Patterning the *C. elegans* embryo

moving beyond the cell lineage

The *Caenorhabditis elegans* embryo undergoes a series of stereotyped cell cleavages that generates the organs and tissues necessary for an animal to survive. Here we review two models of embryonic patterning, one that is lineage-based, and one that focuses on domains of organ and tissue precursors. Our evolving view of *C. elegans* embryogenesis suggests that this animal develops by mechanisms that are qualitatively similar to those used by other animals.

The invariance of the *C. elegans* cell lineage indicates that there is a link between the pattern of cell divisions throughout embryogenesis and the ultimate fate that each cell assumes[1]. How is this remarkable feat accomplished? In this review we focus on two patterning mechanisms that function during gastrulation. The first mechanism establishes anterior–posterior (A–P) polarity between pairs of daughter cells at each cell division. The series of A–P choices throughout embryogenesis is thought to generate a combinatorial code that is critical for establishing different terminal cell fates. This system relies on the cell lineage for embryonic patterning. The second mechanism establishes domains of organ or tissue precursors in the early gastrula. These domains imply that groups of cells are set aside into developmental fields that will give rise to organs or tissues. These domains are composed of cells from many different sublineages, arguing that this patterning system is not lineage-based.

In this review, we describe the observations that led to the lineage and organ/tissue-derived models of development, and we explain how they might function together during embryogenesis. We do not discuss the relative contributions of cell intrinsic or cell signaling processes involved in A–P or organ/tissue patterning, nor do we describe how the left–right axis is established. These subjects are covered in several recent reviews[2–4].

C. elegans embryogenesis

C. elegans embryogenesis can be divided into three periods[1]. During the first 100 min, five divisions give rise to 28 cells, including six 'founder' cells called AB, MS, E, P_4, D and C (Fig. 1). With the exception of the midgut (originating from E) and the germ line (from P_4), no single tissue or organ is made up from descendants of only a single founder cell, and most of the initial 28 blastomeres contribute to many cell types (Fig. 1). The second period begins after the founder cells have been born, when the precursors of the digestive tract gastrulate into the interior of the embryo (the 28-cell stage). Over the next 4 h, six rounds of cell division coupled with cell rearrangement establish a triploblastic embryo with an outer layer of epidermis and a nervous system, a median layer of muscles and other mesodermal derivatives, and an inner tube consisting of the pharynx, midgut and rectum. The third period takes place during the final 8 h. Terminal differentiation and morphogenesis occur, with only a very few additional cell divisions. We focus on the development of the epidermis, pharynx and midgut as much less is known about early myogenesis or neurogenesis[5–7].

Embryonic patterning by cell lineage-based mechanisms

Maternal genes specify the fates of early blastomeres

During the first embryonic period, the body axes are established under the direction of maternally endowed products (reviewed in Refs 2–4). The A–P axis is defined by the position of sperm entry, which is then interpreted by the products of the *par-1–6* genes, four of which encode cortically localized (PAR) proteins, and by the cytoskeleton. During subsequent cleavages, positioning of the mitotic spindle and asymmetric localization of cell-fate regulators are controlled coordinately so that each blastomere develops in response to a stereotyped pattern of cell signals from its neighbors, which are coupled with autonomously acting factors. Mutations that disrupt the cell-fate regulators that function at this early stage alter the development of individual blastomeres. Consequently, all of the descendants that emerge from a given blastomere are affected, independent of the tissues and organs that those descendants will join ultimately. In other words, development before gastrulation is driven by mechanisms that control the behavior of individual sublineages.

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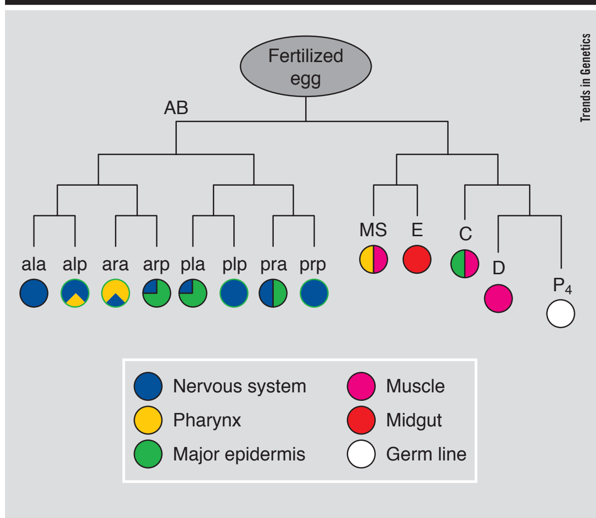
FIGURE 1. Distribution of cell types within the *Caenorhabditis elegans* lineage



