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The GATA-Factor *elt-2* Is Essential for Formation of the *Caenorhabditis elegans* Intestine

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The *Caenorhabditis elegans elt-2* gene encodes a single-finger GATA factor, previously cloned by virtue of its binding to a tandem pair of GATA sites that control the gut-specific *ges-1* esterase gene. In the present paper, we show that *elt-2* expression is completely gut specific, beginning when the embryonic gut has only two cells (one cell cycle prior to *ges-1* expression) and continuing in every cell of the gut throughout the life of the worm. When *elt-2* is expressed ectopically using a transgenic heat-shock construct, the endogenous *ges-1* gene is now expressed in most if not all cells of the embryo; several other gut markers (including a transgenic *elt-2*-promoter::*lacZ* reporter construct designed to test for *elt-2* autoregulation) are also expressed ectopically in the same experiment. These effects are specific in that two other *C. elegans* GATA factors (*elt-1* and *elt-3*) do not cause ectopic gut gene expression. An imprecise transposon excision was identified that removes the entire *elt-2* coding region. Homozygous *elt-2* null mutants die at the L1 larval stage with an apparent malformation or degeneration of gut cells. Although the loss of *elt-2* function has major consequences for later gut morphogenesis and function, mutant embryos still express *ges-1*. We suggest that *elt-2* is part of a redundant network of genes that controls embryonic gut development; other factors may be able to compensate for *elt-2* loss in the earlier stages of gut development but not in later stages. We discuss whether elements of this regulatory network may be conserved in all metazoa. © 1998 Academic Press

Key Words: C. elegans; GATA factor; *elt-2;* gut development.

INTRODUCTION

The adult intestine (endoderm or E lineage) of the nematode *Caenorhabitis elegans* consists of 30–34 nuclei contained in 20 cells, all descended from a single cell (the E cell) of the eight-cell embryo (Sulston *et al.*, 1983). The *C. elegans* gut is a major organ, involved not only in digestion but also in storage and macromolecular synthesis (Kimble and Sharrock, 1983; Blumenthal *et al.*, 1984). Although the development and morphology of the *C. elegans* gut is enormously simpler than in vertebrates or in *Drosophila*, even such a relatively simple organ is likely to be complex. From the rough estimate that the *C. elegans* genome may contain as many as a thousand different transcription factors (McGhee and Krause, 1997), it might be expected that "dozens" (if not many more) of these transcription factors will be involved in gut formation and gut function.

How does the maternally determined "fate" of the E blastomere, the clonal progenitor of the gut, become translated into the zygotic gene activity that persists throughout the life of the worm? To understand the establishment and maintenance of gut-specific transcription patterns, we began by studying the regulation of the *ges-1* gene. *ges-1* codes for a gut-specific carboxylesterase enzyme, which is first expressed when the embryonic gut has only four cells (i.e., the 4E cell stage) and continues to be expressed in a gut-specific manner in all subsequent stages of the life cycle, including the adult (Edgar and McGhee, 1986; Kennedy *et al.*, 1993). Promoter analysis has shown that *ges-1* regulation in the gut centers on a tandem pair of (A/T)GATA(A/G) sites (hereafter referred to simply as GATA sites), approximately 1.1 kbp upstream of the *ges-1* initiation codon (Aamodt *et al.*, 1991; Kennedy *et al.*, 1993; Stroeher *et al.*, 1994; Egan *et al.*, 1995; Fukushige *et al.*, 1996). These GATA sites act as gut-specific enhancers, both in the context of the normal *ges-1* gene and in the context

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of artificial reporter constructs (Egan *et al.*, 1995). The *ges-1* GATA region was used as a probe to isolate a new *C. elegans* GATA factor named *elt-2*, which is related by sequence to endoderm-associated GATA factors found in other organisms (Hawkins and McGhee, 1995).

In the present paper, we show that *elt-2* is expressed in a completely gut-specific manner and that ELT-2 protein is present in all nuclei of the gut lineage, beginning at the 2E cell stage of embryogenesis and continuing into adulthood. Moreover, the loss of *elt-2* function is lethal, with larvae apparently dying from starvation caused by intestinal malformation. The few gut-specific genes that have been analyzed in *C. elegans* all have GATA sites (and hence a factor like *elt-2*) implicated in their control; these genes include *ges-1* (Egan *et al.*, 1995), gut-specific proteases (Ray and McKerrow, 1992; Larminie and Johnstone, 1996), metallothioneins (Freedman *et al.*, 1993), and vitellogenins (Spieth *et al.*, 1985; Zucker-Aprison and Blumenthal, 1989; Spieth *et al.*, 1991; MacMorris *et al.*, 1992).

Recently, Zhu *et al.* (1997) reported that embryos deficient for a particular chromosomal region do not form gut; they suggest that this deficiency removes two (and possibly more) redundant genes that are involved in the earliest zygotic events of gut formation. Partial rescue of gut differentiation markers was used to identify one of these genes as a diverged GATA factor, named *end-1*. *end-1* transcripts are first detected at the 1E cell stage and last detected at the 4E cell stage (Zhu *et al.*, 1997). *elt-2* expression initiates at the 2E cell stage and persists in all later developmental stages. As will be discussed in more detail below, we suggest that *end-1* may be involved in the initiation phase of *elt-2* expression in the early E lineage but that *elt-2* may be a better candidate to control gut-specific genes in later stages of the *C. elegans* life cycle.

MATERIALS AND METHODS

Worm Strains

The following *C. elegans* strains were used: N2, wild type; NL245, *elt-2 (pk46)*; AF1, *szT1[lon-2(e678)]/+*I; *szT1/dpy-8(e1321) unc-3(e151)*X; TY1917, *lon-2(e678) unc-9(e101); yDp12*; JJ532, *pie-1(zu154) unc-25(e156)/qC1 [dpy-19(e1259) glp-1(q339)]*; EU1, *skn-1(zu67)/nT1[unc-?(n754) let-?]*; JJ529, *mex-1(zu121) rol-1(e91)/mnC1 [dpy-10(e128) unc-52(e444)]*; JJ1057, *pop-1(zu189) dpy-5(e61)/hT1* I; *him-5(e1490)/hT1*; and BW1341, *nob-1(ct223); eDp6*. The *ges-1* promoter deletion in strain JM51, now assigned allele *ca13*, is described in Fukushige *et al.* (1996).

Production of Anti-ELT-2 Antibody

Full-length *elt-2* cDNA (pMH1; Hawkins and McGhee 1995) was digested with the restriction endonucleases *Pst*I and *Nsi*I and the resulting 1.38-kb fragment was inserted into the *Nsi*I site of the expression vector pTRXfus (Invitrogen, San Diego, CA). Expression of recombinant *elt-2* was performed according to protocols supplied by the manufacturers. ELT-2 protein was extracted from the bacteria by inclusion body preparation (Way *et al.*, 1990) and purified further using SDS–PAGE; protein was electroeluted in a buffer of 10 mM Tris (pH 8.0), 10 mM glycine, 1% SDS and concentrated using Centricon-30 (Amicon, Beverly, MA) centrifugation columns. Rabbit polyclonal antibodies were raised by injection of ~100 μg of electroeluted ELT-2 recombinant protein, emulsified with an equal volume of Freund's complete adjuvant, followed by repeated boosting at 2-week intervals with ELT-2 protein emulsified with Freund's incomplete adjuvant.

Anti-ELT-2 rabbit antiserum was affinity purified using a GST–ELT-2 column. Bacterially produced GST–ELT-2 fusion protein (corresponding to the entire *elt-2* cDNA) was purified on a glutathione–agarose column and then cross-linked to CNBr–Sepharose 4B (Pharmacia Biotech) following the manufacturer's instructions. Purified antiserum was used for immunocytochemistry at a dilution of 1/3–1/5 in 0.1% Triton, 10% goat serum in PBS (phosphate-buffered saline, pH 7.2). Both embryos and larvae were permeabilized by the freeze-crack method (Siddiqui *et al.*, 1989), followed by fixation in methanol–acetone. Slides were incubated with the purified antiserum, either at 4°C overnight (10–13 h) or at room temperature for 3 h, washed three times for 10 min each with 0.1% Triton–PBS at room temperature, and finally incubated at room temperature for 2–3 h, either with Cy3-labeled goat anti-rabbit antibody or with FITC-labeled donkey anti-mouse IgG (both from Jackson ImmunoResearch Laboratories, Inc.). Finally, slides were washed three times for 10 min each with 0.1% Triton–PBS and mounted in 80–90% glycerol containing antifade solution (Wood, 1988).

Construction of elt-2::lacZ Reporter Gene Constructs

An *elt-2* genomic fragment containing 5144 bp upstream of the *elt-2* ATG codon was subcloned into the *Bam*HI site of the reporter gene expression vector pPD21.28 or pPD96.04 (Mello and Fire, 1996) and the resulting *elt-2::lacZ* reporter construct (see Fig. 6 below) was introduced into wild-type *C. elegans* as described (Mello *et al.*, 1991), using pRF4 to confer a phenotype (Rol) indicating transformation. Transgenic heritable lines were γ-irradiated as described by Egan *et al.* (1995) to produce lines in which the transforming array had been integrated into the genome. After out-crossing three to five times to remove extraneous γ-ray mutations, animals from these lines were stained for β-galactosidase activity as described by Fire (1992).

