ELT-1, a GATA-like transcription factor, is required for epidermal cell fates in *Caenorhabditis elegans* embryos

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Epidermal cells are generated during *Caenorhabditis elegans* embryogenesis by several distinct lineage patterns. These patterns are controlled by maternal genes that determine the identities of early embryonic blastomeres. We show that the embryonically expressed gene *elt-1*, which was shown previously to encode a GATA-like transcription factor, is required for the production of epidermal cells by each of these lineages. Depending on their lineage history, cells that become epidermal in wild-type embryos become either neurons or muscle cells in *elt-1* mutant embryos. The ELT-1 protein is expressed in epidermal cells and in their precursors. We propose that *elt-1* functions at an early step in the specification of epidermal cell fates.

[Key Words: *C. elegans*; hypodermal cells; cell fate specification; lineage patterns; GATA transcription factor]

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Genetic and experimental studies have shown that the early molecular events that lead to hypodermal specification of P₁ descendants are different from the events that specify hypodermis from AB. The maternally expressed gene *pal-1* is required for P₁ to produce hypodermal cells as well as other cell types [Hunter and Kenyon 1996]. In contrast, *pal-1(+)¹* is not required for AB descendants to produce hypodermal cells. *pal-1* encodes a transcription factor with sequence similarity to *Drosophila* CAUDAL [Waring and Kenyon 1991], and the PAL-1 protein is present in the nuclei of P₁ descendants, but not AB descendants [Hunter and Kenyon 1996].

The hypodermal fates of AB descendants are determined by a combination of cell–cell interactions [Priess et al. 1987; Gendreau et al. 1994; Hutter and Schnabel 1994; Mango et al. 1994; Mello et al. 1994; Hutter and Schnabel 1995]. During the two-cell stage of embryogenesis, the P₁ blastomere appears to influence the development of the AB blastomere such that AB acquires the ability to produce both hypodermal and neuronal cell types [Hutter and Schnabel 1995]. In the next few cell divisions, two additional cell–cell interactions determine which specific AB descendants will produce predominantly neurons and which will produce predominantly hypodermal cells. These latter two interactions are both mediated by maternal expression of the *glp-1* gene [Priess and Thomson 1987; Hutter and Schnabel 1994; Mello et al. 1994; Moskowitz et al. 1994]. The GLP-1 protein is a transmembrane receptor similar to *Drosophila* NOTCH and *C. elegans* LIN-12 [Yochem and Greenwald 1989]. GLP-1 is expressed in AB and its daughters, but not in P₁ or in P₁ daughters [Evans et al.

### Key Words

- *C. elegans*
- hypodermal cells
- cell fate specification
- lineage patterns
- GATA transcription factor

Recent studies have identified some of the maternally expressed genes that pattern the early *Caenorhabditis elegans* embryo [for review, see Kemphues and Strome 1997; Schnabel and Priess 1997]. However, there is very little molecular information about the embryonically expressed genes that presumably are regulated by these maternal genes and that lead to tissue-specific differentiation. In the present study, we provide evidence that the embryonically expressed gene *elt-1* functions in the specification of a major tissue in *C. elegans*, the epidermis.

The epidermis consists of a simple layer of cells, one cell thick. The apical surface of each cell is encircled by an adherens junction, so that adjacent cells are linked together; the basal surface overlies a basement membrane. Historically, nematode epidermal cells have been called hypodermal cells because an extracellular cuticle covers the apical surface of these cells. In *C. elegans*, hypodermal cells arise from several different branches of the embryonic lineage. Both blastomeres, AB and P₁, of the two-cell-stage embryo produce several hypodermal cells. The lineage patterns by which AB and P₁ produce hypodermal cells, however, are very different. Whereas most of the nonhypodermal descendants of AB become neurons or pharyngeal cells, most of the nonhypodermal descendants of P₁ become intestinal cells or body-wall muscle cells.

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Thus, the production of hypodermal cells by AB and P₁ is influenced initially by distinct genetic pathways involving GLP-1 and PAL-1, respectively.

Maternally expressed genes like \textit{glp-1} and \textit{pal-1} define identities of early embryonic blastomeres; they initiate the complex patterns of cell division and differentiation that distinguish the blastomeres. These maternal genes must directly or indirectly activate new transcription, leading to blastomere-specific expression of the embryonic genome. In this report we show that mutations in the \textit{elt-1} gene, which was shown previously to encode a GATA-like transcription factor of unknown function (Spieth et al. 1991), is required for the embryo to produce hypodermal cells. We find that the ELT-1 protein is expressed in the early embryo in both AB and P₁-derived hypodermal precursors. We show that cells that produce hypodermis in wild-type embryos produce nonhypodermal cell types in \textit{elt-1} mutant embryos, and that the specific fates of these transformed cells depend on whether they are descendants of AB or P₁. Thus, \textit{elt-1} identifies a point of convergence between the initially distinct pathways AB and P₁ use to produce hypodermal cells.