The early *Caenorhabditis elegans* cell lineage is shown along with the cell types generated from each blastomere (indicated within a circle). The first eight AB great-granddaughters are abbreviated using their last three letters (i.e. ala instead of ABala). Vertical lines represent cells, horizontal lines represent cell divisions. Colors represent the major different tissues and organs formed: epidermis (green), midgut (red), nerves (blue), body wall muscles (pink), germ line (white) and pharynx (yellow). The size of each pie slice is proportional to the number of cells arising from an individual blastomere. Traditionally, epidermal cells are subdivided into 'major' and 'minor' epidermal cells. The major epidermal cells constitute the external epithelium, whereas the minor cells fulfill specialized functions (e.g. anal cells, terminal epithelia in the head and tail, excretory system). 'Minor' epidermal cells are born after major epidermal cells and originate from four distinct lineages that are symbolized in this diagram by circles with a green outline. Founder cells are indicated by MS, E, C, D and P_4.

A–P polarity establishes different cell fates

Recent experiments from several labs support a model of embryonic patterning that depends on a global system of anterior or posterior (A–P) fate specification. According to this model (Fig. 2a), the complex and invariant nature of the *C. elegans* cell lineage depends on up to six binary A–P decisions (i.e. the divisions that occur after the founder cells have been established). At each longitudinal cell division, the anterior or posterior daughter cell activates either anterior or posterior specification genes, which function at that moment of development. Thus, the fate of an individual cell at any point during embryogenesis reflects the identity of its ancestral founder cell modified by the history of cumulative A–P divisions leading up to its birth. For example, the DNA-binding protein SKN-1 is thought to specify blastomere identity for the founder cells MS and E (Refs 2–4). According to the A–P patterning model, the fates of individual MS and E descendants are established by SKN-1 activity acting in conjunction with several rounds of anterior or posterior cell-fate specification. Ultimately this system generates a unique A–P code for each terminal cell fate throughout the embryo.

The maternal genes *lit-1* and *pop-1* repeatedly link A–P cell divisions with cell-fate decisions after the eight-cell stage[8,9]. POP-1, a Tcf/Lef homolog regulated by WNT-dependent signalling in certain cells[10,11], is more abundant in anterior cells compared with their posterior sisters[9,12]. This asymmetry is specific to A–P divisions because transverse divisions result in equal levels of POP-1 in each daughter cell. POP-1 appears to specify anterior identity because anterior daughters assume the fates of their posterior sisters when *pop-1* activity is reduced[9]. Conversely, mutations in *lit* result in posterior daughters adopting the fates of their anterior sisters[8]. *lit-1* might act by repressing POP-1 activity or expression in posterior cells because double *lit-1*:*pop-1* mutants resemble *pop-1* single mutants in at least one A–P decision[8]. The lineage transformations observed for different *lit-1* or *pop-1* mutants suggest that the establishment of A–P polarity in two daughter cells does not depend on wild-type *pop-1* or *lit-1* activity during previous cell divisions. Rather, polarity is reset at each cell division by mechanisms that are not yet undestood[9].

Two recent papers show that *lit-1* codes for a homolog of the MAP kinase-like gene called *Nemo* in *Drosophila* and Nemo-like kinase (NLK) in vertebrates[54,55]. LIT-1 kinase can be activated by components of the wingless signalling pathway such as WRM-1, which is homologous to β-catenin, or MOM-4, a MAP kinase kinase kinase similar to TAK. Activated LIT-1 can phosphorylate POP-1, which may be important for maintaining low levels of POP-1 in posterior cells since POP-1 is distributed symmetrically between anterior and posterior daughters when *lit-1* activity is lost. However, the direct consequence of POP-1 phosphorylation by LIT-1 is not yet understood. The biochemical functions of the LIT-1 and MOM-4 homologs NLK and TAK1 appear to be conserved in vertebrates[56].

