Anterior-posterior patterning within the *Caenorhabditis elegans* endoderm

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**SUMMARY**

The endoderm of higher organisms is extensively patterned along the anterior/posterior axis. Although the endoderm (gut or E lineage) of the nematode *Caenorhabditis elegans* appears to be a simple uniform tube, cells in the anterior gut show several molecular and anatomical differences from cells in the posterior gut. In particular, the gut esterase ges-1 gene, which is normally expressed in all cells of the endoderm, is expressed only in the anterior-most gut cells when certain sequences in the ges-1 promoter are deleted. Using such a deleted ges-1 transgene as a biochemical marker of differentiation, we have investigated the basis of anterior-posterior gut patterning in *C. elegans*. Although homeotic genes are involved in endoderm patterning in other organisms, we show that anterior gut markers are expressed normally in *C. elegans* embryos lacking genes of the homeotic cluster. Although signalling from the mesoderm is involved in endoderm patterning in other organisms, we show that ablation of all non-gut blastomeres from the *C. elegans* embryo does not affect anterior gut marker expression; furthermore, ectopic guts produced by genetic transformation express anterior gut markers generally in the expected location and in the expected number of cells. We conclude that anterior gut fate requires no specific cell-cell contact but rather is produced autonomously within the E lineage. Cytochalasin D blocking experiments fully support this conclusion. Finally, the HMG protein POP-1, a downstream component of the Wnt signalling pathway, has recently been shown to be important in many anterior/posterior fate decisions during *C. elegans* embryogenesis (Lin, R., Hill, R. J. and Priess, J. R. (1998) *Cell* 92, 229-239). When RNA-mediated interference is used to eliminate pop-1 function from the embryo, gut is still produced but anterior gut marker expression is abolished. We suggest that the *C. elegans* endoderm is patterned by elements of the Wnt/pop-1 signalling pathway acting autonomously within the E lineage.

Key words: *Caenorhabditis elegans*, Patterning, Endoderm, E cell, ges-1

**INTRODUCTION**

The entire *C. elegans* endoderm arises from the E blastomere, a founder cell born at the 7-cell stage of the embryo (Fig. 1). The establishment of correct E cell fate has been shown to require (at least) two factors: (1) the presence of the maternally provided *skn-1* product, a putative bZIP transcription factor (Bowerman et al., 1992, 1993), and (2) an inductive interaction that occurs at the 4-cell stage of the embryo between the *P*<sub>2</sub> cell and the EMS cell, the mother of E. The *P*<sub>2</sub>-EMS interaction appears to ‘polarize’ EMS and, in its absence, EMS divides into two MS-like cells, where MS is the normal sister of E (Schierenberg, 1987; Goldstein, 1992, 1993, 1995a). This induction is mediated by maternally provided components of a Wnt signalling pathway (Rocheleau et al., 1997; Thorpe et al., 1997); elements of this pathway, in particular the HMG protein POP-1, have recently been implicated in many anterior/posterior fate decisions during *C. elegans* embryogenesis (Lin et al., 1998).

The zygotic pathway that executes endoderm fate is also becoming defined. At least two (and possibly more) redundant genes mapping to a restricted region on chromosome V are the best current candidates for gut-specifying genes controlled directly by maternal factors; these genes include a GATA factor named *end-1* (Zhu et al., 1997, 1998). Downstream of *end-1* (and possibly directly controlled by *end-1*) lies a second GATA factor, named *elt-2*, which is essential for correct endoderm differentiation (Fukushige et al., 1998).

In triploblastic metazoans, the endoderm layer usually exhibits extensive patterning along the anterior-posterior (or cephalocaudal) axis. In vertebrates, endoderm produces taste buds (Barlow and Northcutt, 1997), thymus, thyroid, lungs, liver, pancreas and gall bladder, and the linings of esophagus, stomach and intestines (Browder et al., 1991). In *Drosophila*, endoderm differentiates into the anterior and posterior midgut, together with the copper cells, large flat cells and iron cells of the middle midgut (Bienz, 1997). In both vertebrates and insects, endoderm patterning appears to involve genes of the homeotic cluster and signals that originate from tissues outside of the endoderm (Roberts et al., 1995; Gualdi et al., 1996; Bienz, 1997; Duluc et al., 1997; Kim et al., 1997).

The *C. elegans* endoderm is a relatively simple uniform tube that lacks a coating of visceral mesoderm. Anterior-posterior patterning of the *C. elegans* gut is subtle but unambiguous. A total of 20 cells are arranged in a series of nine rings called ‘ints’ (Wood, 1988); the anterior-most ring (*int1*) contains four
cells; int2 through int9 each contain two cells (see Fig. 1). Cells of int1 and int2 are mononucleate, cells of int3 through int7 are binucleate and cells of int8 and int9 can be either mononucleate or binucleate (Sulston and Horvitz, 1977). The cells of int1 must attach to the pharyngeal-intestinal valve, the cells of int9 must attach to the rectal-intestinal valve and cells of int5 are in intimate contact with the Z2-Z3 cells of the germline (Wood, 1988). Anterior cells have short microvilli (Sulston and Horvitz, 1977), are associated with an expanded gut lumen and do not express the pho-1 acid phosphatase activity on their luminal surface (Beh et al., 1991).