Background

\textit{C. elegans} embryogenesis begins with a period of rapid cell proliferation. Near the end of this period, cells on the dorsal and dorsal-lateral surfaces of the embryo become visibly different from all other cells (Fig. 1A). The dorsal cells cease division, flatten and spread, and become linked through adherens junctions (Fig. 1B). These cells differentiate as hypodermal cells. Cells on the ventral surface of the embryo undergo one to three additional divisions; almost all of these cells differentiate as neurons or neuronal support cells (Fig. 1A). The hypodermal cells spread ventrally (Fig. 1C) and anteriorly until they enclose the embryo, thus forming a continuous epithelial surface called the hypoderms.

Two basic types of hypodermal cells, major and minor, have been described (Gendreau et al. 1994). The major and minor classes were used originally to distinguish between AB-derived descendants. All P₁-derived hypodermal cells, however, have characteristics of the major class, and, in this paper, are included in the major class. There are 71 major hypodermal cells; these are large cells that are born at the 9th round of embryonic cleavage and that cover most of the body surface. There are 11 minor hypodermal cells; these are relatively small cells that are born at the 10th round of cleavage and that form specialized syncytia at the head and tail. Analysis of the \textit{C. elegans} cell lineage has shown that major hypodermal cells originate from four blastomeres in the 12-cell-stage embryo (Fig. 1D; Sulston et al. 1983). The AB-derived descendants are \textit{ABarp}, \textit{ABpla}, and \textit{ABpra}; the P₁-derived descendant is \textit{C}. Each of these blastomeres undergoes an additional five rounds of cell division before generating major hypodermal cells.

Results

\textit{Isolation of elt-1 mutations}

In screens for recessive, embryonic-lethal mutations affecting tissue development, we found three mutants,
Homozygous phogenesis (Fig. 3, cf. D with C), a process that normally
We found that each of the overlapping cosmids W03E7, T01G2, and T12F9 has rescuing activity. Analysis of the rescuing DNA fragments showed that each contains a gene called elt-1. Because no mutations in elt-1 had been described previously, we tested whether the zu180 embryonic phenotype could be phenocopied by injection of antisense elt-1 RNA into the gonads of wild-type hermaphrodites [Guo and Kemphues 1995]. The injected hermaphrodites produced inviable embryos that appeared identical to zu180 mutants (see below), indicating that zu180 was likely to be a mutation in the elt-1 gene.

elt-1 was identified initially by its similarity to genes that encode GATA-like, zinc-finger transcription factors [Spieth et al. 1991]. This family of transcription factors has been implicated in the specification of tissue type in vertebrates [Orkin 1992]. The predicted ELT-1 protein contains two possible zinc finger domains and is 60% identical in these domains to vertebrate GATA factors, such as GATA-1, 2, and 3 from mouse. We sequenced the elt-1 gene from the zu180 strain and found that it contains a stop codon that would truncate the predicted ELT-1 protein after the first finger domain, confirming that zu180 is a mutation in elt-1.

elt-1 mutants lack hypodermal cells

Homozygous elt-1 mutants do not undergo body morphogenesis [Fig. 3, cf. D with C], a process that normally requires hypodermal cell function [Sulston et al. 1983; Priess and Hirsh 1986]. Major hypodermal cells can be identified on the surface of a wild-type embryo by their large, flat nuclei and prominent nucleoli [Fig. 3C,E]. An elt-1 mutant contains few, if any, candidates for being hypodermal cells by these morphological criteria. Instead, the surface of an elt-1 mutant is covered with numerous cells that, by light microscopy, resemble neurons [Fig. 3D,F]. Internal tissues in an elt-1 mutant, such as the pharynx and intestine, appear to contain the normal numbers and types of cells [Fig. 3, cf. B with A]. Other cell types such as neurons, muscle cells, [see below] and germ cells [data not shown] also are present in an elt-1 mutant. Therefore, mutations in the elt-1 appear to cause a loss of hypodermal cells, but do not affect the ability of the embryo to produce several other tissue types.

We examined elt-1 mutant embryos with molecular markers to confirm the absence of hypodermal cells. In wild-type embryos, adherens junctions surround the apical margins of hypodermal cells, intestinal cells, and some pharyngeal cells; these adherens junctions can be visualized by staining with the antibody MH27 [Priess and Hirsh 1986; Waterson 1988]. Wild-type and elt-1 mutant embryos appear to have identical pharyngeal and intestinal MH27 staining patterns [Fig. 3, cf. G with H]. However, whereas wild-type embryos have ~60 hypodermal cells that stain with MH27 [Fig. 3I], elt-1 mutant embryos contain few or no cells with a hypodermal-like staining pattern [Fig. 3J]. In elt-1 mutants, the few nonpharyngeal or nonintestinal cells that show MH27 staining are small cells of undetermined identity [see Discussion]. For a second hypodermal marker, we used the lacZ fusion construct wls1 that is expressed in wild-type embryos in the lateral group of major hypodermal cells; these 20 cells are called seam cells [Gendreau et al. 1994; see Materials and Methods]. We found that elt-1 mutants...