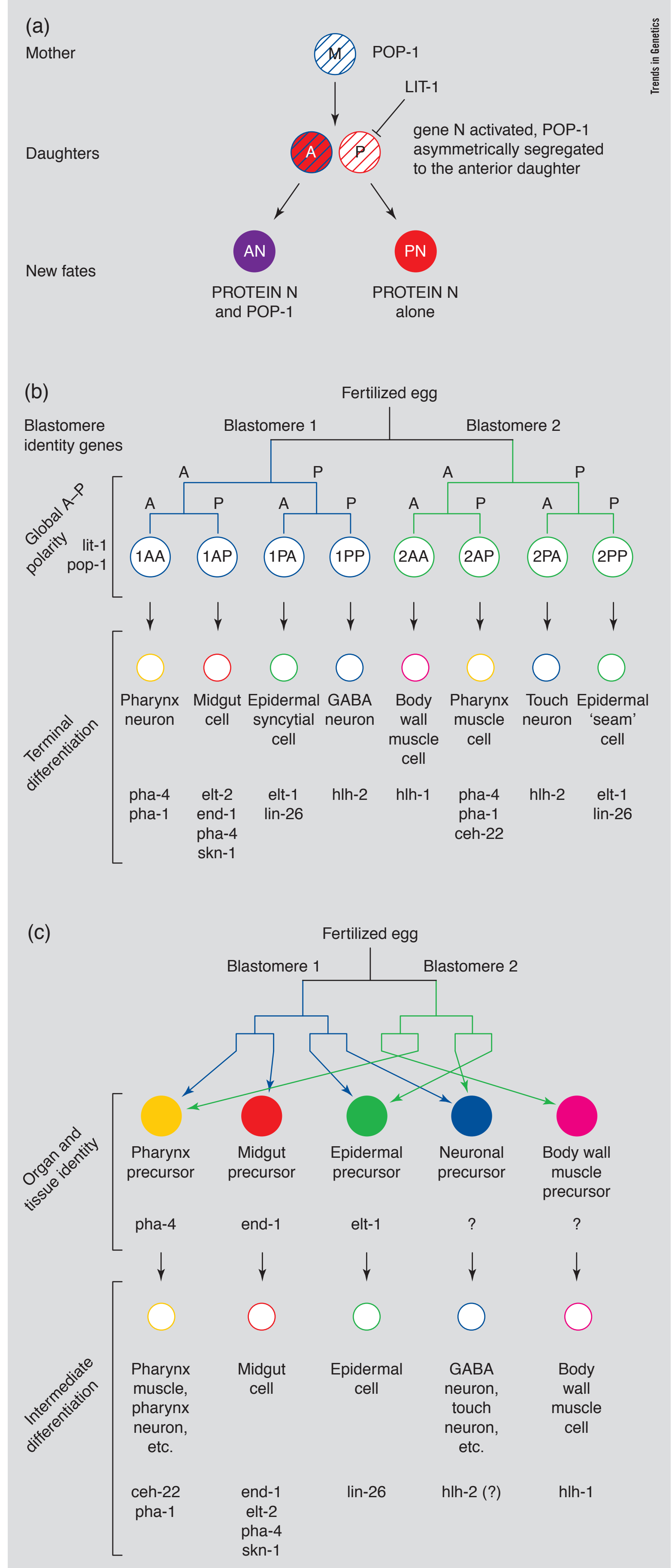
Independent evidence that A–P polarity plays an important role in patterning the *C. elegans* embryo comes from the study of *vab-7*, an *even-skipped* homolog, and of *ceh-13*, a *labial*-like *Hox* gene. The gene *vab-7* is first expressed in the four posterior great-granddaughters of the C blastomere. These cells and their descendants behave as though they have assumed an anterior fate in *vab-7* mutants[13]. The *ceh-13* gene is expressed in all posterior descendants of the AB and E blastomeres, as well as in D (which is the posterior daughter of P_3), beginning at the 28-cell stage. Expression of *ceh-13* in posterior cells depends on the A–P identity and fate of the cell, but not on its position within the embryo[14]. As was recently shown[57], *ceh-13* is not required zygotically for intestine patterning, however its activity is essential for head morphogenesis and the proper positioning of cells in the anterior part of the embryo.

Cells that derive from a single founder cell are grouped into regions

Based on our knowledge of blastomere identity and A–P specification genes, lineage-based mechanisms are essential to construct the worm embryo. Is there additional evidence to support this idea? Using a powerful time-lapse video imaging system, Schnabel *et al.*[15] observed that the descendants of the eight AB great-granddaughters and founder cells MS, E, C, D reproducibly occupy distinct regions in the embryo during the gastrula stage. Whereas the positions of individual cells within a region can vary slightly, the location of each region is invariant from embryo to embryo (Fig. 3a). The authors suggest that these regions define functional units, possibly analogous to segments or compartments in *Drosophila*[15–17]. The difference is that a single blastomere and its descendants comprise an entire positional value in a worm. This hypothesis predicts that removal of one cell early in development will destroy an entire positional field. Thus, the mosaicism of the *C. elegans* lineage could stem from the small number of cells involved, whereas regulative