We have previously described an example of anterior-posterior patterning within the *C. elegans* endoderm that must occur at the level of gene transcription: several small promoter deletions cause the ges-1 esterase gene, which is normally expressed relative to the ges-1 ATG codon. The ges-1ΔB::GFP/lacZ transgene was constructed by inserting the 3.3 kb ges-1ΔB upstream region (~3317 to ~3) into the multiple cloning site (HindIII-Sall) of the vector pPD96.04 (kindly provided by A. Fire, Carnegie Institute of Washington). Transformation was by standard methods (Mello et al., 1991) using the rol-6 marker gene. pJM15-derived transgenes were injected into the ges-1 null strain JM1041. Strains containing the various genetic markers were usually ges-1(+); however, with 3 minutes staining, transgenic ges-1 activity (saturated by this time) can easily be distinguished from the endogenous ges-1 activity (saturated at ~60 minutes). Birefringent gut granules,GES-1 (Edgar and McGhee, 1986), β-galactosidase (Fire et al., 1990) and GFP (Chalfie et al., 1994) were detected by standard means in approximately comma-stage embryos (6-8 hours postfertilization). In the course of this work, the ges-1ΔB-promoter-driven anterior-gut-specific expression pattern has been observed in more than 1000 embryos derived from multiple lines (six heritable lines and three integrated (outcrossed) lines using the ges-1 reporter; two heritable lines and four integrated (outcrossed) lines using the GFP/lacZ reporter).

Although the ges-1 staining procedure is exacting with respect to permeabilization and fixation conditions,GES-1 is the most sensitive of the three reporters; ges-1ΔB directed esterase staining is first detected at the 16-E-cell stage, and remains easily detected in int1 and int2 throughout embryogenesis. In embryos older than the 2-fold stage, we occasionally observe staining in the pharynx and rectum, a feature of most pJM15-derived transgenes (Egan et al., 1995). The β-galactosidase staining procedure is straightforward but is less sensitive than staining forGES-1; β-galactosidase activity can be consistently detected in int1 and int2 cells from the 16- to 20-E-cell stage, with occasional staining in int8 and int9 just before hatching. GFP is the most convenient but also the least sensitive reporter; GFP is only detected in int1 after the 20-E-cell stage. In each experiment, we used the reporter that gave the optimum combination of sensitivity and convenience.

**Strains and alleles**
*C. elegans* culture and genetic methods were as described by Brenner (1974). Bristol strain N2 was used as the wild-type strain. The following mutations, deficiencies and balancers were used: LGI: pop-l(zu189), dpy-5(e61), hT(1;IV); LGIII: dpy-17(e164), ndf(16), lin-39(n1760), mab-5(e1239), unc-32(e189), pie-1(zu154), unc-25(e156), qCl(III); LGIV: him-8(e1489), skn-1(zu67), nT(IV;V); LGV: ges-1(cac6ca7), him-3(e1490), fog-2(q71), stu-3(q265), pha-4(q490), rol-9(sc148).

**Embryo manipulations**
Laser ablations were conducted basically as described (Fukushige et al., 1996). Early embryos were collected and mounted on gelatin slides in egg salts (Edgar, 1995). Blastomeres were identified by position and then laser pulsed until nuclear breakdown or scarring was observed (approximately 20 seconds at 10 pulses/second). Operated embryos were incubated for 12-18 hours at 15°C prior to assaying for gut granules and anterior gut marker expression.

Cytochalasin-D treatment (6 μg/ml) was as described by Edgar (1995) in the Embryonic Growth Media described by Shelton and Bowerman (1996). Embryos were incubated overnight at 15°C prior to assaying for marker expression and DAPI staining.

**Reverse genetics using RNA-mediated interference**
RNA-mediated interference (RNAi) was performed as follows. The two strands of a pop-1 dsDNA clone (generously provided by Dr R. Lin, University of Texas Southwestern Medical Center) were transcribed separately using T3 or T7 polymerase as appropriate; single strands were then annealed in injection buffer for 30 minutes at 37°C (Fire et al., 1998). pop-1 dsRNA injected at concentrations ranging from 10 to 100 ng/μl all produced the same phenotype of arrested embryos containing ectopic gut (Lin et al., 1998); injection

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**MATERIALS AND METHODS**

**Transgene construction and analysis**

All ges-1 deletions described in the current paper derive from the plasmid pJM15, which contains the intact genomic ges-1 gene comprising 3.3 kb of 5' sequence, the complete ges-1 coding region (consisting of eight exons and seven introns spread over 4.5 kb) and 0.7 kb of 3' sequence (Egan et al., 1995). The ges-1ΔB deletion removes bp -1100 to -1050 of the ges-1 promoter; coordinates are

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**Fig. 1.** The *C. elegans* E lineage. A 4-cell embryo is shown at the top of the figure. The ‘polarizing’ Wnt-pathway-dependent interaction between the P2 and the EMS cells is represented as a thick arrow. The mature 20-cell intestine is shown in a left lateral view, along with the lineage derivation of each cell. Selected ‘ints’ are labelled at the bottom of the figure. Division of Ea and Ep is transverse: the ‘left-side’ lineage is shown as solid lines; the ‘right-side’ lineage is shown as broken lines. Adapted from Wood (1988).
of 10 ng/μl resulted in arrested embryos produced over a 1-2 day period; injections at greater than 20 ng/μl caused arrest of the entire brood.