Figure 2. Molecular cloning of elt-1. The zu180 mutation maps near the elt-1 gene on chromosome IV and is complemented by the chromosomal deficiencies edf18 and md17, but not by edf19. zu180 mutants were rescued with the cosmids W03E7, T01G2, T12F9, and the phage BL#Y19, all of which contain elt-1. Rescue was not obtained with W05D7 that does not contain the elt-1 gene. The elt-1 gene is oriented 5' to 3'; exons are boxed; regions encoding zinc-finger domains are shaded. zu180 is a nonsense mutation in tryptophan 283. Map of elt-1 is from Spieth et al. [1991].
carrying this marker do not have detectable lacZ expression (data not shown). Thus, we conclude that elt-1 mutants have a defect in either the differentiation or specification of hypodermal cells.

elt-1 mutant embryos appear by light microscopy to contain more neurons than are visible in wild-type embryos. To extend this observation, we stained elt-1 mutants with the neuron-specific antibody anti-UNC-33; this antibody stains the cytoplasm of apparently all classes of neuronal cells (J. Shaw, pers. comm.). Although we could not accurately count the numerous cells stained with anti-UNC-33 in whole-mount preparations, the elt-1 embryos [Fig. 4B] appear to have many more positively stained cells than do wild-type embryos [Fig. 4A]. This result, combined with the lack of hypodermal markers in elt-1 mutants, suggests that loss of elt-1 activity may cause a transformation of some hypodermal precursors into neuronal precursors.

ABa-derived hypodermal precursors become neuronal precursors in elt-1 embryos

Twelve of the 20 seam hypodermal cells are produced by an AB descendant called ABarp (Sulston et al. 1983; Fig. 1). Because these hypodermal cells are not detectable by seam cell-specific lacZ expression in elt-1 mutants, we examined the development of the ABarp blastomere by light microscopy and video recording. Results of this lineage analysis indicate that the cell division pattern of ABarp in elt-1 mutants is different from the wild-type pattern [Fig. 5A]. The first difference we observe between the wild-type and elt-1 lineage patterns occurs ~220 min postfertilization. In wild-type embryos, four ABarp descendants undergo unequal divisions in which the large...
daughter becomes a hypodermal cell and the small daughter becomes either a neuroblast or a neuronal support cell [Sulston et al. 1983]. In elt-1 mutants, these four ABarp descendants divide equally.

In a wild-type embryo, 22 of the ABarp descendants at 220 min begin to flatten and spread, differentiating as hypodermal cells; these cells do not divide again during embryogenesis [Fig. 5A]. In elt-1 mutants, these ABarp descendants remain rounded instead of flattening, and undergo at least one additional division, producing cells with a neuronal morphology [Fig. 5A; additional divisions indicated by arrow]. The extra cell divisions in elt-1 mutants occur at a time when several blastomeres in wild-type embryos are dividing to produce neurons [Sulston et al. 1983]. The abnormal ABarp lineage in elt-1 mutants, however, is not identical in detail to the lineage pattern of any wild-type blastomere.

To determine whether the additional descendants of ABarp were becoming neurons in elt-1 mutants, we asked if these descendants express the neuronal marker H2O-GFP. This marker consists of a neuronal-specific promoter fused to a gene encoding the green fluorescent protein (GFP) with a nuclear localization signal; wild-type nematodes carrying the H2O–GFP transgene show fluorescence in most neurons [T. Ishihara and I. Katsura, pers. comm.; see Materials and Methods]. We examined the expression of H2O-GFP in operated embryos in which all blastomeres except ABarp and ABalp were killed with a laser microbeam. In wild-type development, cell-cell interactions cause ABalp to adopt a different fate from that of ABarp [Hutter and Schnabel 1994; Mello et al. 1994]. Our ablation strategy, however, prevents these interactions such that both ABalp and ABarp adopt the fate of ABarp. We found that the laser-operated wild-type embryos produce many hypodermal cells as expected, and only about seven H2O-GFP-positive neurons. In contrast, operated elt-1 mutants produce an average of 28 H2O–GFP-positive neurons, and no cells resembling hypodermal cells [Fig. 4C,D; Table 1]. Thus, we conclude that elt-1 function is necessary for the wild-type development of ABa-derived hypodermal cells and that loss of elt-1 activity leads to this blastomere producing neurons instead of hypodermal cells.