FIGURE 2. Two models of embryonic patterning in *Caenorhabditis elegans*

(a) Model of *pop-1* and *lit-1* function (adapted from Ref. 9). In this model, an embryonic mother cell (M) expresses high levels of POP-1 (blue stripes). Factors of unknown nature polarize this cell along the A–P axis, such that when M divides POP-1 is partitioned to the anterior daughter cell (A) but not to its posterior sister (P). Independently of polarization and *pop-1*, new genes (*gene N*; red stripes) are switched on in both daughter cells. Depending on the presence of *pop-1*, or on its absence, the daughter cells will acquire a new fate (purple and red). By a mechanism that is still unclear, the polarization and high POP-1 levels are restored in both daughter cells before they divide (not illustrated). This cycle of events is reiterated at each new longitudinal division, presumably with a different set of *N* genes. Based on genetic and biochemical results, the LIT-1 can phosphorylate POP-1, which could prevent its accumulation or its activity posteriorly. (b) The lineage-based model of embryonic patterning. According to this model, the combinatorial input of genes that specify the identity of blastomeres and of genes that control A–P polarity (among which are *pop-1* and *lit-1*, see panel a) determine whether a cell expresses a certain combination of differentiation regulators that specify particular cell types. For simplicity, we illustrate this model without cell–cell interactions using a hypothetical lineage originating from two blastomeres. This lineage is shown to generate, after two A–P binary switches, a specific type of pharyngeal neuron, a midgut cell, a particular type of epidermal cell, a particular neuron, and so on (for further details see Refs 2–4). Genes such as *vab-7* and *ceh-13* (see text) appear to respond to A–P cues within specific sublineages and could thus implement changes specified by *pop-1* and *lit-1*. (c) The organ/tissue specification model, in which identity genes endow precursor cells with organ or tissue identity. The identity genes are predicted to activate differentiation genes that control the final stages of development. The organ/tissue identity genes are predicted to begin to function between the 50-cell and 80-cell stages (see text). Neuronal and body wall muscle identity genes remain to be identified. Several neuronal differentiation genes have been omitted for clarity (these are reviewed in Ref. 7). The lineage diagram in (c) with its two blastomeres and their descendance is hypothetical.

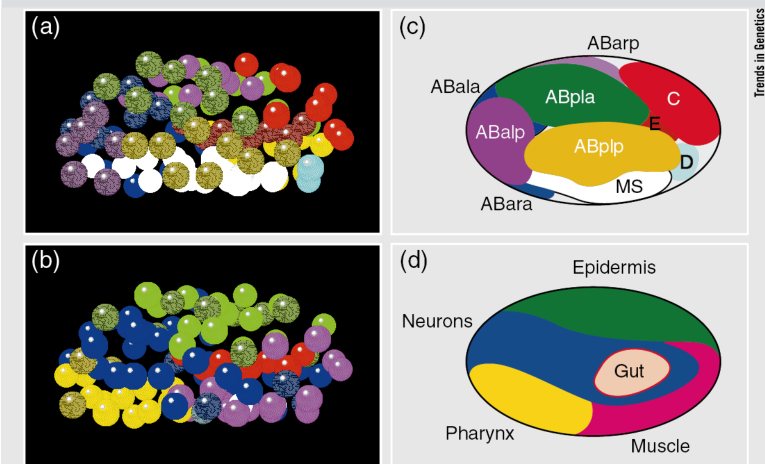


development (in the classical sense) might depend on large numbers of embryonic cells[15–17].

Can lineage-dependent mechanisms fully account for embryonic patterning during gastrulation?

The invariance of the *C. elegans* lineage is perhaps the most remarkable feature of nematode biology. The lineage-based mechanisms reviewed above suggest that a progressive refinement of cell lineage potentials occurs throughout embryogenesis. That is, maternal genes establish the identity of the founder cells as well as the major left–right differences. Subsequently, the A–P system generates a code for each of the founder cell descendants. The combination of founder cell, left–right and A–P identities provides a unique address for each cell in the embryo and suggests that these mechanisms could, in theory, be sufficient to specify every cell in the embryo (Fig. 2b). Does this happen? Recent analysis of the genes and cellular behaviors that occur during gastrulation suggests that the embryo relies on additional patterning schemes, and that these processes are important to build organs and tissues. The need for these mechanisms no doubt stems in part from the polyclonal composition of most of the nematode's tissues and organs. The sublineages that contribute to one tissue or organ cannot be grouped together in a simple way. Thus, if the gastrula-stage embryo relied solely on the A–P and founder cell systems, then the regulatory circuits used to establish a tissue or organ primordium would be extraordinarily complex. For example, transcription of a gene throughout an organ primordium would be predicted to depend on an extensive array of *cis*-acting enhancers. Collectively, these enhancers would

