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. 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.

**C. elegans embryogenesis**

*C. elegans* embryogenesis can be divided into three periods. During the first 100 min, five divisions give rise to 28 cells, including six ‘founder’ cells called AB, MS, E, P4, D and C (Fig. 1). With the exception of the midgut (originating from E) and the germ line (from P4), 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 triplionic 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.

**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.
Perspective

Patterning the C. elegans embryo

308 cell divisions with cell-fate decisions after the eight-cell divisions result in equal levels of POP-1 in each anterior or posterior 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. Conversely, mutations in lit result in posterior daughters adopting the fates of their anterior sisters. 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. 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 understood.

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. 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 homolog similiar to TAK. Activated Lit-1 can phosphorylate POP-1, which is 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.

Independent evidence that A–P polarity plays an important role in patterning the C. elegans embryo comes from the study of sub-7, an even-skipped homolog, and of ceh-13, a labial-like Hox gene. The gene sub-7 is found 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 sub-7 mutants. Two recent papers show that pop-1 genes, lineage-based mechanisms are 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. observed that the descendants of the eight AB great-granddaughters and founder cells MS, E, C, D reproduce occupancy 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. 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 1: Distribution of cell types within the Caenorhabditis elegans lineage](image)
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 lineage-dependent mechanism is 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 is 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
The discovery of zygotic genes that direct the formation of organs and tissues in the gastrula embryo

The cells that produce pharyngeal cells in a wild-type stage organ/tissue identity genes: the winged helix gene end-1 (Ref. 19, 21–24; see Table 1). These regulators correspond to a tissue or an organ (i.e. the E cell). Data from other animals, however, suggest that at least some of the organ/tissue identity genes are expressed at the level of the organ, which is why we include a discussion of midgut development here.}

**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. 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. fox 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 organs/tissues 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: (1) they are established before gastrulation; (2) they are conserved in many species during embryogenesis, from a complete reliance on lineage-based pattern 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 distal marine nematode Enoplus brevis, a species whose initial development is more variable than that of C. elegans.

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 egl-3 for the epidermis and a second GATA factor gene, end-3, 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.

The cells that produce pharyngeal cells in a wild-type embryo do not adopt any wild-type lineage in pha-4 mutants. Thus, there is no simple way to explain the role
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 fate for those that originate from the ABa blastomere and a muscle precursors, which generate the internal epidermis, express pha-4 as soon as these cells become lineally restricted, whereas elt-2 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 markers. Experiments with staged embryos demonstrate that cells respond to these regulators up to the approximately 80-cell stage.

The third characteristic of 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 elt-1 leads many or all cells in the embryo to express pharyngeal or intestinal markers, respectively, at the expense of other cell types. Similarly, ectopic elt-1 expression leads to widespread expression of the epidermal marker elt-1, although it is not yet clear whether elt-1 is sufficient to change the fate of recipient cells. These results suggest that pha-4, elt-1, and elt-2 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.

### TABLE 1. Zygotic genes required to form tissues and organs

<table>
<thead>
<tr>
<th>Organ/Tissue</th>
<th>Gene</th>
<th>Nature of product</th>
<th>Expression</th>
<th>Loss-of-function phenotype</th>
<th>Ectopic expression</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pharynx</td>
<td>pha-4</td>
<td>Possible mouse PFF TF</td>
<td>Pharynx precursors at 10-cell stage until adult</td>
<td>EMB, other cells → 'epidermal'</td>
<td>Other cells → 'epidermal'</td>
<td>20, 21, 23, 26</td>
</tr>
<tr>
<td>Major epidermis</td>
<td>elt-1</td>
<td>Possible mouse PFF TF</td>
<td>Pharynx precursors at 10-cell stage until adult</td>
<td>EMB, other cells → 'epidermal'</td>
<td>Other cells → 'epidermal'</td>
<td>23, 26</td>
</tr>
<tr>
<td>Midgut</td>
<td>elt-2</td>
<td>Possible mouse PFF TF</td>
<td>Midgut precursors at 50-cell stage until adult</td>
<td>EMB, other cells → 'midgut'</td>
<td>Other cells → 'midgut'</td>
<td>23, 27–29</td>
</tr>
<tr>
<td>Body wall muscles</td>
<td>pha-4</td>
<td>Possible mouse PFF TF</td>
<td>Muscle precursors at 50-cell stage until adult</td>
<td>EMB, other cells → 'muscle'</td>
<td>Other cells → 'muscle'</td>
<td>23, 27, 28</td>
</tr>
<tr>
<td>Nervous system</td>
<td>elt-1</td>
<td>Possible mouse PFF TF</td>
<td>Muscle precursors at 50-cell stage until adult</td>
<td>EMB, other cells → 'muscle'</td>
<td>Other cells → 'muscle'</td>
<td>23, 27, 28</td>
</tr>
</tbody>
</table>

