut anatomy is more complex [65,87]. The diagrams are redrawn from previously published works [52,64].

to the apical region. Overall, therefore, a complex network of cell biological functions is essential for the proper targeting of lumen components during embryonic gut development, and maintenance of the lumen throughout larval development and adulthood.

4. Gut specification

The core Gene Regulatory Network that specifies the *C. elegans* gut is summarized in Fig. 5. Specification occurs when the redundant single zinc finger, GATA-type transcription factor genes *end-1* and *end-3* are activated in the early E lineage [42,43]. The *ends* have overlapping function; mutation of both genes abolishes all gut specification, causing E to adopt the fate of the mesectodermal precursor C [42,43]. Activation of the *ends* occurs downstream of the maternal factors SKN-1 and POP-1. SKN-1 is a bZIP/homeodomain transcription factor that specifies E and its sister cell MS [44,45]. SKN-1 activates specification of E in part through activation of the divergent GATA factor genes *med-1* and *med-2* [46]. SKN-1 and MED-1,2 activate *end-1* and *end-3* directly [47–49]. Loss of *skn-1* or the *meds* does not fully block specification due to other regulatory inputs into *end-1,3* activation. Most of this additional activity comes from the TCF-like regulator POP-1. In E, POP-1 activates the *ends* through association with the divergent β -catenin, SYS-1 [50,51]. There is also evidence for a minor contribution to *end-1,3* activation from PAL-1/Caudal [49,52,53].

The role of the END-1 and END-3 factors is ultimately to activate expression of the terminal gut regulator *elt-2*. Loss of *elt-2* is compatible with gut specification, but the gut loses integrity after embryonic development, resulting in larval lethality [54,55]. Another GATA factor gene, *elt-7*, is activated in the early E lineage before *elt-2*. Although loss of *elt-7* alone has no phenotype, loss of *elt-2* and *elt-7* together has a stronger phenotype than loss of *elt-2* alone, indicating that ELT-7 promotes some aspects of gut fate [55]. Complex synergistic interactions occur among the *C. elegans* zygotic gut specification factors, suggesting these have some unique functions [52,56].

The four GATA factors END-1, END-3, ELT-2 and ELT-7 comprise a set of ‘endodermal GATAs’ with similar DNA-binding properties [57,58]. It is likely that the redundancy and structure of the gene network contribute to the robustness of gut specification, such that all these factors make additive contributions to promote timely activation of ELT-2 [52]. Indeed, forced early expression of *elt-2* in the E lineage can rescue simultaneous loss of *end-1*, *end-3*, *elt-2* and

elt-7, supporting the notion that the purpose of END-1,3 and ELT-7 is to activate ELT-2 [57]. The network of GATA factors upstream of *elt-2* may be fairly recently evolved, perhaps by duplication of *elt-2* [59], as only close relatives of *C. elegans* have genes identifiable as *end-1/3* or *med-1/2* orthologues [60].

The endodermal GATAs can function as ‘master regulators’: In the early embryo, forced ectopic expression of any of END-1, END-3, ELT-2 or ELT-7 alone is sufficient to promote widespread endoderm specification [42,54,55,61]. Forced expression of either ELT-2 or ELT-7 can also reprogram some differentiated cells toward an endoderm fate, in a surprising example of ‘transorganogenesis’ [62,63]. These latter results point to a regulative, self-organizing ability of the intestine when generated in an ectopic spatial and temporal context.

There is evidence of broader roles for GATA factors in endoderm in other animals. A small network of endoderm GATA factors also specifies the midgut in *Drosophila* (Fig. 5). In a cascade similar to that of *C. elegans*, the *Drosophila* GATA factor *serpent* (*srp*) activates the GATA factor *dGATAe*, which like *C. elegans* ELT-2 is essential for midgut differentiation [64]. Upstream activation occurs by unrelated mechanisms, and both *srp* and *dGATAe* also expressed outside of the endoderm [65]. In vertebrates, the GATA4/5/6 genes are important for endoderm development [66,67]. Expression of these is complex and occurs in non-digestive tissues such as the heart. Despite these differences, forced overexpression of *C. elegans end-1*, or *Drosophila srp* or *dGATAe* can induce endoderm differentiation in *Xenopus* ectoderm, demonstrating conservation of function [65,68]. Hence, it is possible that a GATA factor was associated with endoderm in a common ancestor of vertebrates and invertebrates.

Perhaps unexpectedly, genes active in the early endoderm also have functions in the adult intestine. A partial compromise of *end-1,3* activation, which delays activation of *elt-2*, causes changes to adult gut metabolism for reasons that are not yet understood [52]. ELT-2 is a central regulator of many gut functions long after gut development is complete, as its loss in adults compromises expression of genes encoding hydrolytic enzymes, and those involved in innate immunity, among many other functions [7,69]. SKN-1 is structurally similar to mammalian Nrf proteins that control protective responses to various stresses, a role it also has in the adult *C. elegans* intestine [70,71]. Finally, the PHA-4/FoxA factor is essential for development of the pharynx, but it is expressed throughout the developing digestive tract and regulates longevity in adults [72–74].