FIGURE 3. Regionalization of *C. elegans* embryos



Regionalization of the 80-cell stage gastrula can be described in two ways. Both panels (a) and (b) display a lateral view of the same approximately 80-cell embryo (anterior to the left, ventral down) and show the positions of all cells (see Ref. 21 for technical details). In (a), cells are color-coded according to their blastomere origin (see Ref. 15) and illustrate regionalization of the embryo based on the cell lineage. In (b), cells are color-coded according to the organs or tissues they will ultimately form (M. Labouesse, unpublished). Color-code: ABala, dark blue; ABalp, dark violet; ABara, light blue; ABarp, light violet; ABpla, dark green; ABplp dark yellow; ABpra, light green; ABprp, light yellow; MS, white; E, dark red; C, light red; D, cyan at the extreme posterior. In addition, the dark green, dark blue, dark yellow and blue/green cells correspond to rare cells that are not yet restricted in their fate. Two different fate maps can be derived from the pictures shown in panels (a) and (b), one that highlights cell lineage origin (c) and one that highlights affiliation to an organ or a tissue (d). For clarity the domains occupied by ABpra and ABprp are not shown in panel (c).

respond to all of the blastomere identity and A–P regulators that exist for each of the different cells within the primordium. To simplify its task, the embryo appears to rely on a second patterning system, described below.

Formation of organs and tissues in the gastrula embryo

The discovery of zygotic genes that direct the formation of the pharynx, epidermis or midgut implicates a model of *C. elegans* embryogenesis that is distinct from A–P patterning. This model was suggested by the observation that a mutation in a single gene can affect an entire organ (for an example see Ref. 18) and, more importantly, by the hypothesis that a unique gene can confer organ identity, first proposed by Mango *et al.*[19] According to this model (Fig. 2c), organs and tissues are established in two stages. First, cells are specified as organ or tissue precursors. These precursors derive from different sublineages, yet they acquire a common identity that is defined by the following characteristics: (1) they cluster together in the same region of the embryo during early gastrulation; (2) they are destined to produce cells of a given tissue or organ and virtually no other cell type; and (3) they homogeneously express a gene that is essential for the specification of the organ or tissue, referred to as 'tissue or organ identity genes' [by analogy with genes specifying blastomere identity (see above) or position identity, e.g. *hox* genes]. During the second stage, the identity genes activate specialized factors that control terminal differentiation of the precursor cells.

A fate map of the gastrula embryo at the 80-cell stage

A key feature of the organ/tissue specification model is that precursors of a given organ or tissue are located within the same region of the embryo. A fate map for pharyngeal, midgut, muscle, epidermal or neural precursors can be constructed as early as the 50- or 80-cell stage, long before any overt differentiation (M. Labouesse, unpublished; Fig. 3b, d). The existence of these domains suggests that they are functionally important, either to establish the precursor cells, or to facilitate their differentiation into a mature structure. Most of these domains are polyclonal because diverse sublineages contribute to each precursor population. Thus, regionalization of the same embryo can be achieved by two different configurations, one that relies on cell lineage (Fig. 3a, c; see above) and one that is based on affiliation to a tissue or organ (Fig. 3b d).

Two observations suggest that the time at which the organ/tissue precursor domains are first observed is biologically significant (50- or 80-cell stage). First, the cell lineage reveals a shift in the developmental potential of individual blastomeres at this stage. During the first 2 h of embryogenesis, most blastomeres are pluripotent and give rise to various cell types that are not functionally related. Many cells at the approximately 80-cell stage, however, produce descendants that contribute exclusively to a single tissue or organ[1] (71% contribute to a single organ or tissue, 18% contribute predominantly to 1, and the remaining 11% contribute equally to >1). Second, as described below, this stage corresponds to the period when organ/tissue identity genes normally are first expressed and active. Moreover, it is the latest time at which blastomeres alter their fates in response to ectopic expression of organ/tissue identity genes. Together, these data suggest that the 80-cell stage is a critical transition point during embryogenesis, from a complete reliance on lineage-based patterning to the addition of an organ/tissue-based strategy. Interestingly, the timing at which the organ/tissue domains are defined and their positions relative to the A–P and dorso-ventral (D–V) axis might be evolutionarily conserved. A similar fate map can be drawn for the distant marine nematode *Enoplus brevis*, a species whose initial development is more variable than that of *C. elegans*[20].

The midgut derives from a single blastomere. For this reason it is impossible to distinguish between events controlling organ identity (i.e. the midgut) or blastomere identity (i.e. the E cell). Data from other animals, however, suggest that at least some of the *C. elegans* midgut regulators might act at the level of the organ, which is why we include a discussion of midgut development here.