Ectopic Expression of Transcription Factors

GATA transcription factors were expressed ectopically by placing the corresponding cDNA sequences under control of a *C. elegans* heat-shock promoter (Stringham *et al.*, 1992). The *elt-2* heat-shock construct was made by inserting the entire 1.6-kb *elt-2* cDNA fragment from pMH1 (Hawkins and McGhee, 1995) into pPD49.78 as well as into pPD49.83, both cut with *Nhe*I and *Eco*RV. As a negative control, a frame-shift mutation was introduced at base pair 323 of the *elt-2* coding region by end-filling the *Eco*RI site; the predicted polypeptide now truncates well upstream of the DNA binding domain (see Fig. 6a below). The *elt-1* heat-shock construct was made by inserting the 1.7-kb *BglII–SmaI elt-1* cDNA fragment isolated from yCpGalELT(−) (Shim *et al.*, 1995) into the vector pPD49.78 (Mello and Fire, 1996) cut with *Bam*HI and *Sma*I. The *elt-3* heat-shock construct contained a 1.3-kb *Kpn*I–*Sma*I cDNA fragment from pElt3 (kindly provided by Dr. J. Gilleard, Wellcome Unit of Parasitology, University of Glasgow) inserted into

pPD49.78 cut with *Kpn*I and *Sma*I. Transgenic worms were produced by injecting a solution containing 100 μg/ml heat-shock construct and 50 μg/ml of pRF4. Integrated transgenic worms were generated by γ-irradiation, followed by outcrossing one to three times as described by Egan *et al.* (1995).

Ectopic ELT protein was expressed in embryos as follows: one- to four-cell embryos, dissected from gravid hermaphrodites, were selected from each integrated transgenic strain, incubated at 20°C for 1.5 h, heat shocked at 33°C for 30 min, and incubated at 20°C overnight prior to assaying for gut granules and *ges-1* activity (Edgar and McGhee, 1986). Two to four independent (integrated) transformed lines were tested for each heat-shock promoter::GATA factor construct.

Production of a Null Mutation in the elt-2 Gene

A strain of *C. elegans* (NL245) in which the Tc1 transposon had inserted into the fifth intron of the *elt-2* gene (after base pair 5948 in GenBank Entry U25175) was identified as described by Zwaal *et al.* (1993). NL245 hermaphrodites were crossed to Lon males of the strain AF1, which contains a reciprocal translocation (*szT1*) to balance the *elt-2* gene on the X chromosome. Two or three cross-progeny were placed on each of 20 plates and incubated at either 16 or 20°C for 1 to 2 weeks, at which point the plates were nearing starvation. Worms were washed from the plate with M9 buffer and washed several additional times with M9. Half the worms were distributed among fresh plates (in numbers listed below) and the other half were used to prepare genomic DNA (Zwaal *et al.*, 1993). Each sample of genomic DNA was tested for a deletion of the *elt-2* gene by nested PCR, using primers indicated in Fig. 6c below. Of the original 20 plates, 2 gave positive signals; only 1 plate was used for subsequent sib selection. The progress of the sib selection can be summarized as follows: second round, 1/30 plates positive; third round, 1/20 plates positive but now with a more intense signal; fourth round, 7/40 plates positive; fifth round, 33/80 plates positive, at which point 80 individual worms were picked to separate plates and 6/80 of these were positive in single-worm PCR. The PCR fragment corresponding to the *elt-2* deletion (assigned allele *ca15*) was cloned and sequenced. In the balanced strain used to initiate the screen, the *mut-2* locus on chromosome I (which is necessary for high frequency of Tc1 excision) is just outside the recombinationally balanced region; thus, *mut-2* is likely to be present at the outset of the experiment but is ultimately lost (as demonstrated by the absence of nicotine-induced twitchers in later population samples; data not shown).

The original balanced *elt-2(ca15)* strain was backcrossed three times to Lon males from the strain AF1. The *elt-2(ca15)* mutation was also balanced by crossing *elt-2(ca15)/szT1* hermaphrodites with *lon-2 unc-9; yDp12* males and isolating a strain that segregated 25% Lon Uncs, signaling that the *yDp12* duplication had been lost. The lethality of *elt-2(ca15)* could be rescued by injection of a 7.5-kb genomic DNA (*Ppu*MI–*Pst*I) fragment containing the entire *elt-2* region (see Fig. 6d below), at a concentration of 1 μg/ml combined with 50 μg/ml pRF4 as a transformation marker. Transgenic *elt-2* strains could only be produced at low frequency and at low DNA concentrations but we suggest that this inefficiency reflects more general features associated with transformation with transcription factors.

Electron Microscopy

L1 larvae of both wild-type and *elt-2(ca15)* homozygotes (produced from rescued transgenic strains) were processed for electron microscopy by standard methods (Wood, 1988). Briefly, larvae were collected using a capillary, washed three times in 0.1 M Hepes, pH 7.4, adjusted to 3% glutaraldehyde, cut in half with a 25-gauge needle, incubated at room temperature between 1 and 2 h, and washed three times in 0.1 M Hepes; the posterior halves were transferred to 1% osmium tetroxide in 0.1 M Hepes, incubated a further 1–2 h at room temperature, washed three more times in 0.1 M Hepes, embedded in 1% agarose, dehydrated by passing through an ethanol series, and embedded in Quetol. Sections were stained in 2% aqueous uranyl acetate, followed by staining with lead citrate, and finally examined in a Zeiss EM902 at 0 EV energy loss.

Miscellaneous

In situ hybridization (Fig. 2 below) was performed essentially as described by Seydoux and Fire (1995), omitting proteinase K treatment. Single-stranded DNA probes were prepared using a 1.6-kb *elt-2* cDNA template that lacked the polyA sequence. Probes were present at 200–250 ng/well; color development ranged from 5 h to overnight; sense-strand controls showed no significant signal. Fluorescent latex beads (0.1 μm diameter) used in the feeding assay (Fig. 7 below) were obtained from Sigma (Product Number L3405). Histochemical staining for *ges-1* esterase activity was performed as described by Edgar and McGhee (1986).

RESULTS

elt-2 Is Expressed in the C. elegans Gut, Beginning at the 2E Cell Stage of the Early Embryo

Rabbit polyclonal antisera were produced against an ELT-2::thioredoxin fusion protein and were then affinity purified; specificity was demonstrated by lack of reactivity in the *elt-2* null mutants (see below) as well as by a single reactive band of the appropriate size detected on Western blots (data not shown). Using this affinity-purified antiserum in immunohistochemistry, ELT-2 protein can first be detected at the 2E cell stage of development, approximately halfway through the cell cycle and following the E-cell ingression associated with gastrulation. A typical example of the earliest stage at which ELT-2 can be detected is shown in Fig. 1A; the corresponding DAPI image of this embryo (Fig. 1B) shows 44–46 total nuclei. All subsequent stages of embryogenesis contain ELT-2 protein in all cells of the gut lineage; examples of 4E, 8E, and 16E cell stages are shown in Figs. 1C–1E, respectively. ELT-2 staining is nuclear, as expected for a presumed transcription factor, and shows no evidence for anterior–posterior polarity. ELT-2 protein persists in the nuclei of all gut cells, in all larval stages, as well as in adults; the presence of nuclear ELT-2 in adult hermaphrodite and male intestines is shown in Figs. 1F and 1G, respectively.

The 5′-flanking region of the *elt-2* gene (including 5.1 kb upstream of the ATG plus the first 32 amino acids of the coding region) was fused to a *lacZ*/GFP reporter construct and a number of independent stably transformed lines produced as described under Materials and Methods. As illustrated in Fig. 2A, reporter gene expres-

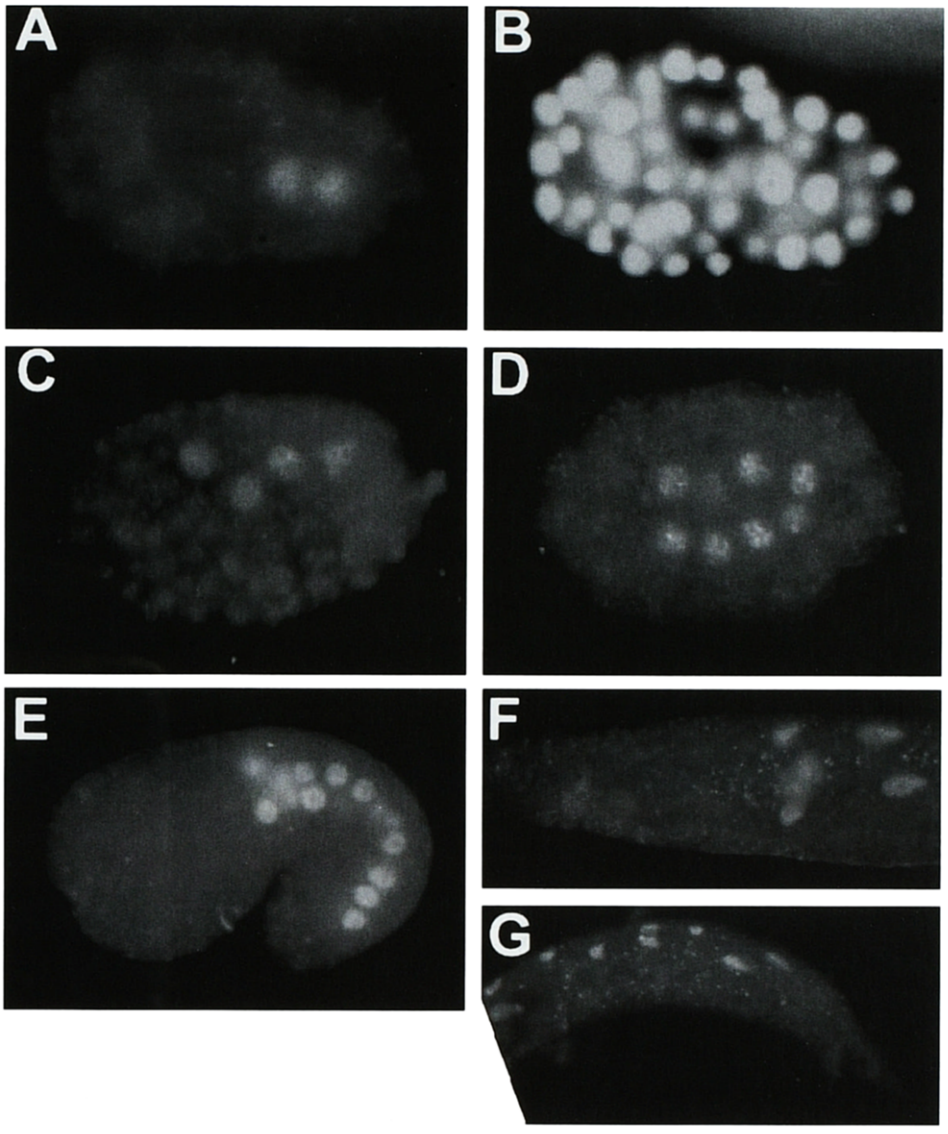

FIG. 1. Immunological detection of ELT-2 protein in wild-type embryos and adults. (A) Anti-ELT-2 staining of N2 embryo at 44- to 46-cell stage, showing the presence of ELT-2 protein in the 2E cells; (B) DAPI staining of the same embryo as in A; (C–E) anti-ELT-2 staining of N2 embryos at the 4E, 8E, and 16E cell stage, respectively; (F and G) anti-ELT-2 staining shown in the anterior intestine of an adult N2 hermaphrodite and in the posterior intestine of an adult N2 male, respectively. In all figures, anterior is to the left; the dimensions of a *C. elegans* embryo are ~30 μm by 60 μm (Sulston *et al.*, 1983).