RESULTS

A modified ges-1 gene as a biochemical marker for anterior endoderm

The ges-1 gene encodes a carboxylesterase expressed in every cell of the gut; ges-1 expression begins at the 4-E-cell stage of embryogenesis and continues throughout the life of the worm (Edgar and McGhee, 1986; Kennedy et al., 1993). Transgenic analysis of the ges-1 promoter has shown that gut-specific ges-1 expression centres on three promoter elements: two GATA sites and a 300 bp region (Δ4) immediately downstream. Removal of any two of these three elements abolishes ges-1 expression in the gut, at the same time activating expression in cells of the pharynx and rectum (Egan et al., 1995; Fukushige et al., 1996). However, removal of any one of these three promoter elements produces a different effect: ges-1 expression is now limited to cells at the gut anterior (Egan et al., 1995).

As shown in Fig. 2, the location of the ges-1 promoter element within the 300 bp Δ4 deletion has now been refined to the 50 base pair ‘B’ region adjacent to the two GATA sites. The ges-1 promoter from which the B region has been deleted is capable of driving anterior-gut-specific expression either of ges-1 itself (ges-1ΔB; Fig. 2b) or of a nuclear-localized GFP/lacZ reporter gene (ges-1ΔB::GFP/lacZ; Fig. 2c,d). As described in more detail in the Materials and Methods section, ges-1ΔB expression is observed from the 16-E-cell stage onward; expression is always intense in int1, is often detected in int2 but at lower levels and can occasionally be detected in int3 and int4, but only after prolonged staining. For the current paper, embryos were examined at the 16- to 20-E-cell stage; thus, typically 2-6 staining cells are observed, depending on whether the final int1 cell division has taken place (see Fig. 1). We now use this transgenic marker to investigate the mechanism of anterior gut formation.

Genes of the C. elegans homeotic cluster are not required for anterior gut marker expression

As noted in the Introduction, genes of the homeotic cluster have been implicated in endoderm patterning in other organisms (Roberts et al., 1995; Biers, 1997; Duluc et al., 1997). To test for a role of homeotic genes in C. elegans endoderm patterning, expression of both the endogenous ges-1 gene and an (integrated) ges-1ΔB transgene were examined in embryos that were homozygous for (apparently null) mutations in lin-39 and in mab-5, as well as in embryos that were homozygous for the deficiency N613, which removes the entire C. elegans homeotic cluster (Wood, 1988; Clark et al., 1993). The results are shown in Fig. 3. In all the mutant embryos examined, endogenous ges-1 is expressed throughout the gut (as are birefringent gut granules; data not shown) and ges-1ΔB is expressed intensely only in the most anterior gut cells. In other words, we see no obvious requirement for homeotic genes in patterning of the C. elegans embryonic gut.

Expression of anterior endoderm markers is autonomous within the E lineage

As noted in the Introduction, endoderm patterning in other organisms involves signals passed between germ layers (Roberts et al., 1995; Gualdi et al., 1996; Biers, 1997; Duluc et al., 1997; Kim et al., 1997). In the current section, we present results from three quite different experimental approaches, all of which show that anterior endoderm in C. elegans does not appear to be induced by non-endodermal cells. An important qualification to this statement should be noted: ‘lineage autonomous’ patterning of the C. elegans endoderm really means autonomous after E cell fate has been established. In a wild-type embryo, this requires the P2-EMS contact that occurs in the 4-cell embryo (see Fig. 1); in the absence of this P2-EMS contact, there is no gut to pattern (Goldstein, 1992).

(a) Laser ablation of non-E blastomeres

To determine if anterior endoderm requires contact with cells from other lineages, laser ablation was used to kill the non-gut progenitors of the first three cell divisions: AB at the 2-cell stage, P2 in the 4-cell stage (after the P2-EMS contact just mentioned), and MS at the 8-cell stage. In combination, these ablations eliminate all non-gut lineages. Fig. 4a-f shows examples of embryos in which the AB, P2 and MS blastomeres

![Fig. 2. Transgenic analysis of the ges-1Δ4 region (Egan et al., 1995). A sequence necessary for expression of ges-1 in the posterior gut resides in the 50 bp B element i.e. deletion of the B region results in anterior-gut-specific expression (b), while deletion of the remainder of the Δ4 region results in wild-type expression (a). The ges-1ΔB promoter can also drive anterior-gut-specific expression of a GFP/lacZ reporter; two focal planes are shown of an embryo expressing ges-1ΔB::GFP/lacZ in the four int1 cells, easily detected against the background of endogenous gut fluorescence (c,d).](image-url)
have been ablated: the distribution of birefringent gut granules reveals the entire gut, and esterase staining reveals ges-1 expression; data are summarized in Table 1. None of these ablations (including simultaneous ablation of AB, P2 and MS) prevent anterior gut marker expression, suggesting that gut patterning is lineage autonomous.

Within the E lineage, ablation of Ep, the posterior daughter of the E cell, also does not prevent the formation of Ea-derived anterior gut (Fig. 4g,h). Thus, there is no evidence for a signal passed between descendants of Ep and descendants of Ea. When the blastomeres that give rise to anterior gut (EMS, E and Ea) are ablated, anterior gut marker expression is eliminated (Table 1; Fig. 4i,j). Thus, there is no evidence for a compensating (regulative) mechanism operating within the E lineage (see also Junkersdorf and Schierenberg, 1992).

We conclude from these laser ablation studies that non-gut cells are not required for the production of anterior endoderm (at least after the 4-cell stage). However, a limitation of this approach is the uncertainty in knowing when (or how) laser ‘ablation’ prevents intercellular signalling.