Organ/tissue 'identity genes'

The unified behavior of organ and tissue precursors is paralleled at the molecular level by the three following candidate organ/tissue identity genes: the winged helix gene *pha-4* for the pharynx, the GATA factor gene *elt-1* for the epidermis and a second GATA factor gene, *end-1*, for the intestine (Refs 19, 21–24; see Table 1). These regulators share three features in common. First, their absence leads to a lack of the organ or tissue primordium and to a cell-fate transformation, the nature of which depends on the gene. In *pha-4* homozygotes, the pharyngeal precursors adopt a non-neuronal ectodermal fate characterized by the synthesis of LIN-26 (Ref. 21). These cells fail to gastrulate properly at the 100-cell stage so that a pharyngeal primordium is never established, and the different cell types normally found within the mature pharynx are not produced[19,21]. The cells that produce pharyngeal cells in a wild-type embryo do not adopt any wild-type lineage in *pha-4* mutants[21]. Thus, there is no simple way to explain the role

TABLE 1. Zygotic genes required to form tissues and organs

Organ/tissue	Gene	Nature of product	Expression	Loss-of-function phenotype	Ectopic expression	Refs
Pharynx						
	pha-4	FKH/HNF-3 TF	Pharynx precursors at 50-cell stage[a] until adult[b]	EMB; no pharynx → PRIM; pharynx → 'ectoderm'	Other cells → 'pharynx cells'	19, 21, 22, 45
	pha-1	Possible bZIP TF	Pharynx precursors at 80-cell stage until comma	EMB; no pharynx DIF	?	18, 26
	ceh-22	HD TF	Subset of pharynx muscles	Poor pharynx function	Activates pharynx muscle genes	31, 33
Major epidermis						
	elt-1	GATA TF	C daughters, precursors of EPID cells at 50-cell stage until comma	EMB; epidermis → muscle/neuron	Other cells → 'epidermal cells'	23, 46
	lin-26	Possible 'Zn-finger TF'	Precursors of EPID cells at 80-cell stage until adult[c]	EMB; AB DIF of EPID cells	Other cells → 'epithelial cells'[d]	27–29
Midgut						
	end-1	Possible 'GATA TF'	Midgut precursor (E) and its daughters until 28-cell stage	None; no midgut if EDR deleted, E → 'C'[e]	Other cells → 'intestinal cells'	24, 25
	elt-2	GATA TF	Midgut precursors at 28-cell stage to adult	LAL; AB midgut DIF	Other cells → 'intestinal cells'	30, 47
	pha-4	FKH/HNF-3 TF	Midgut precursors at 50-cell stage to adult	AB midgut	See above	21, 22, 45
	skn-1(z)	bZIP/HD hybrid domain TF	Differentiated midgut cells	LAL; AB midgut DIF	?	48–50
Body wall muscles						
	pha-1	Possible 'bZIP TF'	Muscle precursors at 80-cell stage until comma	EMB; no apparent muscle phenotype	?	26
	hlh-1	bHLH (MyoD-like)	Muscle precursors at 140-cell stage until adult	LAL; AB muscle DIF	?	51, 52
Nervous system						
	hlh-2	bHLH (DA-like)	Ubiquitous early, then all neuroblasts	Not known precisely, probably EMB	?	53

Abbreviations: AB, abnormal; comma, 'comma' is the stage at which all cells are post-mitotic and the embryo is comma shaped; DIF, differentiation; EDR, endoderm-determining region; EMB, embryonic lethal; EPID, epidermal; HD, homeodomain; LAL, larval lethal; PRIM, primordium; TF, transcription factor (when there is no biochemical demonstration that the protein can bind DNA and/or transactivate, it is mentioned that the protein is a 'possible TF'); *(z)*, zygotic component; →, cell fate transformation.

[a]Refs 21, 22; M. Horner and S. Mango, unpublished.

[b]The *pha-4* gene is also expressed in rectal precursors and rectal cells, where it plays a comparable function[19,21,22].

[c]The *lin-26* gene is also expressed in glia, 'minor epidermal' cells and the somatic gonad, where it plays a comparable function[27–29].

[d]S. Quintin and M. Labouesse, unpublished.

[e]Cells derived from the E blastomere, which normally constitute the midgut, express fates normally expressed by the C blastomere descendants[24].

of *pha-4* in pharynx development using lineage-based mechanisms. In *elt-1* mutants, the 'major' epidermal precursors, which generate the external epidermis, adopt the fates of their closest lineal relatives: a neural fate for those cells that originate from the ABa blastomere and a muscle fate for those that originate from C (see Fig. 1); the fates of epidermal precursors that derive from ABp are not known[23]. Chromosomal deficiencies that remove *end-1* and at least one other redundant gene define an 'endoderm-determining region' (EDR). Loss of the EDR results in a transformation of the E lineage into a C-like lineage, and hence in the production of muscle and epidermis instead of midgut[24].