sion in embryos from all these transformed lines can first be detected at the 2E cell stage of development; this is essentially the same time that ELT-2 protein can first be detected immunologically (compare to Fig. 1A above). Such reporter gene expression persists in the E lineage throughout embryogenesis and is coextensive with *ges-1* activity (see Fig. 2B). Thus, the establishment phase of *elt-2* expression appears to be controlled at the level of transcription initiation. *In situ* hybridization, using *elt-2* cDNA as a probe, is consistent with this conclusion within the limitations of sensitivity of the technique: *elt-2* mRNA is gut specific and is first reliably detected at the 4E to 8E cell stage (Fig. 2C). No *elt-2* mRNA was detected prior to the appearance of ELT-2 protein; in

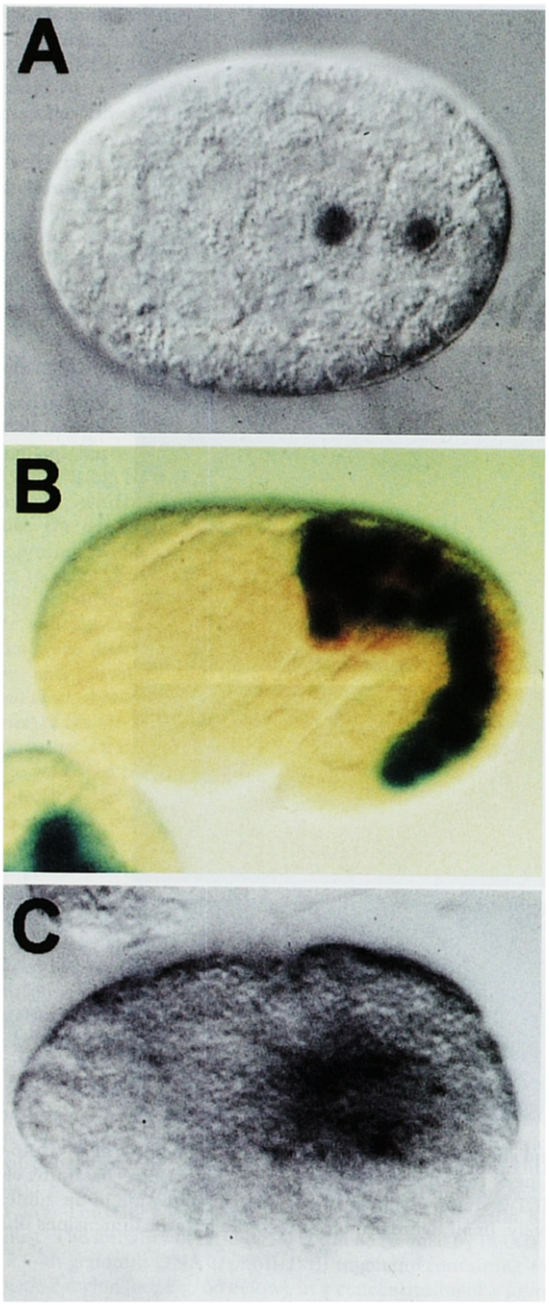

FIG. 2. (A) β-Galactosidase activity detected in the 2E cells of an early embryo transformed with a reporter construct containing 5.1 kb of the *elt-2* promoter fused to *lacZ*; see Fig. 6e for a restriction map; (B) β-galactosidase activity in an embryo from the same transformed strain at the 16–20E cell stage (blue); this embryo was costained for *ges-1* activity (red); (C) *in situ* hybridization to the early N2 embryo using *elt-2* cDNA (antisense) as probe; sense probe showed no significant signal (data not shown). Anterior is to the left.

other words, we could detect no evidence for posttranscriptional control.

elt-2 Expression Accurately Correlates with Gut Identity in Embryos Mutated for Maternal Effect Genes That Specify Blastomere Fate

A number of maternal effect genes have been identified that are necessary for correct gut development (reviewed in Priess, 1994; McGhee, 1995; Kemphues and Strome, 1997; Schnabel and Priess, 1997). The *skn-1* gene codes for a bZIP-like transcription factor necessary for correct fate of the EMS blastomere, the progenitor of both the E lineage and its sister MS lineage (Bowerman *et al.*, 1992, 1993; Blackwell *et al.*, 1994). The *mex-1* and *pie-1* genes encode zinc finger proteins that control the distribution and/or activity of the SKN-1 protein (and other gene products as well) (Mello *et al.*, 1992; Schnabel *et al.*, 1996; Guedes and Priess, 1997). The *pop-1* gene encodes a HMG-like protein that is involved in EMS differentiation, allowing E and MS to adopt different fates (Lin *et al.*, 1995).

What is the relation between the *elt-2* gene, expressed zygotically in the early E lineage, and the maternal effect genes that determine E blastomere fate? We used the anti-ELT-2 antibody to investigate the distribution of ELT-2 protein in embryos produced by homozygous mutant hermaphrodites (Fig. 3). The large majority of the *pop-1(zu189)* embryos examined and all of the *pie-1(zu154)* embryos examined show an expanded region of ELT-2 protein (Figs. 3A and 3B, respectively); this is consistent with the expansion of the gut domain revealed by birefringent gut granules and expression of the *ges-1* gene in these mutant embryos (see Fig. 4 in Fukushige *et al.*, 1996). The effect of *skn-1(zu67)* on gut formation is incompletely penetrant: at 25°C, ~20% of the embryos still form a gut (Bowerman *et al.*, 1992). This incomplete penetrance of *skn-1* is accurately reflected in the pattern of ELT-2 protein in the mutant embryos (assayed at ~22°C). Figure 3C shows three embryos: two embryos represent the 79% of *skn-1* embryos that do not show detectable ELT-2 protein; the third embryo represents the remaining 21% of *skn-1* embryos that do stain for ELT-2.

The most interesting maternal effect gene for the present analysis is *mex-1*. In embryos produced by *mex-1* hermaphrodites, there is an expanded distribution of the SKN-1 protein into anterior blastomeres but not a corresponding expansion of gut (Bowerman *et al.*, 1993, for allele *zu120*; B. Draper and J. Priess, personal communication, for the allele *zu121* used here). As shown in Fig. 3D, the vast majority of *mex-1* mutant embryos (98%) do not show expanded ELT-2 protein outside of the presumptive E-cell-derived gut (which contains 16–20 ELT-2 staining cells). Only a small number of *mex-1* embryos (2%) show a few (4–5) ectopic ELT-2 staining cells. We thus conclude that, at least under