(b) Altered identity of non-gut blastomeres

A second approach to determine if cells outside of the C. elegans homeotic genes are not required for anterior gut marker expression. Wild-type expression of the endogenous ges-1 gene (left column) and an integrated ges-1ΔB transgene (right column) was observed in comma-stage embryos of the following genotypes: wild type (N2) (a,b), lin-39 (c,d), mab-5 (e,f) and nDf16 (g,h). Endogenous GES-1 activity was detected using 30 minute staining; transgenic activity was detected using three minute staining.

Table 1. Anterior gut marker expression following laser ablation of early blastomeres

<table>
<thead>
<tr>
<th>Ablated cell</th>
<th>ges-1ΔB reporter</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB+P2+MS</td>
<td>ges-1</td>
<td>wt 9/11*</td>
</tr>
<tr>
<td>AB</td>
<td>ges-1</td>
<td>18/23</td>
</tr>
<tr>
<td>P2</td>
<td>ges-1</td>
<td>14/14</td>
</tr>
<tr>
<td></td>
<td>lucZ</td>
<td>10/11 8/9</td>
</tr>
<tr>
<td>MS</td>
<td>ges-1</td>
<td>12/14</td>
</tr>
<tr>
<td></td>
<td>lucZ</td>
<td>6/7 20/26</td>
</tr>
<tr>
<td>Ep</td>
<td>lucZ</td>
<td>8/10</td>
</tr>
<tr>
<td>Ea</td>
<td>ges-1</td>
<td>4/24‡</td>
</tr>
<tr>
<td></td>
<td>GFP</td>
<td>1/30</td>
</tr>
<tr>
<td>E</td>
<td>ges-1</td>
<td>0/3</td>
</tr>
<tr>
<td></td>
<td>lucZ</td>
<td>0/10 17/25</td>
</tr>
<tr>
<td>EMS</td>
<td>ges-1</td>
<td>0/8</td>
</tr>
<tr>
<td></td>
<td>lucZ</td>
<td>0/5 11/13</td>
</tr>
</tbody>
</table>

*Fraction of operated embryos expressing the total gut marker (gut granules) that also express ges-1ΔB. For the negative control E and EMS ablations, simply the total number of operated embryos is indicated. Gut granules are detected slightly before ges-1ΔB; for ratios <1, embryos may have arrested between expression times of the two markers.

‡Following prolonged staining of Ea-ablated embryos, occasional faint expression can be detected in Ep-derived gut cells (int4) and are scored as positive. However, such staining occurs only at levels and frequencies equal to those observed in non-ablated control embryos.

Fig. 3. C. elegans homeotic genes are not required for anterior gut marker expression. Total gut, indicated by birefringent gut granules (left column) and anterior gut, indicated by ges-1ΔB expression (right column), were examined in embryos from which the AB (a,b), P2 (c,d), MS (e,f), Ep (g,h), or Ea (i,j) blastomere had been ablated. A GES-1 reporter was used in embryos b, d and f; a β-galactosidase reporter was used in embryos h and j. Data are summarized in Table 1.

Fig. 4. Laser ablation of selected early blastomeres in embryos expressing anterior gut markers. Total gut, indicated by birefringent gut granules (left column) and anterior gut, indicated by ges-1ΔB expression (right column), were observed in embryos of the following genotypes: wild type (N2) (a,b), lin-39 (c,d), mab-5 (e,f) and nDf16 (g,h). Endogenous GES-1 activity was detected using 30 minute staining; transgenic activity was detected using three minute staining.
C. elegans gut influence anterior endoderm patterning is to investigate mutations that change cell identity. The most obvious candidate for a tissue that might influence anterior gut is the adjacent pharynx (including cells of the pharyngeal-intestinal valve attached directly to int1). We thus investigated ges-1ΔB expression in two mutants, pha-4 and skn-1, in which pharynx and pharyngeal-intestinal valve development is eliminated.

Mutations in the zygotic pha-4 gene cause early arrest of pharynx and rectum formation (Mango et al., 1994). pha-4 is the C. elegans homologue of Drosophila forkhead and vertebrate HNF3 genes and has recently been shown to be expressed in all cells of the pharynx (including the pharyngeal-intestinal valve) as well as the rectum (Horner et al., 1998; Kalb et al., 1998). As shown in Fig. 5a,b, the ges-1ΔB transgene shows no change in anterior gut expression when crossed into the pha-4 mutant background (n=43). In other words, normal development of the MS-derived posterior pharynx, the ABa-derived anterior pharynx or the ABp-derived rectum is not required for normal gut patterning. A low level of pha-4 expression that normally occurs in the endoderm itself (Kalb et al., 1998; Horner et al., 1998) also does not appear necessary for anterior gut expression of ges-1ΔB.

The maternally provided SKN-1 factor is required to establish the correct fate of the EMS cell, which gives rise to both gut and pharynx (Bowerman et al., 1992, 1993). The gut defect seen in embryos produced by homozygous skn-1(zu67) mothers is incompletely penetrant; while only 4% of these embryos produce pharynx (at 20°C), ~30% still produce gut (Bowerman et al., 1992). By examining those skn-1 embryos that produce gut but do not produce pharynx, we can test whether anterior gut patterning requires derivatives of the MS cell. The ges-1ΔB transgenic array was crossed into the skn-1 mutant background and embryos analyzed for both gut granule and ges-1 expression. Of the embryos that produced gut granules, half of these (28/56) still expressed the anterior gut marker (Fig. 5c,d). We conclude that normal derivatives of the MS blastomere (which include posterior pharynx and pharyngeal-intestinal valve) are not necessary for anterior gut marker expression. We suggest that skn-1 embryos that express gut granules but do not express ges-1ΔB may simply have arrested gut development before the second (later) marker is expressed.