The second feature shared by the identity genes is that each is sufficient to activate the cognate organ/tissue developmental program in naive blastomeres. Ectopic expression of *pha-4* or *end-1* leads many or all cells in the embryo to express pharyngeal or intestinal markers, respectively, at the expense of other cell types[21,22,25]. Similarly, ectopic *elt-1* expression leads to widespread expression of the epidermal marker *lin-26*, although it is not yet clear whether *elt-1* is sufficient to change the fate of recipient cells[25]. These results suggest that *pha-4*, *elt-1* and *end-1* can initiate significant aspects of pharynx, 'major epidermis' and midgut development. Experiments with staged embryos demonstrate that cells respond to these regulators up to the approximately 80-cell stage[21,25].

The third characteristic of the identity genes is that they are first expressed in organ or tissue precursors, generally as soon as these cells become lineally restricted (Refs 21–24; see Table 1). The identity gene *end-1* is first expressed in the E founder cell, which generates only midgut, whereas *elt-1* and *pha-4* are expressed at slightly later stages when the major epidermal or pharyngeal precursors are born. This early expression, coupled with the loss-of-function and ectopic expression phenotypes, suggests that the identity genes are not terminal differentiation factors that respond to patterning cues laid down by the A–P polarity system (Fig. 2a). Rather, they are an integral part of a mechanism used to construct the triploblastic embryo. An exciting challenge for the future will be to determine how the identity genes are first activated and how the organ and tissue precursor fields are established.

Organ/tissue differentiation genes

How do identity genes work? For midgut, pharynx, epidermis and body wall muscles, differentiation genes have been found that presumably act in a genetic hierarchy in which identity genes activate the transcription of differentiation genes, which in turn regulate the terminal stages of development. Three characteristics distinguish the differentiation genes from the identity genes. First, mutations in the differentiation genes do not prevent organ or tissue formation but rather lead to abnormal differentiation (Table 1). For example, mutations affecting most differentiation genes do not prevent the expression of currently available terminal differentiation markers (Table 1). The exception is PHA-1, an unusual bZIP factor, which results in the loss of three terminal pharyngeal markers when mutated[18,26]. A likely role for the differentiation genes is to confer a subset of differentiation characteristics. For instance, the phenotype of mutations in *lin-26*, which encodes a zinc-finger protein, suggests that this gene could activate an epithelial program within epidermal cells, glia and the somatic gonad[27–29].

A second characteristic of the differentiation genes is that their ectopic expression leads to ectopic expression of the appropriate terminal differentiation markers. At present, it is unclear whether the differentiation genes and the identity genes regulate qualitatively different targets. One possibility is that differentiation genes control terminal markers whereas identity genes control cell-fate regulators. For example, the GATA factor ELT-2 is sufficient to activate the gut esterase gene *ges-1* (Ref. 30). Similarly, ectopic synthesis of the zinc-finger protein LIN-26 leads to widespread expression of a zonulae adherens epithelial marker (S. Quintin and M. Labouesse, unpublished). This regulation might be direct because the differentiation genes encode presumptive transcription factors. Alternatively, the differentiation genes might regulate a subset of identity gene targets, including cell-fate regulators. This scenario implies that the differentiation genes could also control cell fates, but at a later stage in development than the identity genes. This issue will, no doubt, be resolved once we learn more about the identities of target genes for developmental transcription factors.

A third feature of the differentiation genes is that they are first expressed after the identity genes, but before the onset of terminal differentiation (see Table 1). This expression pattern is consistent with these genes being direct targets of the identity genes. For example, *pha-4* activity is both necessary and sufficient for expression of the CEH-22 homeobox protein required for normal differentiation of a subset of pharyngeal muscles[19,22,31].