Abp-derived hypodermal precursors are transformed in elt-1 mutants

The ABp-derived blastomeres ABpra and ABpla produce a total of 36 major hypodermal cells in wild-type em-
precursors in produce H20-GFP-positive cells (Table 1). These results
expression pattern of the neuronal marker H20-GFP in mutants.
was allowed to develop. In an operated wild-type em-
descendants become muscle cells instead of hypodermal
suggest that C does not produce extra neurons in
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found that both Cpa and Caa descendants undergo an extra division in elt-1 mutants, and the
cells produced by these extra divisions do not resemble
hypodermal cells. We isolated the ABp blastomere by
laser-ablation experiments [see Materials and Methods]
and examined the amount of neuronal tissue produced
by H20–GFP expression. In contrast to ABa, ABp does not
appear to produce extra neurons in the elt-1 mutant.
In similar experiments we tested whether ABp produces
muscle cells instead of hypodermal cells in elt-1 mutants
by use of the muscle-specific marker hlh-1-GFP (K. Dej,
S. Xug, and A. Fire, pers. comm.), and found that ABp
does not produce extra muscle cells. We conclude that
elt-1 is required for the specification of major hypoder-
cmal cells from the ABp blastomere. In elt-1 mutants,
however, ABa and ABp descendants have different trans-
formations in cell fate.
C-derived hypodermal precursors become muscle
precursors in elt-1 mutants
In wild-type development, the C blastomere produces 13
major hypodermal cells and 32 muscle cells [Sulston et
al. 1983]. Two C granddaughters, Cpa and Caa, almost
exclusively produce hypodermal cells, whereas the other
two granddaughters, Cpp and Cap, produce only muscle
cells. We examined the lineage of C in elt-1 mutants and
found that both Cpa and Caa descendants undergo an
additional round of division, producing cells that do not
resemble hypodermal cells in morphology [Fig. 5C shows
the lineage of Cpa in both wild-type and elt-1 mutant
embryos]. To test whether the abnormal Cpa and Caa
descendants are becoming neurons, we examined the ex-
pression pattern of the neuronal marker H20–GFP in
laser-operated embryos in which only the C blastomere
was allowed to develop. In an operated wild-type em-
byro, the C blastomere produces numerous hypodermal
cells, but no H20–GFP-positive cells (Table 1). We found
that the C blastomere in an operated elt-1 embryo does
not appear to produce hypodermal cells but also does not
produce H20–GFP-positive cells (Table 1). These results
suggest that C does not produce extra neurons in elt-1
mutants.
To test the possibility that the abnormal Cpa and Caa
descendants become muscle cells instead of hypodermal
cells, we examined the expression of the muscle marker
hlh-1-GFP in elt-1 mutants. To examine the develop-
ment of only Cpa and Caa, we killed all other blasto-
meres with a laser microbeam. Cpa and Caa blastomeres
from wild-type embryos [n = 19] produce hypodermal
cells but do not produce muscle cells, as expected. In
contrast, when either Cpa or Caa is isolated in elt-1 mu-
tants [n = 3 and 5, respectively], each blastomere pro-
duces an average of 13 muscle cells. Thus, C, ABa,
and ABp blastomeres all require elt-1 to produce hypodermal
cells. In the absence of elt-1 function, however, C pro-
duces muscle cells instead of hypodermal cells while
ABa produces neurons.
Cell fate transformations in elt-1 mutants are affected
by lineage-specific factors
Why do C-derived hypodermal precursors become
muscle precursors in elt-1 mutants while the ABa and
ABp-derived precursors adopt nonmuscle fates? The C-
derived precursors in elt-1 mutants could respond to
muscle-determining factors that are normally sup-
pressed by elt-1(+) activity. These could be position-spe-
cific extrinsic factors [Schnabel 1995], or lineage-specific
intrinsic factors [Hunter and Kenyon 1996]. To ask
whether the posterior positions of C-derived precursors
affect their pattern of development in elt-1 mutants, we
constructed and examined mex-3;elt-1 double mutants.
In mex-3 mutants, the lineage-specific transcription fac-
tor PAL-1 is misexpressed in AB descendants, causing
these anterior blastomeres to adopt C-like fates and pro-
duce hypodermal cells and muscle cells [Fig. 6C; Draper
et al. 1996; Hunter and Kenyon 1996]. If the transformed
fates of hypodermal cells in the elt-1 mutant are caused
by position-specific factors, many of these anterior blas-
tomeres in mex-3;elt-1 mutants would produce neurons,
as do ABa descendants in elt-1 mutants. If the trans-
formed fate depends on lineage-specific factors, however,
these anterior blastomeres would be expected to produce
muscle cells, as do C descendants in elt-1 mutants. We
found that the anterior region of the mex-3;elt-1 double
mutant lacks hypodermal cells, and instead consists al-
most entirely of muscle cells [Fig. 6E] with very few neu-
ron [Fig. 6F].

ELT-1 is expressed in hypodermal cells and their
precursors
To determine when and where the ELT-1 protein is ex-
pressed, antibodies were generated against two regions
outside the zinc finger domains of ELT-1 [see Materials
and Methods]. Antisera against both regions of ELT-1
gave identical staining patterns. This pattern is not de-
ected in wild-type embryos stained with preimmune
sera, or in elt-1(zu180) mutants stained with anti-ELT-1
sera [see Materials and Methods]. The 71 major hypoder-
cmal cells are born ~260 min postfertilization [about the
365-cell stage]; these cells are readily identified in em-
byros stained for adherens junctions [Fig. 7B]. At this
stage, ELT-1 is detected in the nuclei of all 71 major
hypodermal cells, and is not detected in any other cell
type [Fig. 7A,B]. Two categories of cells that do not stain

Table 1. Number of neurons produced by isolated blastomeres

| Blastomeres     | Wild type | elt-I(zu180) |
|-----------------|-----------|-------------|
| ABa and ABa p   | 7 ± 2 (n = 48) | 28 ± 7 (n = 17) |
| C               | 0 ± 0 (n = 15) | 0 ± 0 (n = 11) |

