# pha-4, an HNF-3 homolog, specifies pharyngeal organ identity in Caenorhabditis elegans

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**To build complex organs, embryos have evolved mechanisms that integrate the development of cells unrelated to one another by cell type or ancestry. Here we show that the *pha-4* locus establishes organ identity for the *Caenorhabditis elegans* pharynx. In *pha-4* mutants, pharyngeal cells are transformed into ectoderm. Conversely, ectopic *pha-4* expression produces excess pharyngeal cells. *pha-4* encodes an *HNF-3* homolog selectively expressed in the nascent digestive tract, including all pharynx precursors at the time they are restricted to a pharyngeal fate. We suggest that *pha-4* is a key component of a transcription-based mechanism to endow cells with pharyngeal organ identity.**

The digestive tract consists of an epithelial tube that is spatially and functionally regionalized. In simple animals, such as *Hydra,* the gut contains only a few specialized cells and is divided into only two regions: the mouth and gut sac. In more complex animals, the digestive tract is composed of many different cell types and compartments. How is this complicated organ built? To tackle this question, we are studying foregut, or pharynx, development in the experimentally tractable organism *Caenorhabditis elegans.*

The nematode pharynx is a large neuromuscular organ used for feeding. Like the foregut compartments of more complex animals, the pharynx is composed of many different cell types such as muscles, glands, neurons, epithelia, and valves (Albertson and Thomson 1976). Two early blastomeres, ABa and MS, generate the pharynx

(Sulston et al. 1983). Each blastomere gives rise to multiple cell types within the pharynx, and each uses a different genetic program to produce pharyngeal cells (for review, see Schnabel and Priess 1997). The ABa-derived pathway depends on intercellular signaling mediated by the GLP-1 receptor, whereas pharynx production from MS appears to be cell autonomous. Two predicted transcription factors, *skn-1* and *pop-1,* are necessary for the MS developmental program, including the production of pharyngeal cells. Recent studies have begun to elucidate the gene networks controlling *glp-1, skn-1,* and *pop-1* (for review, see Han 1997); however, the mechanisms that act downstream of these genes, to mediate pharyngeal organogenesis, are largely unknown.

The genetic pathways that generate the ABa and MS cell lineages converge on the *pha-4* locus. Animals lacking zygotic *pha-4* activity fail to produce pharynx cells from either ABa or MS (Mango et al. 1994a). In addition, these mutants lack a rectum. The rectum is descended from a third blastomere called ABp and does not derive from the pharyngeal cell lineages. Most other cells appear to be generated normally in *pha-4* mutant embryos, including cells from ABa and MS that are not part of the pharynx. Thus, the *pha-4* locus is critical to generate a group of cells related by function (the digestive tract) rather than by cell lineage (ABa, MS) or cell type (pharynx neuron, pharynx muscle, etc.). This phenotype reflects a transition during embryogenesis from maternal genes, like *glp-1, skn-1,* and *pop-1,* that control entire cell lineages and broad domains of axial patterning, to zygotic genes, like *pha-4,* that regulate the formation of specific organs, tissues, and cell types.

In the present study we address two potential mechanisms by which *pha-4* could function. First, *pha-4* could establish an organizing center that is critical for development of the entire pharynx. By analogy, loss of the anchor cell during *C. elegans* vulval development results in no vulva being formed (Greenwald 1997). Similarly, mutations in *WT-1* or c-*ret* lead to the absence of the kidney in mouse embryos (Kreidberg et al. 1993; Schuchardt et al. 1994). Each of these phenotypes reflects a loss in cell signaling. For vulval development, signaling occurs between cells in two different organs, whereas in kidney formation *WT-1* and c-*ret* are necessary for epitheliomesenchymal interactions among different tissues within the developing kidney primordium.