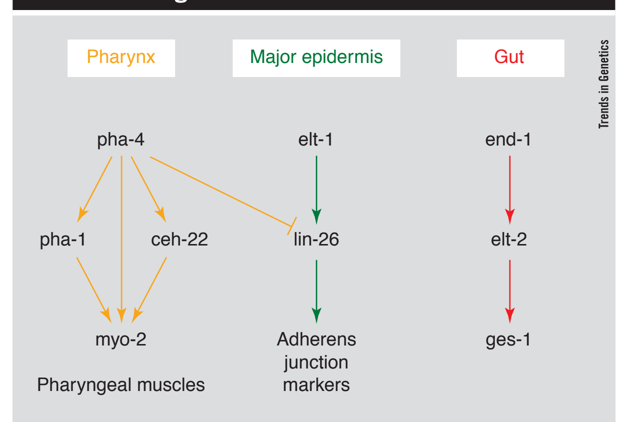
The data described here provide a simple model for how tissues and organs are constructed from a hierarchy of transcriptional regulators. In the midgut, a linear pathway appears to function: *end-1* activates the GATA factor *elt-2*, which controls the late genes[4,24,25,30]. Similarly, in epidermal cells, *elt-1* probably activates *lin-26*, which might regulate epithelial genes[23,29,32]. The pathway for pharynx formation is not linear. While *pha-4* can activate *ceh-22* directly, it also directly controls at least one terminally expressed gene, namely pharyngeal myosin[22,31,33]. Moreover, a parallel pathway for pharyngeal muscle formation exists as neither *ceh-22* nor *pha-1* is essential for producing pharyngeal muscles, whereas the double-mutant combination leads to a complete absence of this cell type[31] (see Fig. 4 for a summary of interactions).

Combining the lineage and organ/tissue specification models

A–P fate specification, acting in combination with blastomere identity genes, provides an elegant system for patterning the *C. elegans* embryo. However, the existence of a fate map and the discovery of the organ/tissue identity genes implies another level of organization beyond cell lineage. We suggest that both strategies are important for building a worm. But, how do they work? One possibility is that the two modes of development co-evolved to allow a smooth transition from lineage-based patterning mechanisms, which act early to establish the body axes, to organ-derived patterning, which takes the future function of cells into account. For example, the position of the founder cells and their descendants could determine the future location of the organ/tissue precursors. In addition, one role of the blastomere identity or A–P polarity genes might be to activate the expression of organ/tissue identity genes in the appropriate cells during early gastrulation. If so, it is likely that other factors are involved as well because the organ/tissue identity genes are not expressed in simple A–P or sublineage patterns. With the exception of the midgut[4], the nature of the factors and whether they include the pregastrula maternal genes, are unknown.

Once the organ/tissue fields are established, the A–P system can function combinatorially with the organ/tissue regulators to provide patterning information within a homogeneous primordium. For example, Schroeder and McGhee have used an asymmetrically expressed *ges-1* reporter construct to show that *pop-1* activity distinguishes anterior from posterior midgut[34]. Characterization of the *cis*-acting regulatory sequences of zygotically expressed genes might enable us to determine which mechanisms are prevalent at a given time. For example, enhancer elements that respond directly either to cellular A–P polarity (e.g. they bind POP-1) or to organ/tissue-wide regulation (e.g. they are recognized by PHA-4, ELT-1 or END-1) might be found. In addition, the recently generated *C. elegans* microarray chip (S. Kim, pers. commun.) might help answer these questions, as all of these regulators are predicted to control mRNA levels.

FIGURE 4. Pathways involved in generating specific tissues and organs



A summary of the interactions between zygotically expressed genes. Most of these interactions are inferred from genetic data, including ectopic expression experiments. Positive interactions are symbolized by an arrow, negative interactions by a line with a bar. This diagram is not exhaustive as the study of zygotic genes involved in generating organs or tissues is just beginning.

A comparison between *C. elegans* and other species

The existence of a fate map suggests that nematodes use developmental strategies similar to vertebrates and *Drosophila*. In all of these animals, polyclonal groups of cells are allocated to domains that will generate organs and tissues[35–37]. Moreover, two of the identity genes discussed here play a similar functional role in other species, for example, *pha-4* (or *forkhead* or *HNF3*) for pharynx or foregut development[21,22,38] and *end-1* (or *elt-2* or *serpent*) for the midgut[24,30,39]. In general, studies on pharyngeal, epidermal and midgut development in *C. elegans*, myogenesis in mice[40], and neurogenesis in flies and mice[41,42] suggest that a similar strategy is involved in generating organs and tissues in all species. In each case, identity genes, acting before differentiation genes, are essential to specify an integrated pool of precursors.

How far can one extend the similarity between nematodes and either insects or vertebrates? The recent completion of the *C. elegans* genome[43] has established that most of the major developmental pathways known to act in other metazoans are found in *C. elegans* (with the notable exception of Hedgehog, Toll)[44]. However, it is unclear to what extent these pathways play the same biological role in a worm. The study of embryogenesis in multiple organisms will teach us about the different strategies involved in forming an animal and how evolutionarily conserved molecular pathways are used to bring about different body plans.

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