We conclude from these genetic ablation experiments that correct pharynx development is not required for production of the adjacent anterior endoderm. A possible limitation to this approach is that the mutant cells could still convey some positional information.

(c) Expression of anterior gut markers in ectopic gut cells

Two C. elegans maternal effect lethal mutations, pie-1 and pop-l, have been described in which a non-gut lineage is transformed into a gut lineage (Mello et al., 1992; Lin et al., 1995). Such mutant embryos allow us to determine if anterior gut markers can be expressed in the ectopic gut, a novel setting in which the normal cell-cell contacts have been altered.

In embryos produced by homozygous pie-1 mothers (where pie stands for pharynx and intestinal excess), the P2 blastomere exhibits the fate of the EMS blastomere, thereby producing excess ectopic gut in the embryo posterior (Mello et al., 1992). As shown in Fig. 6b, these embryos express the ges-1ΔB

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**Fig. 5.** Anterior gut marker expression in pharynx-defective mutants. Gut granules (left column) and ges-1ΔB expression (right column) were examined in pha-4 homozygotes (a,b) and in progeny of skn-1 homozygotes (c,d). These embryos produce no pharynx or pharyngeal-intestinal valve but anterior gut marker expression was observed in all the pha-4 embryos (n=43) and in 50% of skn-1 embryos (n=56) that produced gut.

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**Fig. 6.** Anterior gut marker expression in ectopic gut. The progeny of pie-1 (a-f) or pop-l (g-l) homozygotes produce both endogenous and ectopic gut. Gut granules (first and third columns) and ges-1ΔB::lacZ expression (second and fourth columns) were examined in these embryos following no ablation (first row), ablation of the progenitor of the ectopic gut, P2 in pie-1 embryos (c,d) and MS in pop-l embryos (i,j), or ablation of the progenitor of the endogenous gut, EMS in pie-1 embryos (e,f) and E in pop-l embryos (k,l). Non-ablated ‘guts’ all express anterior gut markers. See Table 1 for further data.
transgene in 4-8 cells; laser ablation experiments demonstrate
that both the endogenous and the ectopic guts contribute to this
staining pattern, each "gut" expressing in 2-4 of their anterior-
most cells (Fig. 6d,f; Table 1).

In embryos produced by homozygous pop-1(zu189) mothers
(where pop-1 stands for posterior pharynx defective), the MS
blastomere (which normally produces posterior pharynx)
instead exhibits the fate of its sister cell E, thereby producing
an ectopic gut as an anterior extension of the normal gut (Lin
et al., 1995). (As will be emphasized in a later section, pop-
1(zu189) mutants lack maternal pop-1 activity but zygotic pop-
1 is still functional (Lin et al., 1998)). As shown in Fig. 6h, the
expression of the ges-1ΔB transgene in the pop-1(zu189)
background has two components: 2-4 staining cells near the
anterior of the endogenous gut and 2-4 staining cells near the
anterior of the ectopic gut. Laser ablation studies confirm that
both the endogenous and ectopic guts express the anterior gut
markers (Fig. 6j,l, Table 1).

Thus, in both pie-1 and pop-1 maternal effect mutants, both
endogenous and ectopic guts are capable of producing anterior
gut cells in roughly the correct proportion and in roughly the
correct location. These results suggest that normal cell-cell
contacts are not necessary for anterior gut specification.

Overall, we interpret the results obtained with the three
different experimental approaches to mean that, once the E cell
has been born, anterior fate within the E lineage is in fact
determined autonomously.

**Cytochalasin blocking of cell division reveals the
dynamics of endodermal patterning**

Cytochalasin D prevents cell division in the early embryo by
disrupting the actin cytoskeleton; DNA synthesis and gene
expression continue (Edgar and McGhee, 1988). Examination of
the ges-1ΔB expression patterns in embryos blocked at various
stages of development allows us to approach the following
questions. Does the ges-1ΔB expression pattern observed in
blocked embryos agree with the pattern predicted for a lineage-
autonomous mechanism of endodermal patterning? Does the
ges-1ΔB expression pattern suggest segregation of a
transcriptional repressor away from the gut anterior or
segregation of a transcriptional activator toward the gut anterior?
How does gut polarity reflect the detailed cell division
choreography that occurs in the normal E lineage (Fig. 1)?

The results are shown in Fig. 7 and summarized in Table 2.

In blocked embryos with more than one E cell, ges-1ΔB
esterase activity is seen in only a subset of all the cells that
express gut granules, i.e. expression of the ges-1ΔB transgene
reveals patterning within the gut lineage. In embryos blocked
at the 4-cell stage, expression of the ges-1ΔB transgene is
rarely detected in the EMS cell (Fig. 7a,b) and only faint
expression is observed in the E cell of embryos blocked at the
8-cell stage (Fig. 7c,d). The first significant expression of ges-
1ΔB is detected in embryos blocked with 2 E cells, where
expression is consistently observed in Ea but not in Ep (Fig.

<table>
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<th>faint</th>
<th>intense</th>
<th>(n)</th>
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<td>60</td>
<td>40</td>
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<td>(5)</td>
</tr>
<tr>
<td>E</td>
<td>28</td>
<td>58</td>
<td>14</td>
<td>(36)</td>
</tr>
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*Percentage of embryos expressing the total gut marker (gut granules) that also express ges-1ΔB in the indicated pattern in the given cells.
†Total number of embryos examined.
§Staining is more intense in anterior cells than in posterior cells.
¶Staining is equal in anterior and posterior cells.