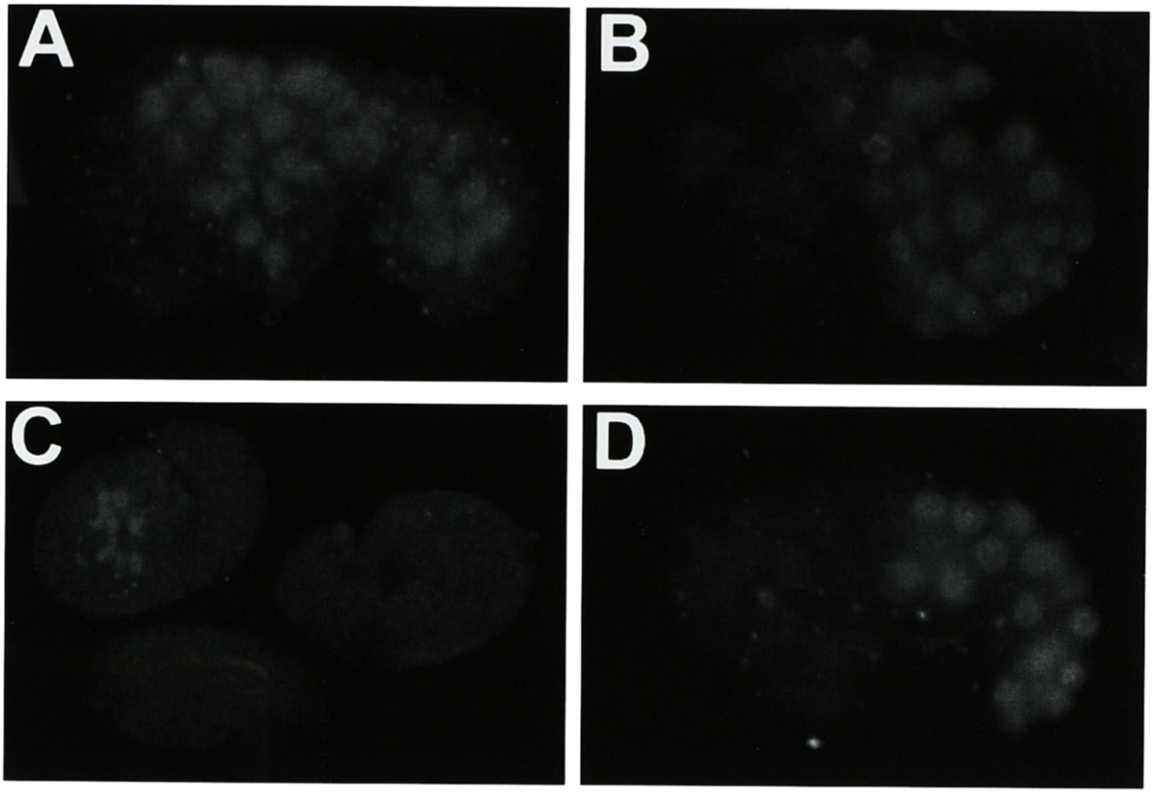

FIG. 3. Anti-ELT-2 antibody staining of embryos produced by *C. elegans* hermaphrodites homozygous for maternal effect mutations affecting gut. Embryos represent the terminal phenotypes produced by overnight incubation. (A) A typical *pop-1(zu189)* embryo, showing extra ELT-2 staining cells at the anterior of the normal gut; 126 embryos were examined, of which 62 (49%) showed 40 ELT-2 staining cells, a further 52 (41%) showed >30 ELT-2 staining cells but which could not be counted accurately, and a final 12 embryos (10%) showed only the 20 gut cells expected for a wild-type embryo. (B) A typical *pie-1(zu154)* embryo, showing extra gut cells at the embryo posterior; 67 embryos were examined, all of which showed more ELT-2 staining cells (average = 26.5 ± 1.7) than a wild-type embryo (16–20 E cells, depending on stage). (C) Three embryos produced by homozygous *skn-1(zu67)* hermaphrodites, one representing the 92/434 = 21% of *skn-1* embryos that do produce gut (and stain for ELT-2) and two representing the remaining 342/434 = 79% of *skn-1* embryos that do not produce gut. (D) A typical *mex-1(zu121)* embryo, showing ELT-2 staining in the normal gut but showing no detectable ELT-2 staining in the embryo anterior. A total of 409 *mex-1* embryos were examined of which only 8 (2%) showed ELT-2 staining (in 4–5 cells) outside the presumptive E-cell derived gut.

the present conditions, SKN-1 is not sufficient for *elt-2* expression.

Ectopic Expression of elt-2 Activates ges-1 Expression in Most, If Not All, Cells of the Early Embryo

The *elt-2* cDNA was placed downstream of two different *C. elegans* heat-shock promoters, hsp16-2 and hsp16-41, characterized by Stringham *et al.* (1992) and arranged in convenient constructs as described by Mello and Fire (1996); the two promoters gave essentially identical results. Heat-shock protocols were optimized with respect to embryo stage and with respect to the duration and intensity of the heat shock. The strongest response, uncomplicated by high levels of death in wild-type control embryos, was produced by selecting one- to four-cell-stage embryos and incubating them for 90 min at 20°C, for 30 min at 33°C, and then overnight at 20°C, prior to staining for *ges-1* activity.

When the above heat-shock regime is applied to wild-type untransformed embryos, the majority (>95%) develop apparently normally and proceed to hatch. In the minority (<5%) of the embryos whose development is arrested by the heat shock, *ges-1* expression remains restricted to a contiguous domain of cells (Fig. 4A), the presumptive gut as judged by coextensive birefringent gut granules (data not shown). Dramatically different results are obtained when

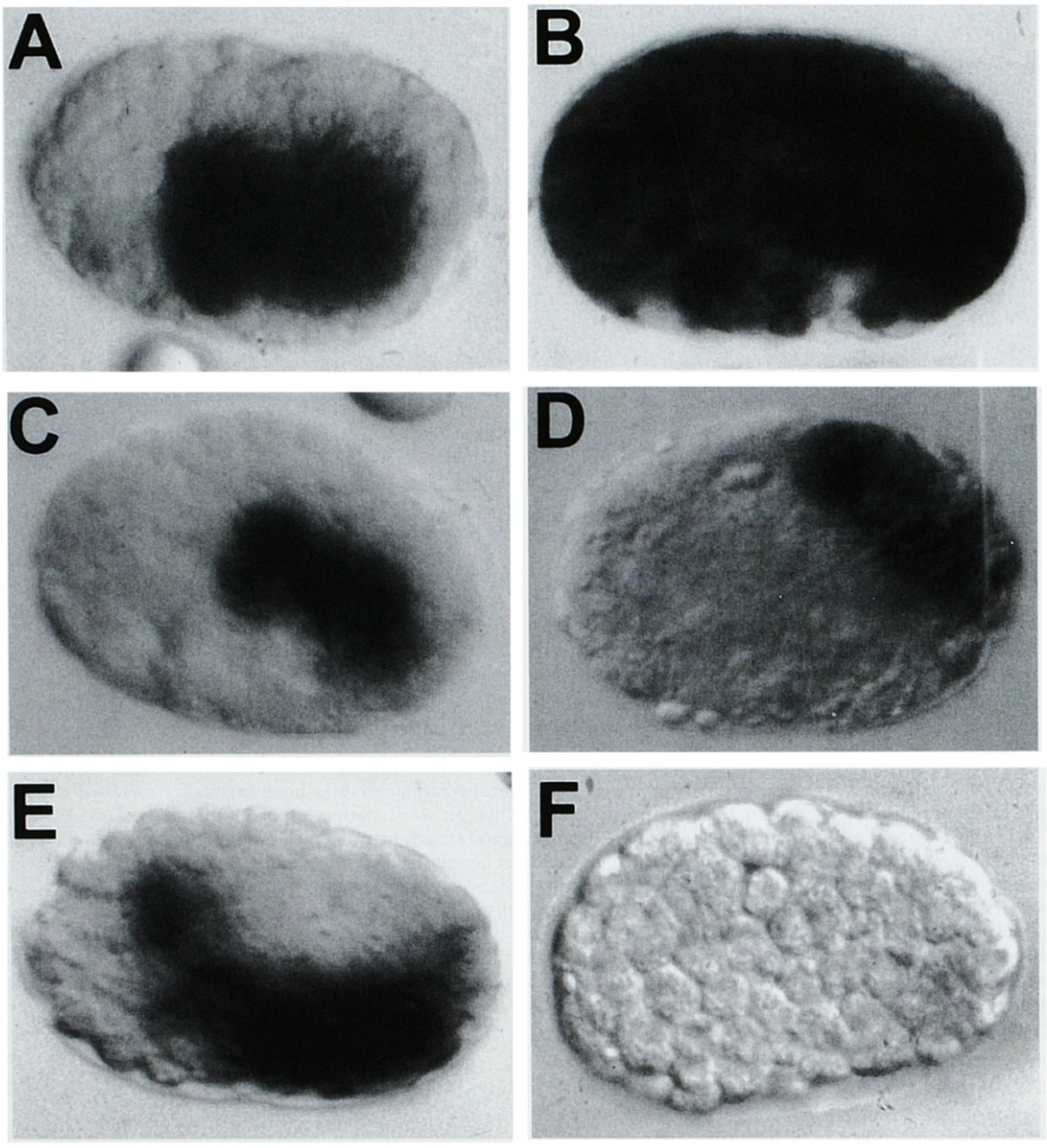

FIG. 4. Ectopic expression of *elt-2* cDNA drives ectopic expression of *ges-1*. (A) Heat shocked and arrested N2 control embryo; <5% of wild-type untransformed embryos are arrested by the optimized heat-shock protocol but in such arrested embryos, GES-1 esterase activity (Edgar and McGhee, 1986) remains restricted to presumptive gut cells; (B) embryos transformed with heat-shock promoter::*elt-2* cDNA construct express *ges-1* in essentially all cells of the embryo following heat shock; (C) control embryos transformed with a heat-shock promoter::frame-shifted *elt-2* cDNA construct express *ges-1* only in presumptive gut cells following heat shock; (D) and E) embryos transformed with, respectively, heat-shock promoter::*elt-1* and heat-shock promoter::*elt-3* cDNA constructs arrest after heat shock but GES-1 activity remains restricted to presumptive gut cells; (F) JM51 [*ges-1(ca13)*] embryos contain a promoter deletion of the endogenous *ges-1* gene (Fukushige *et al.*, 1996); following transformation with the heat-shock promoter::*elt-2* cDNA and subsequent heat shock, these embryos arrest but do not show any esterase activity.

the heat-shock regime is applied to the heat-shock promoter::*elt-2* cDNA transformed embryos. First, essentially 100% of the embryos arrest prior to morphogenesis (>500 embryos examined). Second, essentially all of these arrested embryos stain heavily for *ges-1* in most, if not all, cells of the embryo (Fig. 4B). In comparable embryos left

unstained for *ges-1*, we verified by immunohistochemistry that *elt-2* is indeed widely expressed in the heat-shocked embryos (see below). As a control, several strains of worms were produced containing the heat-shock promoter driving an *elt-2* cDNA containing a frameshift mutation upstream of the DNA binding domain; after heat shock, embryos from these strains show neither a high level of embryonic arrest nor expression of *ges-1* outside the presumptive gut (Fig. 4C). We conclude that ectopic expression of the *elt-2* gene can indeed drive ectopic expression of *ges-1*.

It is an important question to ask whether the activation of ectopic *ges-1* expression is specific for *elt-2* or whether any GATA factor might have the same effect. Strains of worms were produced containing the heat-shock promoter fused to cDNAs of two *C. elegans* GATA factors, neither of which are expressed in the gut: *elt-1* (Spieth *et al.*, 1991b; Page *et al.*, 1997) and *elt-3* (kindly provided by Dr. John Gilleard, University of Glasgow). When the same heat-shock protocol is applied to these embryos, there is a high incidence of embryonic arrest (~100%; several hundred embryos examined), indicating that the constructs are indeed expressed ectopically; however, neither the *elt-1* nor the *elt-3* construct directed *ges-1* expression outside of the presumptive gut cells (Figs. 4D and 4E).