A second possible explanation for *pha-4* function is that the protein could act within individual pharyngeal cells to establish pharynx identity. That is, *pha-4* might be analogous to *pax-6* and the network of transcription factors that initiate eye formation (Desplan 1997). Loss-of-function mutations in either *eyeless, sine oculis, eyes absent,* or *dachshund* leads to a complete absence of the eye in *Drosophila.* Conversely, each of these genes, either alone or in combination, is capable of inducing ectopic eyes. These phenotypes suggest that this group of factors synergize to define eye identity during development.

In this report we present evidence that *pha-4* encodes an *HNF-3* homolog that is expressed in all pharyngeal precursors and establishes their fate. We suggest that *pha-4* and the *Drosophila* eye genes represent a new class of developmental regulator that specifies organ identity. Given that *HNF-3* genes have been implicated in gut development in other organisms (Kaufmann and Knochel 1996), we propose that specification of organ identity within the digestive tract may be a conserved feature of *HNF-3* proteins.

## Results and Discussion

### pha-4 *encodes the* HNF-3 *homolog* Ce-fkh-1 *and is expressed in the developing digestive tract*

To understand the molecular mechanism of *pha-4* function, we identified the gene by a combination of positional cloning and candidate gene approaches. Previous analyses showed that *pha-4* is located within the YAC Y79B4 (S. Mango, unpubl.). YAC Y79B4 contains the predicted winged helix transcription factor *Ce-fkh-1,* which was an excellent candidate to encode *pha-4* based on map position and mRNA expression (Azzaria et al. 1996; S. Mango, unpubl.). Three lines of evidence demonstrate that *pha-4* corresponds to *Ce-fkh-1.* First, extrachromosomal arrays carrying either the cosmid F38A6, which carries *Ce-fkh-1,* or a 16.7-kb PCR fragment spanning the *Ce-fkh-1* locus can rescue homozygous *pha-4* mutants (Fig. 1A). Second, antisense *Ce-fkh-1* RNA injected into wild-type hermaphrodites causes embryos and larvae to arrest with a weak *pha-4* phenotype (see Materials and Methods). Third, three EMS-induced *pha-4* alleles harbor mutations within *Ce-fkh-1* (Fig. 1B). The *Ce-fkh-1* locus generates three mRNAs, which are predicted to encode three proteins of 506, 441, and 410 amino acids (Azzaria et al. 1996). The allele *pha-4(q490)* carries a nonsense mutation at amino acid 99 relative to the largest *pha-4* isoform and is predicted to truncate all three proteins severely. Therefore, this allele, which behaves like a null allele in genetic tests (Mango et al. 1994a), is likely to be a molecular null as well. The other two sequenced alleles, *n2498* and *q487,* encode nonsense mutations at
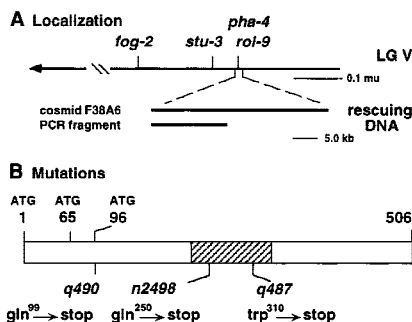


**Figure 1.** *pha-4* encodes the *HNF-3* homolog *Ce-fkh-1.* (*A*) The genetic (*top*) and physical (*bottom*) location of *pha-4.* (*B*) Three *pha-4* protein isoforms are indicated by ATG. The DBD is hatched. Each mutation depicted was induced by EMS (Mango et al. 1994a) and is the result of a C → T transition, as expected.
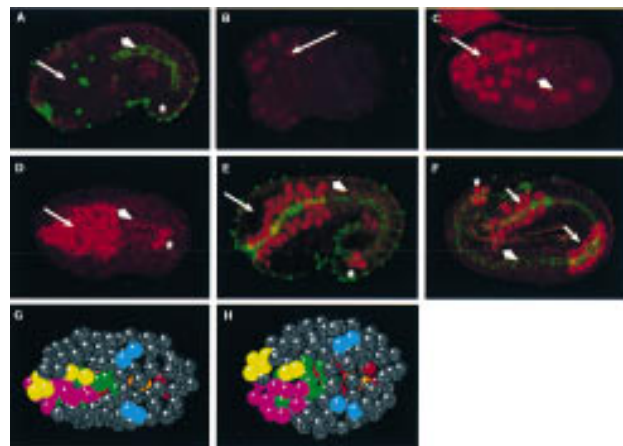