Blastomeres were isolated, allowed to develop, and examined
for H20–GFP expression. Details of blastomeres isolation are in
Materials and Methods.
Epidermal development in the C. elegans embryo

sors and in a subset of the 32 AB descendants (Fig. 8A). The developmental time at which staining first is detected for each hypodermal lineage is indicated by an asterisk beside the wild-type lineages in Figure 5. Because there is natural variability in the position of cells at the 44-cell stage (Schnabel et al. 1997) and because additional variability is introduced by our fixation technique, we have not yet been able to identify all of the AB descendants at this stage with certainty. However, the positions and number of cells that express ELT-1 at this stage correlate well with the positions and number of hypodermal precursors present at this stage (Table 2, Sulston et al. 1983). ELT-1 is expressed in the AB descendants through the successive rounds of cell division until

for ELT-1 should be noted. First, ELT-1 is not detected in the nuclei of minor hypodermal precursors, and is not detected in the nuclei of their descendants later in embryogenesis. Second, ELT-1 is not detected in the nuclei of the nonhypodermal sisters of major hypodermal cells. As the embryo undergoes morphogenesis, ELT-1 levels appear to decrease in the dorsal and ventral hypodermal nuclei but persist at relatively high levels in the lateral, or seam, nuclei (Fig. 7C, E, G). ELT-1 can be detected in these hypodermal seam nuclei throughout the remainder of embryogenesis (data not shown).

The ELT-1 protein first is detected at ~100 min postfertilization at the 28-cell stage of embryogenesis. ELT-1 is detected in the nuclei of all four C granddaughters but is not detected in any other nuclei (data not shown). Thus, at the 28-cell stage, ELT-1 is present in the two C granddaughters that produce hypodermal cells as well as the two granddaughters that produce only muscle cells. In subsequent stages, ELT-1 can no longer be detected in the C-derived muscle precursors, but is detected in the C-derived hypodermal precursors. In the 44-cell embryo, ELT-1 is detected in the C-derived hypodermal precursors and in a subset of the 32 AB descendants (Fig. 8A). The developmental time at which staining first is detected for each hypodermal lineage is indicated by an asterisk beside the wild-type lineages in Figure 5. Because there is natural variability in the position of cells at the 44-cell stage (Schnabel et al. 1997) and because additional variability is introduced by our fixation technique, we have not yet been able to identify all of the AB descendants at this stage with certainty. However, the positions and number of cells that express ELT-1 at this stage correlate well with the positions and number of hypodermal precursors present at this stage (Table 2, Sulston et al. 1983). ELT-1 is expressed in the AB descendants through the successive rounds of cell division until

Figure 6. Immunofluorescence micrographs of body-wall muscle cells (left) and neurons (right) in wild-type (A, B), mex-3 (C, D), and mex-3;elt-1 (E, F) embryos. Embryos 6 hr after fertilization at 22°C were fixed and stained with mAb 5.6 to visualize body-wall muscle cells and with anti-UNC-33 to visualize neurons. mex-3 embryos generate more muscle cells (C), but produce less neurons (D), than do wild-type embryos (A and B, respectively). mex-3;elt-1 embryos produce even more muscle cells than mex-3 single mutants (cf. E with C) and only a few neurons (F). Arrows point to individual neurons; a neuron has cytoplasmic staining surrounding the nonstaining nucleus.

Figure 7. Expression pattern of the ELT-1 protein midway through embryonic development. Immunofluorescence micrographs of wild-type embryos stained with anti-ELT-1 sera (left), and with mAb MH27 (right) to outline major hypodermal cells. (A, B) Dorsal view of an embryo ~260 min postfertilization. The major hypodermal cells have just been born, and the majority of these cells reside on the dorsal surface. ELT-1 is present in the nuclei of all four C-derived muscle cells but not detected in any other nuclei (data not shown). Thus, at the 28-cell stage, ELT-1 is present in the two C granddaughters that produce hypodermal cells as well as the two granddaughters that produce only muscle cells. In subsequent stages, ELT-1 can no longer be detected in the C-derived muscle precursors, but is detected in the C-derived hypodermal precursors. In the 44-cell embryo, ELT-1 is detected in the C-derived hypodermal precursors and in a subset of the 32 AB descendants (Fig. 8A). The developmental time at which staining first is detected for each hypodermal lineage is indicated by an asterisk beside the wild-type lineages in Figure 5. Because there is natural variability in the position of cells at the 44-cell stage [Schnabel et al. 1997] and because additional variability is introduced by our fixation technique, we have not yet been able to identify all of the AB descendants at this stage with certainty. However, the positions and number of cells that express ELT-1 at this stage correlate well with the positions and number of hypodermal precursors present at this stage (Table 2, Sulston et al. 1983). ELT-1 is expressed in the AB descendants through the successive rounds of cell division until
the terminal division of the hypodermal cells (Fig. 7A). Thus, ELT-1 is expressed in blastomeres that generate major hypodermal cells and in the major hypodermal cells.