We have previously produced a strain of *C. elegans* (JM51) in which a 1.1-kb region including the regulatory GATA sites has been deleted from the promoter of the endogenous chromosomal *ges-1* gene by means of transposon insertion and imprecise excision (Fukushige *et al.*, 1996). When the transgenic heat-shock promoter::*elt-2* cDNA construct was crossed into this *ges-1* promoter deletion strain and the heat-shock protocol repeated, such embryos arrest but show no significant esterase expression in any cell (Fig. 4F). In other words, *elt-2* induction of ectopic esterase activity must act through the 5′-flanking region of the *ges-1* gene.

We conclude that *elt-2* is sufficient to direct *ges-1* expression in nongut cells of the early embryo and, by implication, can also direct *ges-1* expression in the embryonic gut.

Ectopic elt-2 Causes the Ectopic Expression of Other Gut-Specific Genes

We investigated three other markers of early gut differentiation: birefringent gut granules, the gut-specific antigen recognized by the monoclonal antibody MH33 (Francis and Waterston, 1985), and the *elt-2* gene itself. First, Fig. 5A demonstrates that heat-shocked embryos transformed with the heat-shock promoter::*elt-2* cDNA construct do indeed contain ELT-2 protein in most if not all cells of the embryo. Similar heat-shocked embryos show widespread expression of birefringent gut granules (Fig. 5B) and MH33 reactivity (Fig. 5C). As with the ectopic *ges-1* expression described above, ectopic MH33 expression is specific for *elt-2:* in heat-shocked embryos from the control strain transformed with the heat-shock promoter::*elt-1* cDNA, MH33 reactivity remains restricted to the presumptive gut area (Fig. 5D).

To investigate whether *elt-2* can autoregulate itself, we used crosses to combine the extragenic array containing the heat-shock promoter::*elt-2* cDNA with an independent array containing the *elt-2* promoter fused to the *lacZ*/GFP reporter. The standard heat-shock protocol produces intense β-galactosidase staining throughout these doubly transgenic embryos (Fig. 5E), indicating that *elt-2* can positively regulate its own promoter. Figure 5F shows a control embryo, transformed only with the reporter gene; in the minority of these embryos that are arrested by the heat shock, reporter gene expression remains confined to presumptive gut cells.

The ability of heat-shock-induced *elt-2* expression to activate the above gut differentiation markers is highly efficient during the first half of embryogenesis, i.e., during the stages when these markers are normally expressed. However, ectopic gut marker expression is not observed when the same heat-shock experiments are conducted at stages later than early morphogenesis, either when monitoring the above four markers or when monitoring expression of the *pho-1* gene, a gut-specific acid phosphatase enzyme normally expressed late in embryogenesis (Beh *et al.*, 1991). This inability to induce late marker expression could have several explanations: for example, the heat-shock response itself is known to be weaker in late embryos (Stringham *et al.*, 1992; see also Egan *et al.*, 1995) or perhaps embryonic expression patterns have now become stable and resistant to perturbation. We discuss elsewhere the more general problem of whether blastomere fates can be transformed by ectopic expression of transcription factors such as *end-1* and *elt-2* (J. Zhu, T. Fukushige, J. D. McGhee, and J. Rothman, in preparation).

A Null Mutation in the elt-2 Gene Causes L1 Lethality with Aberrant Gut Development

Zwaal *et al.* (1993) established a method based on the insertion and imprecise excision of the transposon Tc1 by which, in principle, any sequenced *C. elegans* gene can be mutated. Using resources provided by R. Plasterk (Amsterdam), we isolated a strain of worms (NL245) in which a Tc1 transposon had inserted into the fifth intron of the *elt-2* gene, as diagrammed in Fig. 6b. Such worms showed no obvious gut phenotype, not unexpected for transposon insertions into introns (Rushforth and Anderson, 1996). Attempts to isolate strains derived from NL245 hermaphrodites in which the transposon had excised imprecisely were never successful, despite PCR screening of many hundreds of individual populations.

To increase the chances of isolating an *elt-2* mutant despite its predicted lethality and (possibly) to enhance transposon excision frequency (Maryon *et al.*, 1996) by altering homologous chromosome pairing in the *elt-2* region, the *elt-2* Tc1 insertion was balanced over the reciprocal translocation *szT1* (*I; X*). Twenty populations were established and 2 showed a strong excision band upon PCR screening. A clonal strain of worms carrying one of these

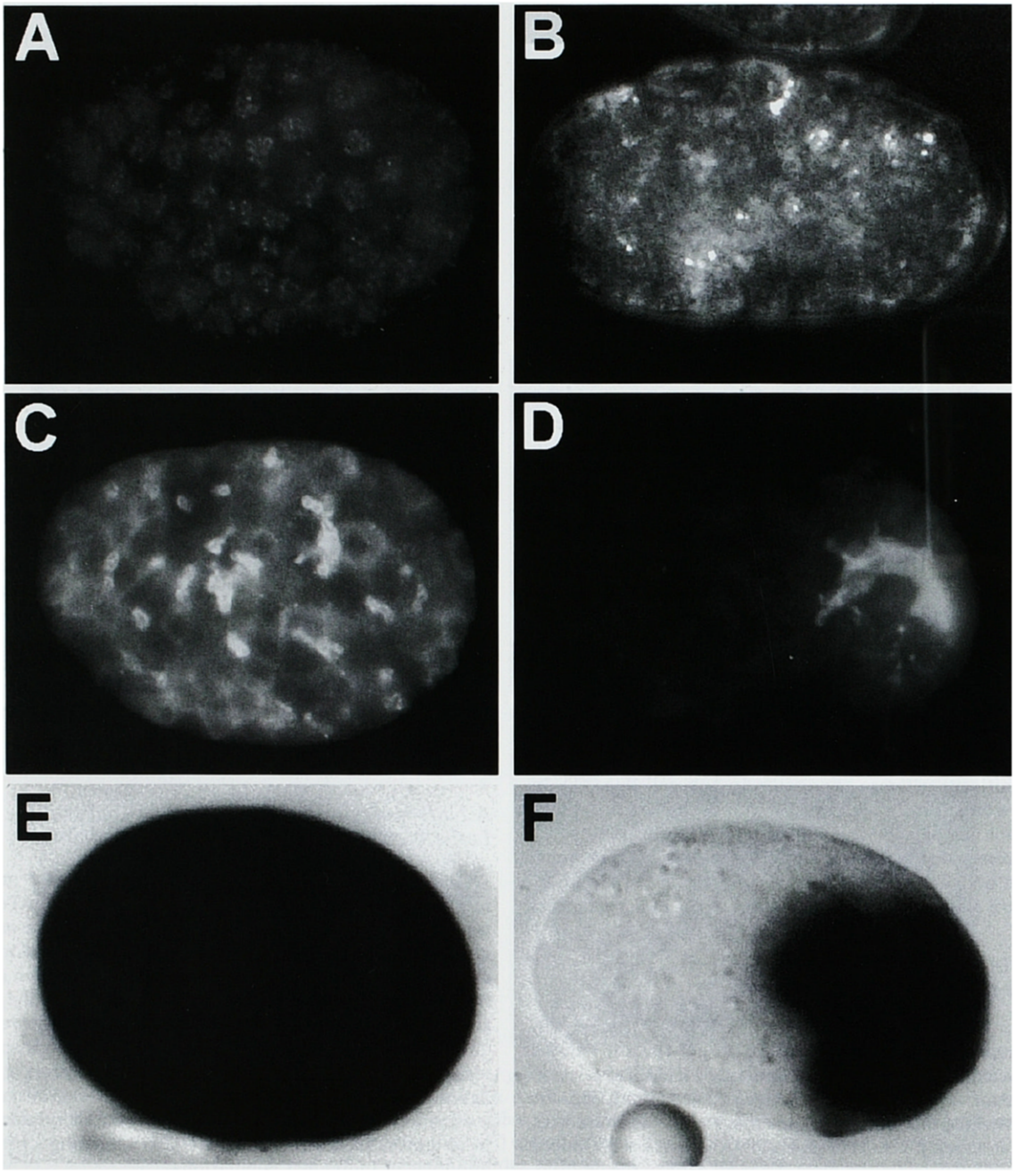

FIG. 5. Expression of other gut markers in embryos transformed with the heat-shock promoter::*elt-2* cDNA. (A) Anti-ELT-2 staining throughout a heat-shocked embryo transformed with the heat-shock promoter::*elt-2* cDNA construct; (B) birefringent gut granules throughout a similar embryo; (C) reactivity to monoclonal antibody MH33 throughout the same embryo shown in A; (D) MH33 stained heat-shocked embryo from the control strain transformed with a heat-shock promoter::*elt-1* cDNA construct; (E) heat-shocked embryo from a strain doubly (and independently) transformed with the heat-shock promoter::*elt-2* cDNA and with the *elt-2* promoter::*lacZ/GFP* reporter (see map in Fig. 6e); β-galactosidase activity is expressed in essentially all cells of the embryos; (F) in one of the minority of heat-shock-arrested embryos produced by a strain transformed only with the *elt-2* promoter::*lacZ/GFP* reporter, β-galactosidase activity is confined to the presumptive gut cells.