<table>
<thead>
<tr>
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<td>75</td>
<td>10</td>
<td>(59)</td>
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<td>82</td>
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<td>(38)</td>
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<td>8E</td>
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<td>85</td>
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</tr>
<tr>
<td>16E</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>(31)</td>
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Fig. 7. Anterior gut marker expression in cytochalasin-blocked wild-
type embryos. Early embryos were treated with cytochalasin D and
incubated overnight prior to examining for gut granules (left column)
and ges-1ΔB expression (middle column). Schematics in the right
column summarize these expression patterns; arrows indicate
anterior/posterior divisions, asterisks indicate transverse divisions.
Marker expression was examined in cytochalasin-blocked embryos at the
following stages of gut development as indicated on left: EMS
(a,b), E (c,d), 2E (e,f), 4E (g,h), 8E (i,j) and 16E (k,l). Data are
summarized in Table 2.
Fig. 8. Anterior gut marker expression in a pie-1 embryo cytochalasin-blocked at the equivalent of the 2-E-cell stage. Gut granules (a) mark total gut; ges-1ΔB expression (shown in two focal planes, b and c) occurs in the anterior daughters of both the endogenous and ectopic gut lineages. As in Fig. 7, the schematic on the right summarizes this expression pattern. See Table 3 for further analysis.

The next cell division in the E lineage is transverse and gives rise to 4 E cells; as shown in Fig. 7g,h, ges-1ΔB expression is roughly equal in the two (left-right) daughters of Ea but is essentially undetectable in the two daughters of Ep. The next division is oriented anterior-posterior and gives rise to 8 E cells; as shown in Fig. 7i,j, the anterior-most gut cells produced by this division (Ea(l/r)a, which will give rise to int1 and int3) stain intensely; the posterior daughters of this same division (Ea(l/r)b, which will give rise to int2 and int5) occasionally stain weakly. All Ep descendants show no detectable staining. In embryos blocked with 16 E cells (Fig. 7k,l), int1 cells (Ea(l/r)a) stain intensely while int2 cells (Ea(l/r)b) occasionally stain weakly, consistent with the mature (unblocked) wild-type staining pattern. Again, none of the more posterior cells stain.

The above patterns of ges-1ΔB expression in blocked embryos are in contrast to the expression patterns observed for general gut markers, such as gut granules, endogenous ges-1 and the control (undeleted) ges-1 transgene. At the 2- to 8-cell stage of the embryo, expression of these general gut markers is detected in all blastomeres that will ultimately give rise to gut; thereafter, the markers are expressed in all gut cells (Lauffer et al., 1980; Cowan and McIntosh, 1985; Edgar and McGhee, 1986; as well as data not shown).

In summary, the pattern of ges-1ΔB expression in cytochalasin-blocked embryos is completely consistent with lineage-autonomous cell fate decisions within the E lineage: every progenitor of anterior gut expresses the marker. Progenitors such as Ea give rise to both expressing and non-expressing cells; the fact that such progenitor cells express ges-1ΔB in blocked embryos supports a model in which an activator is segregated into the anterior cell at each division rather than a repressor segregated away from the anterior.

Even ectopic guts appear to be patterned by a similar process; Fig. 8b,c shows two focal planes of a cytochalasin-blocked ges-1ΔB pie-1 embryo; ges-1ΔB expression is detected in the anterior daughter of E (i.e. Ea) as well as in the anterior daughter of P3 (which would normally be the D cell). Further data are summarized in Table 3.

Zygotic pop-1 is required for anterior gut fate

A recent paper on the role of the C. elegans pop-1 gene in anterior-posterior patterning (Lin et al., 1998) suggested to us that pop-1 may be involved in the formation of anterior gut. The evidence is as follows. A POP-1 monoclonal antibody was used to show that, during virtually every anterior-posterior division in C. elegans embryogenesis, higher levels of POP-1 (or strictly speaking, a particular POP-1 epitope) are present in the anterior cell relative to the posterior cell. Of particular interest to the present study, POP-1 distribution in the gut lineage was asymmetric when the gut had 2 cells, symmetric when the gut had 4 cells and asymmetric when the gut had either 8 or 16 cells. Thus increased levels of POP-1 correlate with anterior gut cell fate as revealed in the cytochalasin-blocked ges-1ΔB embryos shown in Fig. 7. In addition, POP-1 asymmetry in the E lineage was shown to be independent of cell contact after the initial P2-EMS induction, just as we have found for anterior gut fate.

Lin et al. (1998) showed that pop-1 was required for correct patterning within the AB lineage. They found that, in embryos produced by hermaphrodites homozygous for the pop-1 mutant allele zu189 (as used in Fig. 6 of the current paper), only maternal POP-1 was absent; zygotic (apparently normal) POP-1 was expressed by the 24-cell stage. In pop-1(zu189) embryos, anterior daughters of early divisions in the AB lineage took on the fate of their posterior sisters but later defects in the lineage pattern were rescued by zygotic POP-1. When both maternal and zygotic POP-1 were eliminated with RNA-mediated interference (RNAi), all AB descendants took on the fate of the posterior-most cell (Lin et al., 1998). Thus pop-1 is required for anterior fate within the AB lineage. We now apply this same analysis to the problem of anterior fate within the E lineage.