Discussion

elt-1 functions in the specification of the hypodermal cell fate

In this study we have shown that the elt-1 gene, which encodes a GATA-like transcription factor, is essential for the development of major hypodermal cells in the C. elegans embryo. elt-1 mutants lack cells with morphological and molecular characteristics of major hypodermal cells: In elt-1 mutant embryos, no cells resembling major hypodermal cells are visible by light microscopy, or are detected with antibodies that recognize the adherens junctions of hypodermal cells. elt-1 mutants also fail to express a marker specific for seam cells, the lateral group of major hypodermal cells. Other cell types, such as pharyngeal, intestinal, neuronal, and muscle cells are present in elt-1 mutants, indicating that elt-1 mutations do not affect the development of most other tissues.

elt-1 may not be required for the development of the minor hypodermal cells that normally form the specialized hypodermis at the termini of the body. elt-1 mutants contain a few small cells of undetermined identity that are surrounded by adherens junctions. These cells may be minor hypodermal cells, but alternatively could be neuronal support cells that contain adherens junctions and that normally link to the hypodermis. ELT-1 protein is not detected in minor hypodermal cells or their precursors in wild-type embryos, consistent with the hypothesis that elt-1 activity is not required for the development of these cells.

In principle, genes required for hypodermal development could function at one of several distinct steps in embryogenesis. Such genes could function at the beginning of embryogenesis to determine the identity of early blastomeres that ultimately produce hypodermal cells, they could function later in development to specify the hypodermal cell fate, or they could be required simply to maintain the differentiated state of hypodermal cells. Several maternally expressed genes have been described in C. elegans that function in specifying the identity of early blastomeres [Priess 1994]. Mutations in these genes often cause the affected blastomere to adopt a pattern of cleavage and differentiation similar, or identical, to that of a blastomere located elsewhere in the embryo. For example, mutations in the gene glp-1 can cause ABp to adopt a pattern of development characteristic of A Ba [Hutter and Schnabel 1994; Mango et al. 1994; Mello et al. 1994; Moskowitz et al. 1994]. In elt-1 mutant embryos, the ABarp, ABpla, and ABpra blastomeres have abnormal lineages; however, these lineage patterns are not identical to any wild-type lineage pattern. Although these blastomeres fail to generate hypodermal cells, other cell types normally generated by these blastomeres are present in elt-1 embryos. In normal embryogenesis, ABarp, ABpla, and ABpra blastomeres produce major hypodermal cells and are the only blastomeres that produce the four specialized touch neurons. In elt-1 mutant embryos, these four touch neurons are present [B. Page, unpubl.], indicating that elt-1 is required for only a subset of the cell types generated by these blastomeres.

We propose that elt-1 activity is required for the specification, rather than for the differentiation or maintenance, of major hypodermal cells. In contrast, the gene lin-26, which also is required for hypodermal development in C. elegans embryos, appears to function after the hypodermal cell fate is specified. Cells with several characteristic features of hypodermal cells are produced in lin-26 mutant embryos, however, these cells appear to degenerate [Labouesse et al. 1994]. We have shown that mutations in elt-1 cause precursors that normally produce hypodermal cells to produce neurons or muscle cells instead. This result suggests that in wild-type embryogenesis these precursors are not irrevocably committed to the hypodermal fate prior to elt-1 function. Thus, elt-1 likely functions at a time when blastomeres are pluripotent, as expected for a gene involved in specifying cell fate. Molecular studies support the hypothesis that elt-1 functions upstream of lin-26. Chromosomal deficiencies of the elt-1 region have been shown to prevent the wild-type expression pattern of the LIN-26 protein [Chanal and Labouesse 1997], as do mutations in elt-1 itself [B.P. unpubl.].

When does elt-1 function to specify the hypodermal cell fate? The ELT-1 protein is detected in differentiated hypodermal cells as well as in precursors of hypodermal cells. We have evidence that in some hypodermal lineages elt-1[+] functions before the birth of the hypodermal cell. In wild-type embryos, these lineages generate hypodermal cells by an unequal cell division, the large daughter differentiates as a hypodermal cell and the

Table 2. Number of ELT-1-positive cells compared with the number of major hypodermal precursors

| Approximate developmental time [min] | Total number of cells | ELT-1-positive cells | Hypodermal precursors |
|-------------------------------------|-----------------------|----------------------|---------------------|
| 100                                 | 51 ± 5                | 18 ± 4 [n = 6]       | 14                  |
| 170                                 | 85 ± 8                | 22 ± 5 [n = 9]       | 23                  |

Developmental time after fertilization at 20°C. The number of hypodermal precursors is predicted from the wild-type lineage [Sulston et al. 1983].

[Diagram of ELT-1 and nuclei (DAPI) with Figure 8. Expression pattern of the ELT-1 protein at the 51-cell stage. Dorsal/lateral view of a wild-type embryo stained with anti-ELT-1 (A) and with DAPI (B) to visualize nuclei. Dorsal/lateral, but not ventral, nuclei show high levels of ELT-1.]

[Table showing the number of ELT-1-positive cells compared with the number of major hypodermal precursors with approximate developmental time, total number of cells, ELT-1-positive cells, and hypodermal precursors for 100 and 170 minutes.]

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small daughter adopts a nonhypodermal cell fate. In elt-1 mutants, these divisions produce daughters of equal size, indicating that elt-1 functions at or prior to these divisions. Thus, elt-1 may function within a few cell divisions before the hypodermal cells are born.