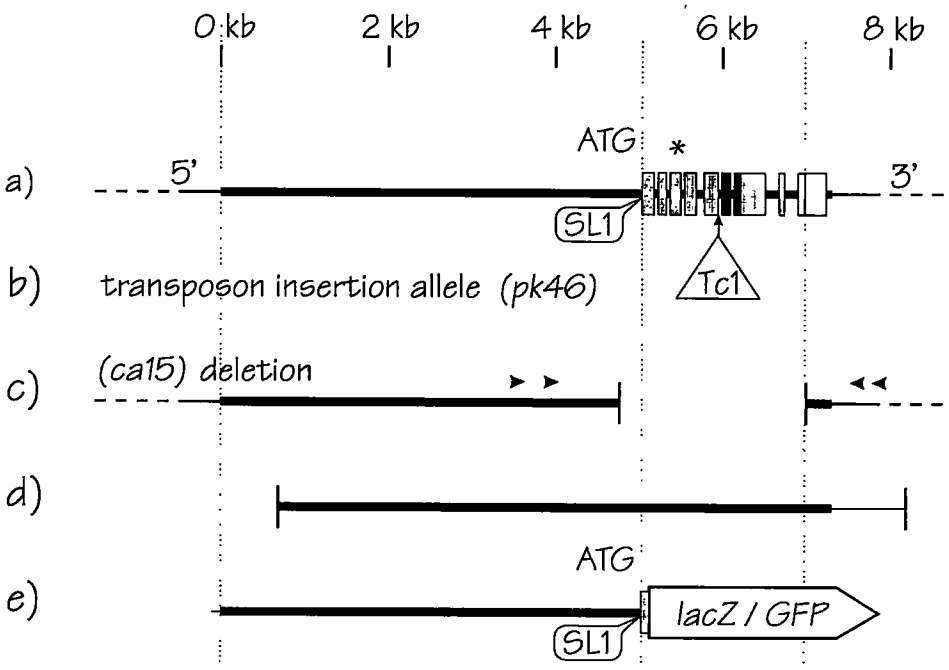

FIG. 6. The *C. elegans elt-2* gene. (a) Diagram of the chromosomal *elt-2* gene (located ~2 map units to the right of center of the X chromosome). The thicker horizontal line represents the 7304-base pair sequence in GenBank Entry U25175. The position of the ATG translation initiation codon and the addition site of the SL1 *trans*-spliced leader are indicated. Protein coding exons are shaded gray; the 3′ UTR is shown as an open box; the DNA binding domain (the zinc finger plus 25-amino acids downstream) is shown in black. The asterisk indicates the position in the protein coding sequences where a frameshift was introduced into the heat-shock construct used in Fig. 3. Scale in kilobases is shown at the top of the figure. (b) *elt-2(pk46)* contains a Tc1 transposon insertion at the beginning of the fifth intron. (c) The *elt-2(ca15)* deletion allele produced by imprecise excision of the Tc1 insertion in *elt-2(pk46)* removes the entire *elt-2* coding sequence. The arrowheads represent the positions of PCR primers used to detect this imprecise excision event (see Materials and Methods). (d) Representation of the ~7.5-kb genomic fragment used for transgenic rescue of *elt-2(ca15)* lethality. (e) Structure of the transgenic reporter gene used to detect *elt-2* expression patterns in Figs. 2A, 2B, 5E, and 5F. 5144 base pairs of the *elt-2* promoter are fused to a *lacZ* or *lacZ/GFP* reporter, at a *Bam*HI site corresponding to 32 amino acids downstream of the *elt-2* ATG codon.

deletions [*elt-2(ca15)*] was easily isolated by PCR screening and sib selection (see Materials and Methods).

The *elt-2(ca15)* deletion completely removes the *elt-2* coding region between 265 base pairs upstream of the ATG initiation codon and 16 base pairs downstream of the termination codon (Fig. 6c); this mutation undoubtedly confers a null phenotype. The deletion-containing strain was out-crossed three times and then balanced as *elt-2(ca15)/lon-2 unc-9*. As will be described in the following paragraph, animals homozygous for the *elt-2(ca15)* mutation die as L1 larvae; this lethality could be rescued by transformation with a 7.5-kb genomic fragment of the *elt-2* gene (Fig. 6d).

Approximately one-quarter of the progeny from the heterozygous *elt-2(ca15)/lon-2 unc-9* hermaphrodites arrest as L1 larvae with a "wasted gut" appearance, as shown in Fig. 7A. PCR was used to show that these individuals do indeed correspond to homozygous *elt-2* mutants (data not shown). The gut phenotype can be identified at hatching but becomes more prominent as the mutant animals attempt to feed and the bacterial food builds up as a solid bolus just behind the pharynx. This is illustrated in Fig. 7B, which shows a mutant larva that fed for 12 h on a mixture of *Escherichia coli* and fluorescent 0.1-μm latex beads. At first, the beads do not penetrate beyond the pharyngeal intestinal valve but, apparently as a result of the unremitting pumping of the pharynx, beads later penetrate into the gut lumen corresponding to the int-1 cells and int-2 cells; beads and food have not been seen to penetrate further. In contrast, a wild-type larva (Fig. 7C) rapidly ingests the beads and *E. coli*, both of which can be seen to flow freely back and forth throughout the intestinal lumen as the animal moves and feeds (Fig. 7D); the concentration of beads in wild-type larvae never accumulates much beyond that shown in the figure and beads can be seen to be ejected at defecation, an event never observed in the *elt-2* mutants. The collection of beads and food in the anterior gut of the *elt-2* mutant is not simply a result of constipation and lack

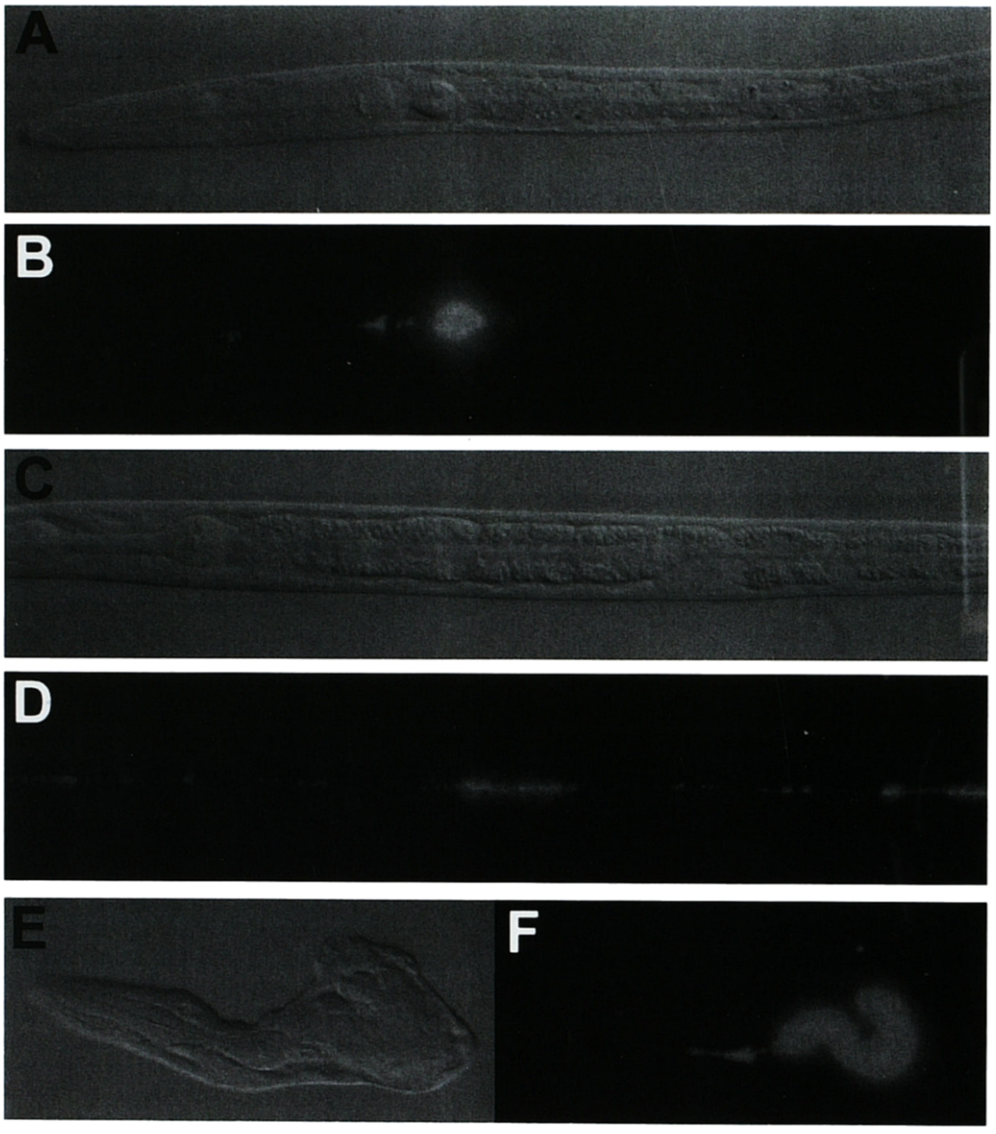

FIG. 7. Terminal phenotype of *elt-2(ca15)* mutants. (A, B) *elt-2(ca15)*; (C, D) wild-type L1 larva control; (E, F) *nob-1* control, as described in text; (A, C, E) Nomarski images; (B, D, F) fluorescent images to show fluorescent latex beads (0.1 μm diameter) ingested with *E. coli* food, filling the intestines in wild-type and *nob-1* larvae but collecting at intestine anterior in *elt-2* mutants.

of food flow. That is, *nob-1(ct223)* embryos are missing their entire posterior regions, including their anus (L. Edgar, personal communication), and yet their remaining guts fill with beads with no apparent obstacles (Figs. 7E and 7F).