Abbreviations: AB, abnormal; comma, ‘comma’ is the stage at which all cells are post-mitotic and the embryo is comma shaped; 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.

2. The elt-1 gene is also expressed in ‘ectodermal’ cells and the seminal gland, where it plays a comparable function.
3. The elt-2 gene is also expressed in glia, ‘major epidermal’ cells and the seminal gland, where it plays a comparable function.
4. Cells derived from the E blastomere, which normally contribute to the mesoderm, express elt-1 normally expressed by the C blastomere descendants.

Perspective
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 identity genes. First, mutations in the differentiation genes do not prevent organ or tissue formation but rather lead to abnormal differentiation (Table 1). The second is that PHA-4, an unusual bZIP factor, which results in the loss of three terminal pharyngeal markers when mutated\(^{29,30}\). A likely role for the differentiation genes is to constitute a subset of differentiation characteristics. For instance, the phenotype of mutations in \(\text{lin-26}\), 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 \(\text{ELT-2}\) is sufficient to activate the gut esterase gene \(\text{ges-1}\), which controls the late genes \(\text{pha-1}\), \(\text{pha-4}\) and \(\text{ceh-22}\) (Ref. 30). Similarly, ectopic synthesis of the zinc-finger protein \(\text{LIN-26}\) leads to widespread expression of a zonulae adherens epithelial marker (S. Quentin and M. Laboisse, unpublished). This regulation might be direct because the differentiation genes encode presumptive transcription factors. Alternatively, the differentiation genes might regulate a subset of identity genes, 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 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, \(\text{pha-4}\) activity is both necessary and sufficient for expression of the CEBH-22 homeobox protein required for normal differentiation of a subset of pharyngeal muscles\(^{29,30}\).

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: \(\text{end-1}\) activates the GATA factor \(\text{ELT-2}\), which controls the late genes\(^{29,30}\). Similarly, in epidermal cells, \(\text{elb-1}\) probably activates \(\text{lin-26}\), which might regulate epithelial genes\(^{29,30}\). The pathway for pharynx formation is not linear. While \(\text{pha-4}\) can activate \(\text{celb-22}\) directly, it also directly controls at least one terminally expressed gene, namely pharyngeal myosin\(^{29,30}\).

Moreover, a parallel pathway for pharyngeal muscle formation exists as neither \(\text{celb-22}\) nor \(\text{pha-1}\) is essential for producing pharyngeal muscles, whereas the double-mutation combination leads to a complete absence of this cell type\(^{39}\) (see Fig. 4 for a summary of interactions).
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 each species, polyclonal groups of cells are allocated to domains that will generate organs and tissues11,12,13,14. Moreover, two of the identity genes discovered for the midgut 24,30,39. In general, studies on pharyngeal, mesoderm. The existence of a fate map suggests that nematodes use developmental strategies similar to vertebrates and Drosophila. 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 genome13 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)34. However, it is unclear to what extent these pathways play the same basic role. The study of embryogenesis in multiple organisms will teach us about the different strategies involved in forming an animal and how evolutionarily conserved pathways are used to bring about different body plans.

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References