Patterns of cell differentiation in elt-1 mutants

We propose that ABa, ABp, and C descendants adopt different nonhypodermal fates in elt-1 mutants because these blastomeres normally generate hypodermal cells by distinct genetic pathways. We have shown that in elt-1 mutants, the C blastomere generates muscle cells instead of hypodermal cells, whereas the ABa blastomere generates neurons instead of hypodermal cells. These results indicate that hypodermal specification in the C lineage normally may involve a choice between hypodermal and muscle cell fates, whereas hypodermal specification in the ABa lineage may involve a choice between hypodermal and neuronal cell fates.

The maternally expressed gene pal-1 appears to promote both hypodermal and muscle fates in C descendants (Hunter and Kenyon 1996). The PAL-1 protein, a transcription factor related to Drosophila CAUDAL, is expressed in C descendants, but not AB descendants. If PAL-1 is expressed inappropriately in AB descendants, as is the case in mex-3 mutants, AB descendants adopt a cell fate similar to C and produce muscle cells and hypodermal cells (Draper et al. 1996). We have shown here that AB produces muscle cells instead of hypodermal cells in mex-3;elt-1 double mutants, as does the C blastomere in elt-1 mutants. At present, we do not know what cell type the ABp blastomere generates instead of hypodermal cells in elt-1 mutants. These nonhypodermal cells, however, do not appear to be muscle cells or neurons. Why is the cell fate transformation of ABp descendants different from that of ABa in elt-1 mutants? ABp undergoes a GLP-1/APX-1 mediated cell interaction that ABa does not; this interaction results in ABp undergoing a pattern of development that differs from ABa (Mango et al. 1994; Mello et al. 1994). In glp-1;elt-1 mutants, we find that ABp produces excess neurons instead of hypodermal cells, a cell fate transformation similar to ABa in elt-1 single mutants (B. Page, unpubl.). Mutations in glp-1 and mex-3 have no affect on the positions of early embryonic blastomeres. Thus, the different transformations in cell fate seen in elt-1 mutants are a consequence of the different pathways used to establish the identities of the early blastomeres.

Control of elt-1 expression

ELT-1 protein is detected in the nuclei of a contiguous group of ABa, ABp, and C descendants on the dorsal surface of early embryos. The similar positions of the cells that express ELT-1 suggest the possibility that simple positional cues could contribute to elt-1 expression. Our current understanding of cell type specification in C. elegans, however, suggests that lineage-specific mechanisms could also determine gene expression patterns. For example, the homeobox gene mab-5 normally is expressed in a simple pattern in the posterior of the embryo (Costa et al. 1988). Yet, even if cells that normally express mab-5 are mispositioned to the anterior of the embryo, these cells still express mab-5 (Cowing and Kenyon 1996). Little is known at present about how complex, lineage-specific patterns of expression are achieved in the C. elegans embryo. Promoter analysis of the muscle-specific hlih-1 gene, which is expressed in multiple lineages, suggests that distinct cis-acting promoter elements can activate hlih-1 expression in a lineage-dependent manner (Krause et al. 1994). It will be of interest to determine whether the expression of the elt-1 gene is regulated similarly by separate, lineage-specific elements. For example, elt-1 expression in the C, but not ABa or ABp, lineage might be directly regulated by PAL-1, since expression of PAL-1 appears to precede and overlap with that of ELT-1 in C descendants.

Part of the simple pattern of ELT-1 expression on the dorsal surface of the embryo requires the loss of ELT-1 expression in cells that leave the dorsal surface during development. We have observed that all four granddaughters of the C blastomere initially express ELT-1. ELT-1 expression is maintained in the descendants of the two C granddaughters that are hypodermal precursors; these descendants remain on the dorsal surface of the embryo. In contrast, the two C granddaughters that are muscle precursors produce descendants that leave the surface and that lose ELT-1 expression. It should be possible to test in future experiments whether loss of elt-1 expression is a prerequisite for these C descendants to become internalized and to differentiate as muscle.

Although the early embryonic development of C. elegans and Drosophila seems very different at the cellular level, there is evidence of some common molecular features. The posterior-specific PAL-1 protein is closely related to the posterior-specific CAUDAL protein in Drosophila (Waring and Kenyon 1991; Hunter and Kenyon 1996). Similarly, the VAB-7 protein, which is required for proper embryonic patterning in some lineages in C. elegans is related to the pair-rule EVE protein in Drosophila (Ahringer 1996). In this respect, it is intriguing that the elt-1 gene of C. elegans and the pannier gene of Drosophila encode similar GATA-like transcription factors (Ramain et al. 1993). Wild-type Drosophila embryos express high levels of the pannier mRNA in the dorsal ectodermal cells (Winick et al. 1993). Severe mutations in pannier cause embryonic lethality, and the arrested embryos appear to lack epidermal cells. It is thus possible that pannier and elt-1 have evolutionarily related functions in epidermal tissue specification. It will be interesting to determine whether other components of the pathways leading to hypodermal/epidermal specification are similar in the two organisms.