Both the Nomarski and the fluorescent images shown in Figs. 7A and 7B, respectively, suggest that the gut lumen of the *elt-2* mutant larvae is somehow blocked and this blockage is confirmed by electron microscopy. Homozygous *elt-2* mutant larvae (along with wild-type control larvae) were picked several hours after hatching on plates without food. Figures 8A and 8B represent a cross-section of a starved wild-type larva, showing the granule-filled intestine and the well-developed microvilli, terminal web, and glycocalyx. In contrast, sections of mutant worms (Figs. 8C and 8D) show an aberrant and indistinct gut lumen filled with what appear to be components of a poorly assembled brush border. Microvilli are sparse, irregular, and less than half as long as in wild-type larvae (Figs. 8B and 8D). A more

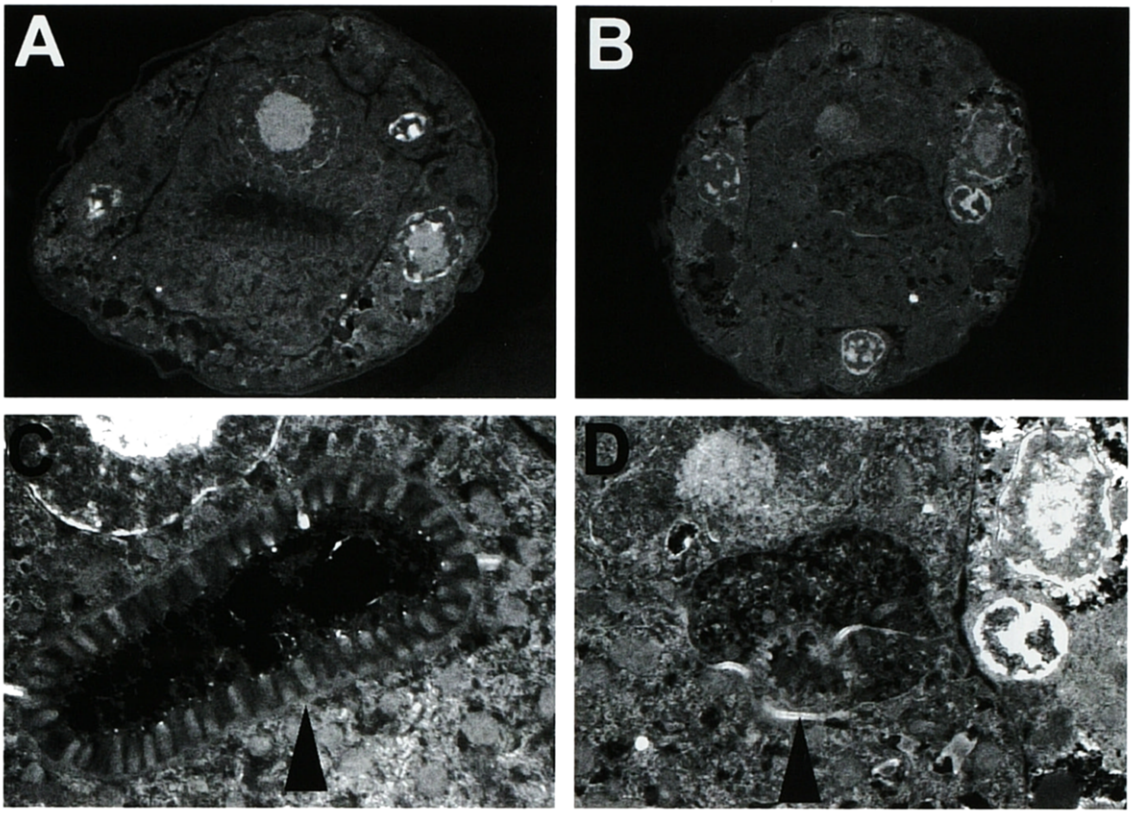

FIG. 8. Electron microscope images of cross-sections from starved L1 larvae from (A, C) wild type and (B, D) *elt-2(ca15)*. C and D are higher power views of the lumen regions from A and B, respectively. C shows the dense regular microvilli (arrowhead) and homogenous glycocalyx material found in the lumen of a starved L1; D shows short and irregular microvilli (arrowhead), along with granular material found in the lumen of the *elt-2* mutant. The diameter of an L1 larva is ~10–15 μm.

complete analysis of the mutant ultrastructure will be reported elsewhere.

We suggest that the proximate cause of *elt-2* lethality is starvation: food cannot penetrate into the intestine because the lumen is blocked by aberrant assembly of the brush border.

Is the ges-1 Gene Expressed in elt-2 Mutant Embryos?

elt-2 mutant embryos still stain for *ges-1* esterase in the gut. Essentially all embryos (>500 examined) produced by the heterozygous *elt-2(ca15)/lon-2 unc-9* strain stain for gut esterase; if the *elt-2* mutants did not express *ges-1*, 25% of the embryos would have been expected to remain unstained but such unstained embryos were not detected. In addition, we selected adult hermaphrodite *elt-2* homozygotes that were mosaic in the germline for the rescuing *elt-2* transgenic array; these adults produce >90% mutant embryos (verified both by phenotype and by PCR) but essentially all of these embryos stain for *ges-1* esterase, for gut granules, and for MH33 antigen (Figs. 9A–9C), but not for ELT-2 (Fig. 9D). Therefore, although *elt-2* can be sufficient for *ges-1* (and MH33 and gut granule) expression, it is not necessary; the implications of these results will be discussed below.

DISCUSSION

elt-2 Is a Gut-Specific GATA Factor

In the present paper, we describe the properties of the *C. elegans* gut-specific GATA factor *elt-2*. ELT-2 protein first appears at the 2E cell stage of gut development; this is at a point when the overall embryo has ~45 cells, one cell cycle after the gut becomes clonally established. ELT-2 protein is present in all gut nuclei of all subsequent stages of *C. elegans* development, including the adult. In embryos produced by *skn-1*, *pop-1*, *pie-1*, and *mex-1* mothers, ELT-2 staining is coincident with gut differentiation.

The phenotype of the *elt-2* mutant is L1 lethality with a

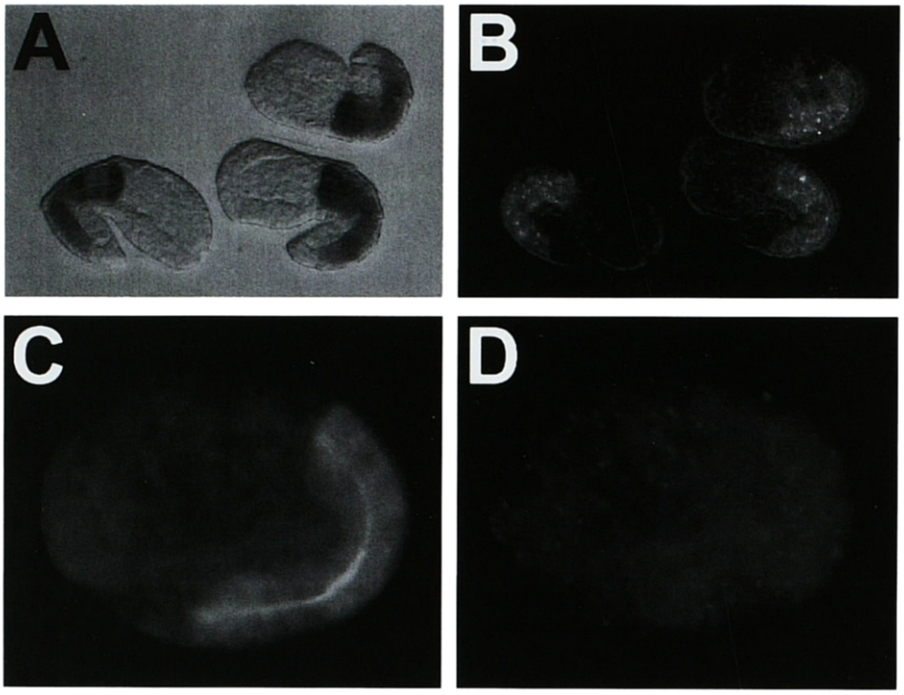

FIG. 9. Gut marker expression in homozygous *elt-2(ca15)* embryos. (A) GES-1 esterase activity; (B) birefringent gut granules in the same embryos as A; (C) reactivity with monoclonal antibody MH33; (D) same embryo as C, showing the absence of reaction with anti-ELT-2 antibody. The essentially background ELT-2 staining of this last embryo should be contrasted with the intense gut signal present in a wild-type embryo at the same stage of development (Fig. 1E).

malformed intestine; the gut lumen is blocked, presumably leading to starvation. Thus, *elt-2* is clearly necessary for correct gut formation and gut function in later embryogenesis. However, *elt-2* mutants still possess a recognizable intestine with the normal complement of 20 cells. Either *elt-2* does not function in the earliest stages of E lineage development or early *elt-2* functions are redundant and can be replaced by the activities of other genes. The multiple genes (including the *end-1* GATA factor) recently implicated by deficiency mapping to be involved in early gut development also appear to be redundant; single gene mutations have not yet been identified and indeed may have no phenotype (Zhu *et al.*, 1997). Thus, the earliest stages of gut formation may be controlled by a network of redundant genes and any early phenotype of the *elt-2* mutant may be masked by other members of this network.

What Controls elt-2?

Figure 10 summarizes our provisional view of genes lying upstream and downstream of *elt-2* within the E lineage developmental pathway. During the first few cycles of the E lineage, when *elt-2* expression is initiated, maternal control of gene expression is being replaced by zygotic gene control. Since there is no reason to assume that this transition is abrupt, *elt-2* could be directly controlled by both maternal and zygotic genes.

The prime candidate for a maternally derived transcription factor that might control *elt-2* directly is the *skn-1* product, shown to be necessary for the correct fate of the EMS blastomere (Bowerman *et al.*, 1992, 1993). Our preliminary analysis of the *elt-2* promoter shows that the *elt-2* gut-specific enhancer contains a cluster of consensus SKN-1 binding sites (Blackwell *et al.*, 1994) and, moreover, SKN-1 protein (kindly provided by K. Blackwell, Harvard) can bind to these regions *in vitro* (our unpublished results). On the other hand, it was shown above that *mex-1* embryos [in which SKN-1 protein is present in cells of the AB lineage (Bowerman *et al.*, 1993)] do not express *elt-2* ectopically, indicating that the presence of the SKN-1 factor is not sufficient for *elt-2* expression under these conditions. Furthermore, levels of maternally derived SKN-1 protein reach a peak at the four-cell stage of the embryo and have declined by the 2E cell stage when ELT-2 first appears (Bowerman *et al.*, 1993). Thus, at the moment, it is not clear whether *skn-1* or some other maternally derived transcription factor is directly involved in *elt-2* activation.