**Figure 2.** PHA-4 is expressed in the developing digestive tract. (*A–F*) PHA-4 (red) and MH27 adherens junction (green) staining. (*A*) *pha-4(q490)* null embryos. (*B*) 4E stage embryo (expression in ABaraaaa/p, ABaraapa/p, ABarapaa/p, ABalpaaa/p, ABalpapa/p, all MS great-granddaughters, midgut). (*C*) 8E stage embryo (expression in MSaaaa/p, MSaapa, MSpaaa/p, MSpapa, ABa descendants, midgut). (*D–E*) Midstage embryos [97 ± 3 PHA-4+ cells in the head (expression in all pharyngeal cells, pharynx–intestinal valve cells, and arcade cells, based on cell position and double staining ($n$ = 3)], rectal cells (vir, rep, KK′), and midgut. (*F*) Terminal embryo (expression in entire pharynx, midgut, and rectum). (Arrow) Pharynx; (arrowhead) midgut; (*) rectum. Nuclear positions in *pha-4*(+) (*G*) and *pha-4*(–) (*H*) embryos at the 8E stage, ventral view. Pharyngeal cells are colored according to their lineal ancestry (yellow, ABalp; pink, ABara; green, MS). Midgut cells are red and rectal cells are blue. The embryos in *B* and *C* are rotated relative to the embryos shown here. Anterior is left (*A–H*); each embryo is ~50 μM.

positions 250 and 310, respectively. These data show that *pha-4* is encoded by *Ce-fkh-1.*

To determine when and where PHA-4 protein is expressed, we designed a polyclonal antibody that recognizes the PHA-4 carboxyl terminus. This antibody is specific, as no staining is observed in *pha-4(q490)* null embryos (Fig. 2A) or after preincubation with the immunizing peptide (Materials and Methods). We observe PHA-4 in the nuclei of cells destined to form the digestive tract. At the 4E stage, weak expression is detected in 8 MS great-granddaughters and 10 ABa descendants (ABaraaaa/p, ABaraapa/p, ABarapaa/p, ABalpaaa/p, ABalpapa/p) that each produce pharyngeal cells, as well as nonpharyngeal cells (Fig. 2B; we have staged embryos according to the number of E-derived midgut cells; see Fig. 3A for a diagram of the cell lineage). We also detect faint expression in the four midgut precursors. Brighter staining is observed in 6–8 MS-derived and 16–18 ABa-derived cells after the next cell division (MSaaaa/p, MSaapa/p, MSpaaa/p, MSpapa/p; Fig. 2C). Soon thereafter, at the 8–12E stage, PHA-4 is first observed in the rectal precursors. Expression is maintained in all pharyngeal, midgut, and rectal cells throughout embryogenesis. A similar pattern has been seen by J. Kalb, K. Lau, B. Goszczynski, T. Fukushige, D. Moons, P. Okkema, and J. McGhee (pers. comm.). In summary, PHA-4 expression
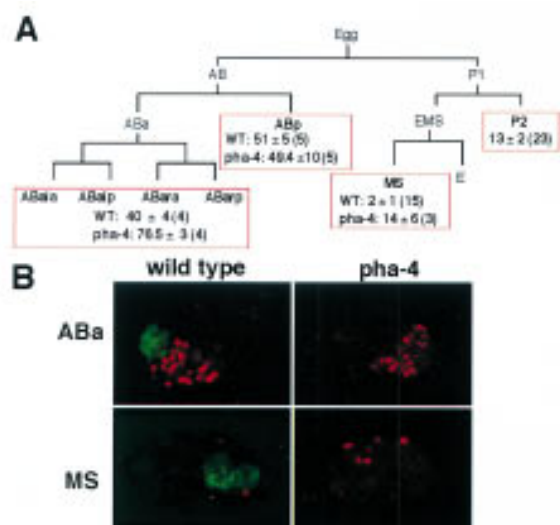
**Figure 3.** *pha-4* embryos produce extra ectodermal cells. (*A*) Partial cell lineage depicting the number of LIN-26+ cells produced by isolated blastomeres. In parentheses is the number of ablated embryos analyzed. (*B*) Example of LIN-26 (red) and 3NB12 pharynx muscle (green) staining in operated embryos.