5. Control of the cell/nuclear divisions in the E lineage

Despite a highly stereotyped pattern of cell divisions, the *C. elegans* gut is surprisingly tolerant of changes in the number of gut cells. Two Cdc25 orthologs are known to influence nuclear and cell divisions with the E lineage. Gain-of-function mutations in *cdc-25.1* cause an increase in embryonic cell divisions specifically in the gut, resulting in embryos that carry greater than 30 gut nuclei, and in adults, up to 60 [75,76]. In the embryo, the supernumerary nuclei correspond to extra cells (M.M. and Gina Broitman-Maduro, unpublished observations). In adults, it is likely that the additional nuclei (beyond those found in the embryo) are the result of nuclear division, just as occurs in the wild type [15]. The Cdc25 ortholog CDC-25.2 is required for all nuclear divisions after the E16 stage [77]: Loss-of-function *cdc-25.2* embryos hatch with a 16-cell gut that also fails to undergo both the binucleation and endoreduplication that occur in the wild type. In both of these cases, specification of endoderm fate is not affected. More surprisingly, even with fewer cells (i.e. 16) or too many (greater than 50) the gut seems to form relatively normally with little other phenotypic consequences, at least in the laboratory. Exami-

nation of the gut primordium in *cdc-25.1* gain-of-function mutants suggests that organogenesis accommodates the extra cells, sometimes producing what appear to be extra intestinal rings (M.M. and Gina Broitman-Maduro, unpublished observations). In mutant backgrounds affecting morphogenesis it is also possible for rings to consist of three or four cells outside of int1 [13]. In mutants that partially compromise specification, the number of gut cells also varies among surviving embryos and adults [52,56]. Together, these results suggest that the mechanisms that drive *C. elegans* gut morphogenesis are not strictly dependent on the number of cells made by the E cell, and point to further mechanisms that are robust in promoting formation of a functional intestine.

6. Future questions

With a recent high-resolution description of the cellular events that occur in gut morphogenesis, newer *in vivo* markers and methods that allow identification of transcripts from single cells, the field is poised to investigate new questions about *C. elegans* gut development [13,78,79]. Some asymmetrically expressed factors are apparent in the gut primordium, and in the surrounding cells, but most of these have not yet been connected to the core endoderm specification network or specification mechanisms in other tissues [13,21]. It should now be possible to define the gene regulatory network that drives all steps of gut development, starting with a cell-by-cell description of the transcriptome. Similarly, regional functionalization of the intestine could be studied by examining the transcriptome of cells in each of the nine rings. It would also be of interest to identify pre-patterning factors that specify such differences in the earliest stages in the primordium, extending earlier findings [21,29]. Open-ended screens have likely missed many factors that operate in the early gut, presumably due to redundancy or because such factors are used in other developmental contexts. Tissue-specific knockout methods may be a way to identify requirements for such factors; alternatively, focused screens to enhance compromised but viable backgrounds may identify new factors as has been recently done [80].

Although the early gut specification network is well-understood, the evolution of the network itself has been less studied. It is known that the contributions of SKN-1 and POP-1 are different in the related nematode, *C. briggsae*, and that the *med* genes experience rapid gene duplication [81,82]. As pointed out above, outside of close relatives of *C. elegans* (including *C. briggsae*), there appear to be no recognizable *med* or *end* genes at all, raising the question of how the more widely conserved *elt-2* gene becomes activated in these other species [60,83].

Another area for exploration is to understand mechanisms that promote the robust formation of the intestine. Beyond the reproducibility of the cell lineage, *C. elegans* has evolved ways to suppress phenotype variation across the embryo due to environmental stress or physical deformation of the egg [84,85]. As described above, the gut primordium forms a functional intestine even when the number of gut cells has been changed drastically. With new markers and methods, the mechanisms by which the gut primordium accommodates such changes can now be examined.

7. Conclusions

For the past 25 years, the *C. elegans* gut has been a useful model for organogenesis due to such advantages as its defined cell lineage, transparency, rapid development, ease of genetic analysis, and a well-established core gene regulatory network. With new methods to follow development and to establish the transcriptomes of individual cells, future work promises to make further advances that will reveal, at high spatiotemporal resolution, how a single

cell gives rise to an entire germ layer. As many aspects of gut development, such as polarization and morphogenesis, involve similar mechanisms in other systems, it is likely that these newer findings will continue to be relevant to development in many animals.

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