The recently described *end-1* GATA factor (Zhu *et al.*,

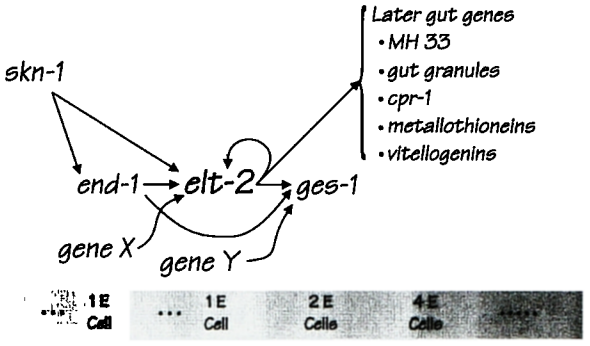

FIG. 10. The proposed position of the *elt-2* gene within the regulatory network controlling *C. elegans* endoderm formation. The development time at which each gene is first expressed is (roughly) aligned with the corresponding number of cells in the E lineage, shown at the bottom of the figure. Each of the suggested participants in the pathway (and associated uncertainties) is described more fully in the text.

1997) is an excellent candidate for the initial activation of *elt-2* in the early E lineage. *end-1* transcripts are first detected at the 1E cell stage, i.e., one cell cycle prior to *elt-2* initiation, and are last detected at the 4E cell stage (although the distribution of END-1 protein is as yet unknown). Moreover, the same region of the *elt-2* promoter that contains the cluster of SKN-1 binding sites (see above) also contains a cluster of WGATAR sites. One interesting possibility is that the maternally derived SKN-1 factor and the zygotically derived END-1 factor cooperate in *elt-2* activation (Fig. 10). A further complication is that other genes within the *itDf2* region may also participate in *elt-2* activation (depicted as "gene X" in Fig. 10); it has been argued that mutation of any one of these several genes would still allow gut to form (Zhu *et al.*, 1997) and hence *elt-2* to be expressed. In any event, *elt-2* is likely to lie not in the first wave, but in the second wave of zygotically expressed transcription factors involved in gut development (Fig. 10).

Does elt-2 Control ges-1?

Because *elt-2* was cloned by virtue of binding to functionally important GATA sites within the *ges-1* promoter, our first question is whether *elt-2* does indeed directly activate *ges-1*. We suggest that it does but the evidence is not yet unambiguous. The ectopic expression of *elt-2* shows that *elt-2* is sufficient to direct *ges-1* expression in essentially every embryonic cell and by implication also in the gut. This activation shows specificity, in that two other *C. elegans* GATA factors are not capable of *ges-1* activation. Yet *elt-2* null embryos still express *ges-1*.

One interpretation of *ges-1* expression in the *elt-2* mutants is that *elt-2* does not in fact regulate *ges-1* and that the observed *elt-2*-directed ectopic *ges-1* expression is due to some experimental particularity, for example, overexpression associated with the transgenic heat-shock promoter. However, because of the *elt-2* specificity of *ges-1* activation, because ELT-2 protein has been demonstrated to bind to the *ges-1* promoter to form a complex indistinguishable from the (single) complex detected in embryonic nuclear extracts (Stroeher *et al.*, 1994; Hawkins and McGhee, 1995), and because we believe that it is unlikely to be a coincidence that *elt-2* expression is E lineage specific and initiated immediately prior to the initiation of *ges-1* gene expression, we favor a different interpretation: that *elt-2* does indeed bind directly to the *ges-1* promoter but that other genes can compensate in the early embryo for lack of *elt-2* function. This situation is reminiscent of the role of vertebrate GATA-1 factor in globin gene control during erythropoiesis: all evidence indicates that GATA-1 controls globin genes directly (see, for example, Evans and Felsenfeld, 1989; Martin and Orkin, 1990); however, in definitive erythroid precursors derived from GATA-1 knockout ES cells, globin genes are still expressed at normal levels (Weiss *et al.*, 1994).

Could it be *end-1* that actually controls *ges-1*? Levels of *ges-1* mRNA [normalized to total poly(A)+ RNA levels] increase approximately 10-fold between L1 larval stages and adulthood (Kennedy *et al.*, 1993). *end-1* expression appears transient and *end-1* transcripts are last detected in the same cell cycle at which *ges-1* expression begins (Zhu *et al.*, 1997); as noted earlier, END-1 protein has not yet been detected. In contrast, ELT-2 protein persists from the 2E cell stage through to adulthood. Thus, while *end-1* might be involved in the earliest stages of *ges-1* activation (Fig. 10), *end-1* appears unlikely to be involved in later stages. Furthermore, since gut is still likely to form in the absence of *end-1* function (Zhu *et al.*, 1997), there may yet be another gene (shown as "gene Y" in Fig. 10) directly involved in early *ges-1* control.

In GATA factor knockouts in mice, the effects of losing one GATA factor might be partially compensated by up-regulation of a second GATA factor: mice deficient in GATA-1 show increased levels of the GATA-2 factor (Weiss *et al.*, 1994) and mice deficient in GATA-4 show increased levels of GATA-6 (Kuo *et al.*, 1997; Molkentin *et al.*, 1997). In contrast to these two examples, we could find no evidence in the *elt-2* mutant for enhanced expression of an *end-1*::*lacZ*/GFP reporter (our unpublished observations). Although these experiments must be repeated once an antibody to END-1 becomes available, we tentatively conclude that *elt-2* is not involved in *end-1* repression (Fig. 10).

In summary, we believe that *elt-2* remains a good candidate for being directly involved in *ges-1* control, although other factors must also be involved, especially at the earliest stages. Many of the above arguments can also be used to suggest that *elt-2* may regulate at least the later expression of the genes that produce gut granules and the MH33 antigen. It will be an important future problem to

delineate the precise roles of *elt-2* and *end-1* within the regulatory network governing endoderm formation.

What Later Gut-Specific Genes Might Be Controlled by elt-2?

The malformed gut produced in *elt-2* mutants certainly suggests that important structural genes lie under *elt-2* control, either directly or indirectly. In larval stages, GATA sites (and hence a factor such as ELT-2) have been implicated in the gut-specific regulation of a cysteine protease gene (Ray and McKerrow, 1992; Larminie and Johnstone, 1996) and a metallothionein gene (Freedman *et al.*, 1993). As noted earlier, GATA sequences have been implicated in control of vitellogenin gene expression in *C. elegans* (Spieth *et al.*, 1985, 1991; Zucker-Aprison and Blumenthal, 1989; MacMorris *et al.*, 1992, 1994). We propose that ELT-2 could be the factor conferring gut specificity to the expression of *C. elegans* vitellogenin genes and we have noted previously (Egan *et al.*, 1995) that the GATA region controlling *ges-1* matches at 13 bp/13 bp to a promoter region that strongly influences expression of the *vit-2* gene (MacMorris *et al.*, 1992, 1994). ELT-2 is also present in the adult male gut (see Fig. 1G), where vitellogenin synthesis never occurs; hence, other factors must be present that confer sex and stage specificity (Shen and Hodgkin, 1988).

GATA Factors in a Conserved Pathway of Gut Development

The past few years have seen striking examples of regulatory gene conservation across vast evolutionary distances. Prominent examples include the cluster of homeotic genes expressed in ectoderm derivatives (McGinnis and Krumlauf, 1992; Salser and Kenyon, 1994; Sharkey *et al.*, 1997) and muscle regulatory genes expressed in mesoderm derivatives (Weintraub, 1993; Yun and Wold, 1996). At the same time, relatively little is known about conserved pathways of endoderm development.

The transcription factors GATA-4, GATA-5, and GATA-6 have all been shown to be strongly expressed in vertebrate guts (Laverriere *et al.*, 1994; Jiang and Evans, 1996) and GATA-4 knockout mice show aberrant endoderm formation (along with prominent defects in heart formation) (Soudais *et al.*, 1995; Kuo *et al.*, 1997; Molkentin *et al.*, 1997). In *Drosophila*, the *serpent* gene, previously shown to play a critical role in gut development (Reuter, 1994), has been identified with the GATA factor ABF (Abel *et al.*, 1993; Rehorn *et al.*, 1996; Sam *et al.*, 1996). Sequence comparisons of the zinc finger DNA-binding domains of all currently known GATA factors indeed show a loose clustering of *elt-2* together with GATA-4, 5, 6, and *serpent* into a possible subclass of gut-associated GATA factors (Rehorn *et al.*, 1996). We have reported previously (Hawkins and McGhee, 1995) that the level of amino acid sequence identity between the DNA binding domains of *elt-2*, *serpent*, and GATA-4, 5, 6 lies in the range of 67–77%. The *end-1* factor shows considerable sequence divergence; within the DNA binding domains, the level of amino acid identity between *end-1* and the set of *elt-2*, *serpent*, and GATA-4, 5, 6 genes is approximately 42%.

Although sequence relatedness of the DNA-binding domains and the common expression patterns in the early gut at least raise the possibility that *elt-2* and the *Drosophila serpent* gene could be homologs, it is important to emphasize that the two genes also show obvious differences. *serpent* mutations have far more drastic consequences for early *Drosophila* development than *elt-2* mutations have for early *C. elegans* development. *serpent* is expressed in several tissues outside of the *Drosophila* gut and *serpent* levels in the gut decline during embryogenesis (Abel *et al.*, 1993; Rehorn *et al.*, 1996; Sam *et al.*, 1996); in contrast, *elt-2* expression appears completely gut specific and ELT-2 protein is present in all stages of the *C. elegans* life cycle.

In summary, GATA-4, 5, 6, *serpent*, and *elt-2* appear to have sufficient properties in common to suggest a conserved GATA-factor-dependent pathway of gut formation. Yet, differences between the properties of the various genes are just as obvious as the similarities. In the future, the notion of a conserved pathway of gut regulation will have to be tested more stringently than by simply noting sequence similarities: possible tests would be to attempt rescue of various GATA factor mutants with their predicted homologous genes or to demonstrate that GATA factors interact with similar genes in the guts of all metazoans.

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