Since lack of maternal pop-1 (as produced by allele zu189) still allows anterior gut to be formed (Fig. 6), we propose that the production of anterior gut within the E lineage requires only zygotic pop-1. To test this model, RNAi was used to

**Table 3. ges-1ΔB expression in cytochalasin-blocked pie-1 embryos**

<table>
<thead>
<tr>
<th>Cell</th>
<th>% Gut granules</th>
<th>% Esterase</th>
<th>(n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EMS</td>
<td>60</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>E</td>
<td>100</td>
<td>67</td>
<td>6</td>
</tr>
<tr>
<td>2E</td>
<td>100</td>
<td>100§</td>
<td>17</td>
</tr>
<tr>
<td>4E</td>
<td>100</td>
<td>100§</td>
<td>7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cell</th>
<th>% Gut granules</th>
<th>% Esterase</th>
<th>(n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>“EMS”</td>
<td>93</td>
<td>29</td>
<td>14</td>
</tr>
<tr>
<td>“E”</td>
<td>83</td>
<td>67</td>
<td>6</td>
</tr>
<tr>
<td>“2E”</td>
<td>100</td>
<td>70§</td>
<td>20</td>
</tr>
</tbody>
</table>

*The stage of ectopic gut development is described by the corresponding stage of endogenous gut development.

§Percentage of embryos expressing the total gut marker (gut granules) in either endogenous or ectopic gut progenitor(s) that express gut granules or ges-1ΔB in given cell(s).

$\text{Marker expression higher in anterior daughters than posterior daughters.}$
eliminate both maternal and zygotic contributions (Lin et al., 1998). Injection of pop-1 dsRNA (Fire et al., 1998) results in the production of arrested embryos with approximately double the wild-type number of gut cells (Fig. 9). Almost all of these embryos (299/318 = 94%) fail to express ges-1ΔB in the gut (Fig. 9b). Although the endogenous ges-1 gene is still expressed (Fig. 9e), the anterior-to-posterior gradient of endogenous ges-1 activity observed in wild-type embryos is now abolished (see below). We conclude that (zygotic) pop-1 is required for the lineage-autonomous production of anterior gut.

**DISCUSSION**

**Homeotic genes in endoderm patterning**

The role of homeotic genes in anterior/posterior patterning of the ectoderm and mesoderm has been well documented in many organisms, including *C. elegans* (Krumlauf, 1994; Kenyon et al., 1997). In general, the role of homeotic genes in endoderm patterning has been less well characterized, except in *Drosophila* where it has been shown that endodermal patterning involves Ubx expression in the visceral mesoderm and *labial* expression in the endoderm (Bienz, 1997). In vertebrates, a number of Hox genes are expressed in endoderm (Duluc et al., 1997; Walters et al., 1997) and two Hox knockouts (*Hoxa5* and *Hoxa3*) have been described to have endoderm defects (Aubin et al., 1997; Manley and Capecchi, 1998).

In the present study, expression of an anterior-gut-specific transgene resulting from a 50 bp deletion within the ges-1 promoter (see Fig. 2 above) was not changed when all known genes of the *C. elegans* homeotic cluster were deleted. It should be noted that both *mab-5* and *celh-13* (the candidate *C. elegans labial* homolog) are expressed asymmetrically in the *C. elegans* endoderm (Cowin and Kenyon, 1992; Wittmann et al., 1997) but we detect no requirement for these genes in endoderm patterning. Thus, the *C. elegans* endoderm presents a contrasting (and undoubtedly simpler) situation than endoderm formation in either insects or vertebrates.

**Endoderm patterning in *C. elegans* is lineage autonomous**

In both insects and vertebrates, patterning of the endoderm has been shown to involve regionalized interactions between endoderm and mesoderm (Roberts et al., 1995; Gualdi et al., 1996; Bienz, 1997; Kim et al., 1997). We used three quite different experimental approaches to show that, in contrast, patterning within the *C. elegans* E lineage appears to be completely lineage-autonomous. Asymmetric expression of the *celh-13* gene in the embryonic E lineage has also been shown to be lineage autonomous (Wittmann et al., 1997), as has the propagation of POP-1 asymmetries (Lin et al., 1998). Thus, we are left with the question: how does an anterior gut cell, which is capable of expressing ges-1ΔB, become different from a posterior gut cell, which is incapable of expressing the same gene? In the E cell of the 8-cell embryo, there must be some spatial cue that differentiates the E cell anterior from the E cell posterior. This cue could be induced by the P2-EMS contact or could be an ‘intrinsic’ feature of all cell divisions that split off from the germ line. The cue is unlikely to lie in the posterior of the E lineage, because Ep cannot be ablated without inhibiting anterior gut marker expression (see Fig. 4 above). One location for the anterior endoderm cue that comes to mind is the anterior cortex of Ea; presumably such a cortical domain would be segregated to the anterior daughter at each anterior-posterior division but would be segregated equally to both daughters of a transverse division. A number of striking cortical asymmetries have been described in early *C. elegans* embryos (Kemphues and Strome, 1997). A second possibility is that the anterior cue could be produced by the specific behaviour of the centrosomes. In the early *C. elegans* embryo, cell divisions segregating somatic founder cells from the germ line, as well as cell lineages in which successive mitotic spindles align in the same direction, involve a 90° rotation of the centrosome-nucleus complex, following capture of microtubules associated with one of the centrosomes by a site on the cell cortex (White and Strome, 1996). One could imagine a mechanism by which this differential behaviour of the two centrosomes provides a coordinate system within a lineage. Indeed, the PIE-1 protein, a factor required to repress gene expression in the germline, has been reported to associate with centrosomes during cell division and subsequently disappear from the somatic daughter (Mello et al., 1996).