defines the digestive tract during embryogenesis. The initiation of bright expression at the 8E stage corresponds to the time when the pharyngeal precursors are born. These cells are destined to produce only pharyngeal cells. However, they are not restricted to any particular cell type within the pharynx, consistent with *pha-4*'s proposed global role in specifying pharynx identity (Sulston et al. 1983; Mango et al. 1994a).

### pha-4 *is required to establish the pharyngeal precursors*

We used lineage analysis to show that *pha-4* function is required by the 8E stage in development. Previous studies demonstrated that *pha-4* mutants lack a pharynx (Mango et al. 1994a). Here we investigate the *pha-4* mutant phenotype during early developmental stages and show that *pha-4* function is required for the earliest signs of pharyngeal development during embryogenesis. In the wild type, the pharyngeal precursors congregate at the ventral midline, where they ingress, beginning at the 4–8E stage (Fig. 2G). In *pha-4* embryos, ingression is delayed or aborted so that most of these cells remain on or near the ventral surface. In addition, whereas wild-type pharyngeal precursors cluster into a column as they gastrulate, this process usually fails in *pha-4* mutants (Fig. 2H). All other embryonic blastomeres behave normally at this time, including midgut cells (4/4 embryos lineaged completely to the 8E stage). Thus, *pha-4* activity is required for the proper migration and possibly adhesion of the pharyngeal precursors by the 4–8E stage.

In the absence of *pha-4* function, pharyngeal cells adopt an ectodermal fate. Staining with an antibody that recognizes LIN-26, a zinc-finger protein expressed in epidermis and neuronal support cells (Labouesse et al. 1994, 1996), shows that excess numbers of LIN-26+ cells are

made in *pha-4* mutants (M. Labouesse and S. Mango, unpubl.; see also Chanal and Labouesse 1997). To determine the source of these cells, we allowed individual blastomeres to develop after all other blastomeres were killed with a laser microbeam. This experiment demonstrated that excess LIN-26+ cells were made by isolated ABa and MS blastomeres, whereas their sister cells, ABp and P2, were unaffected (Fig. 3). Therefore, cells that would become part of the pharynx in a wild-type embryo, appeared to be transformed into LIN-26+ ectodermal cells in a *pha-4* mutant. We confirmed this conclusion by following the late cell lineages of ABa and MS in *pha-4* embryos. The terminal cell divisions and programmed cell deaths were altered in the cell lineages that would normally generate pharyngeal cells, suggesting that these cells no longer had pharynx identity. For example, in wild-type embryos, ABaraapapaa and ABaraapppaa divide to generate one daughter that dies and one pharyngeal neuron (Sulston et al. 1983). In *pha-4* embryos, ABaraapapaa and ABaraapppaa arrested without undergoing the terminal cell division (2/2 embryos followed). In addition, no programmed cell deaths were observed for ABalpaaapap, ABarapaaapp, or MSaaappa (2/2 embryos examined for each cell).