Materials and methods

Strains and alleles

The Bristol strain N2 was used as the standard wild-type strain. The genetic markers and deficiencies used in this paper are...
listed by chromosome as follows: linkage group I (LG1): bli-3(e767), ced-1(e1735), egl-30(ad805), mex-3(zu166). LGIV: daf-14(m77), dpy-13(e184), dpy-20(e1282), emb-11(g4), fem-3(e1996), let-51(s41), let-55(s45), let-657(s1254), unc-24(e138), unc-43(e408), eDF18, eDF19, mDf7, nT1. LGX: lin-2(e1309), mex-3(zu166) was provided by B. Draper (this laboratory). The strain JR126 contains a seam-cell specific lacZ fusion expression construct that is integrated into the genome (Gendreau et al. 1994); this strain was provided by J. Rothman (University of California, Santa Barbara). The H2O–GFP neuronal marker used in this study was designed and integrated into the genome by T. Ishihara and I. Katsura (National Institute of Genetics, Mishima, Japan). All other mutant strains listed were obtained or are available from the C. elegans Genetic Stock Center. The basic methods for worm culture and genetics were performed as described by Brenner (1974).

Isolation of elt-1 alleles

The elt-1(zu180), (zu220), and (zu228) mutations were isolated independently in screens for nonconditional embryonic-lethal mutations. lin-2 mutant larvae were used as the starting strain for mutagenesis as described previously (Press et al. 1987), except that F1 animals were scored for dead eggs instead of F2 animals. Larvae were placed on microscope slides and viewed by Nomarski optics to score tissue differentiation in the inviable embryos.

Genetic analysis

elt-1(zu180) was positioned on chromosome IV by standard linkage tests [Brenner 1974]. zu220 and zu228 were shown to be alleles of elt-1 by complementation tests. elt-1(zu180) was mapped to the right arm of chromosome IV between fem-3 and daf-14 by standard three- or four-factor analysis. The mapping data are available from the C. elegans stock center.

Cloning of elt-1

The genomic DNA between fem-3 and daf-14 has been cloned and ordered into overlapping sets of cosmids and yeast artificial chromosomes (Coulson et al. 1986; 1988). Tom Barnes generously sent us a collection of strains carrying extrachromosomal arrays that were generated by coinfection of cosmids in the fem-3 daf-14 region. These arrays were crossed into the strain elt-1(zu180)unc-43(e408)/unc-24(e138)dp-20(e1282). One set of arrays rescued the embryonic lethal phenotype of elt-1(zu180). This array was generated by the injection of the following collection of cosmids: F13C8, C48A2, R05H9, C33A12, T01G2, T12H5, F38E11, F56H11, and T05A1. We microinjected these cosmids individually into the gonads of elt-1(zu180)unc-43(e408)/unc-24(e138)dp-20(e1282) hermaphrodites using the procedures of Mello et al. (1991).

ELT-1 immunostaining

Rabbit polyclonal antibodies were raised against two different ELT-1 specific peptides: [ELT-1C] ELDOQSGVWGMKNTQP-MLMTPT and [ELT-1D] SNFYPNSIEDQLEYKTC. Embryos were fixed and stained as described previously [Lin et al. 1995]. Antibodies against both peptides give identical staining patterns. To examine the staining pattern in elt-1(zu180) mutants, embryos of various stages were stained with anti-ELT-1. The hypodermal cells stained by anti-ELT-1 in wild-type embryos are absent in terminal stage elt-1 embryos. Thus, we examined early embryos (100–200 cells) for ELT-1 staining prior to hypodermal cell differentiation. Only 4% of embryos isolated from N2 hermaphrodites lacked anti-ELT-1 staining (n = 71). In contrast, 23% of embryos from elt-1(zu180) heterozygous hermaphrodites, lacked anti-ELT-1 staining (n = 112). The 23% of embryos not stained with anti-ELT-1 correlates with the 25% expected to be elt-1(zu180) homozygotes.

Analysis of embryos

A Zeiss Axiosplan microscope equipped with DIC (Nomarski) optics and epifluorescence was used for microscopy. Embryos were prepared for light microscopy following the method of Sulston et al. (1983). Embryos were processed for fluorescence microscopy as described in Bowerman et al. (1992, 1993). Photographs were taken with Kodak Technical Pan film and developed in Kodak HC110 developer. Laser ablation experiments were performed at 22°C with a VSL-337 laser attached to a Zeiss Axioscope microscope as described in Avery and Horvitz (1989). The procedure for isolating ABaP and ABaP was as follows: at the 4-cell stage, blastomers ABp, EMS, and P2 were killed with a laser microbeam. Subsequently, ABa underwent a left/right division and these descendants, ABal and ABar, divided anterior/posterior. The two anterior granddaughters of ABa then were killed with a laser microbeam. The blastomere C was isolated by killing ABa, ABp, and EMS at the 4-cell stage. After P2 divided into C and P3, P3 was killed. In all of these experiments, the nonablated blastomeres were allowed to develop for 20 hr at 15°C, and the number of cells expressing the H2O–GFP marker was determined by use of epifluorescence. Lineage analysis was performed with the four-dimensional system described in Draper et al. (1996).

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