**Wnt/HMG signalling in endoderm patterning in *C. elegans***

As we noted above, the P2-EMS contact early in the cell cycle of the 4-cell embryo polarizes EMS, enabling it to divide into the (anterior) MS cell and the (posterior) E cell (Schierenberg, 1987; Goldstein, 1992, 1993, 1995a,b). This interaction has
been shown to involve a *C. elegans* Wnt pathway (Rocheleau et al., 1997; Thorpe et al., 1997). Although Wnt pathways show both similarities and differences in different organisms (Cadigan and Nusse, 1997), a common final step of all such pathways appears to involve a HMG protein that differentially regulates transcription. In *C. elegans*, this HMG protein appears to be the product of the *pop-1* gene (Lin et al., 1998).

How are *pop-1* and other elements of the Wnt pathway connected to the hypothetical positional cue for anterior gut discussed in the previous section? We have shown that the establishment of E lineage polarity is independent of *pop-1*, since we see expression of the anterior gut marker in embryos produced by *pop-1*(zu189) mothers, i.e. in embryos that lack maternally provided POP-1. In contrast, we have shown that anterior gut marker expression is completely dependent on (zygotic) *pop-1* function. Because Lin et al. (1998) have shown that POP-1 asymmetries in many (possibly most) *C. elegans* lineages depend on a Wnt signalling pathway, we anticipate that anterior gut marker expression (and hence endoderm patterning in general) will also depend on elements of a Wnt signalling pathway. It is an open question whether this pathway functions in establishing the anterior gut cue, in propagating the cue or in interpreting the cue.

**Why should a deleted *ges-1* gene reflect anterior-posterior endoderm patterning?**

The *ges-1ΔB* expression pattern provides evidence that anterior-posterior patterning at the transcriptional level exists within the *C. elegans* endoderm. At the same time, our results raise the question: if the normal (undeleted) *ges-1* gene is expressed in all cells of the gut, why should a deleted transgene be expressed only in the gut anterior? We suggest that the ability of *ges-1* to respond to anterior-posterior patterning within the gut derives from the fact that *ges-1* is normally expressed in a gradient, high at the anterior, low at the posterior. (Although it is usually GES-1 enzymatic activity that is detected in a gradient, a similar gradient is detected using transgenic reporters and thus is likely to reflect levels of *ges-1* transcription.) Our previous analysis has shown that the *ges-1* promoter contains three principal elements: a tandem pair of GATA sites and an element called D4 (Egan et al., 1995), refined in the present paper to the 50 bp B region (see Fig. 2). Removal of any one of these three elements causes anterior gut expression, i.e. abolishes expression in the posterior gut. We propose that such a deletion weakens the *ges-1* promoter in such a way that only the most strongly expressing cells, i.e. the anterior cells, retain detectable activity.

Anterior expression of *ges-1ΔB* correlates with high levels of POP-1, raising the possibility that POP-1 is directly involved in activating the *ges-1ΔB* promoter. An alternative model is that POP-1 acts indirectly by activating factors associated with anterior gut ‘fate’, which in turn interact with *ges-1ΔB* directly. If POP-1 is assumed to interact with the consensus binding site of the LEF-1/TCF class of HMG proteins to which it is most closely related, then there are several reasons for favouring this second alternative. First of all, the sequence of the B region does not contain a preferred LEF-1/TCF binding site. Secondly, a factor that binds to the B element can be detected in embryonic nuclear extracts using an electrophoretic mobility shift assay but this binding is not competed by the preferred LEF-1/TCF binding sequence. Thirdly, binding of a different factor in the extract to the preferred LEF-1/TCF sites is not competed by the B element (our unpublished results).

Although POP-1 may not directly control *ges-1ΔB* expression in the anterior gut, we suggest that the factor that is involved may be, like POP-1, an architectural protein. Multimers of the B region do not appear to act as an independent enhancer element (our unpublished results) and the B sequence contains an inverted pair of potential binding sites for SRY (Heinemeyer et al., 1998), a member of a different class of HMG proteins (Baxevanis and Landsman, 1995). Thus, our current working model is that the normal gradient of *ges-1* activity is engendered by a gradient of a second HMG protein, acting in concert with a lineage-specific uniformly expressed enhancer-binding factor, such as the GATA factor ELT-2 (Hawkins and McGhee, 1995; Fukushige et al., 1998). In any event, the *ges-1ΔB* transgene provides an unusual opportunity to explore the molecular mechanisms by which a promoter is able to sense anterior-posterior position within a cell lineage.

**How general is the mechanism of anterior-posterior endoderm patterning?**

Finally, we wish to point out interesting parallels between the position-sensitive expression of the *ges-1* gene in *C. elegans* endoderm and gene regulation within the vertebrate digestive tract. A number of genes are expressed in a gradient along the cephalocaudal axis of the vertebrate intestine (reviewed in Traber and Silberg, 1996). In one of the most intensely investigated cases, that of the gene encoding intestinal fatty acid binding protein, it has been shown that not only is IFABP expressed differentially along the intestinal axis but, like the *ges-1* gene, certain promoter deletions can alter the position of this expression (Simon et al., 1995). We also point out that several HMG proteins (eg, hTCF4) are distributed in a gradient along the intestinal axis (Korinek et al., 1998) and that some of these molecules have also been implicated as downstream effectors in a WNT pathway (Korinek et al., 1997). Thus, we suggest that control of the *ges-1* gene in the *C. elegans* endoderm may operate by some of the same mechanisms that control endoderm gene expression in all (triploblastic) metazoans.

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