The new cell lineage patterns observed in *pha-4* embryos did not resemble a different branch of the wild-type lineage. Normally, individual sublineages can be identified by the total number of cell divisions within the lineage (e.g., 8, 9, or 10 for AB-derived blastomeres), the pattern of programmed cell deaths and the morphology of individual cells. In *pha-4* embryos, AB-derived cells divided nine times and had few programmed cell deaths, unlike any specific sublineage in wild-type embryos. Thus, *pha-4* mutant cells lost their pharyngeal lineage characteristics without adopting a pattern that mimicked another wild-type lineage. This result is consistent with *pha-4*'s involvement in establishing pharyngeal fate rather than a particular cell lineage.

### pha-4 *can specify pharyngeal fate*

Expression of ectopic *pha-4* is sufficient to confer pharynx identity to embryonic blastomeres. We used a heat shock promoter (Jones et al. 1986; A. Fire, pers. comm.) to express PHA-4 throughout the embryo. By Nomarski optics, all manipulated embryos arrested with a large pharynx-like organ, bounded by a basement membrane. In addition, these embryos failed to undergo normal morphogenesis and contained fewer epidermal cells or body wall muscles. We confirmed these observations by staining for pharyngeal muscles and marginal cells, which are generated by multiple branches of the lineage tree in wild-type animals (Sulston et al. 1983). Most heat-treated embryos contained at least 50% more pharyngeal muscle cells (Fig. 4A,D) and three times more pharyngeal marginal cells (Fig. 4B,E) compared to the wild type. Conversely, the number of LIN-26+ epidermal and neuronal support cells was strongly reduced after heat shock, as was the number of body wall muscles (Fig. 4C,F; Materials and Methods). These effects were only observed with an intact *pha-4* gene and were not seen
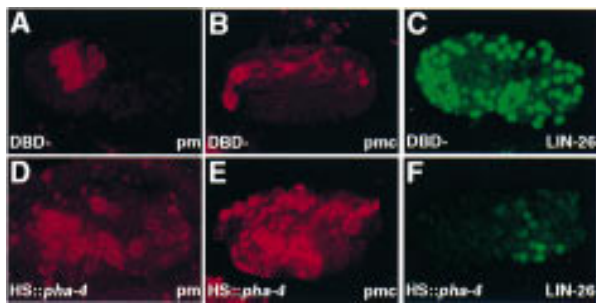
**Figure 4.** Ectopic PHA-4 specifies pharyngeal identity. Embryos expressing full-length *pha-4* (*D–F*) or *pha-4* missing the DBD (*A–C;* DBD–) under control of the heat shock promoter, were stained with the pharyngeal muscle antibody 3NB12 (*A,D;* pm), the pharyngeal marginal cell antibody αIF (*B,E;* pmc), or anti-LIN-26 ectodermal antisera (*C,F;* Labouesse et al. 1996).

after heat shock of wild-type embryos or embryos carrying a control plasmid expressing a modified *pha-4* construct that lacks the DNA binding domain (Fig. 4).

Not all embryonic cells were transformed into pharyngeal cells by ectopic *pha-4* (Materials and Methods; Fig. 4). The incomplete transformation probably reflects two phenomena. First, cells may only be competent to respond to exogenous *pha-4* for a brief period of time. For example, we observed the strongest effects when *pha-4* was induced by the mid-4E stage. Induction after the 8E stage, on the other hand, gave rise to healthy L1 larvae with no obvious defects (*n* > 25 embryos for each stage; data not shown). A second reason for the partial transformation is that PHA-4 activity may be modulated by positive and negative cofactors that are each present in only a subset of embryonic blastomeres. Existence of cofactors could also explain the apparent paradox that ectopic *pha-4* can confer pharyngeal identity, but the endogenous gene is normally expressed in the midgut and rectum. We propose that one or more cofactors cooperate with PHA-4 to promote pharynx development, another dampens PHA-4 activity in midgut precursors, and a third functions with PHA-4 to establish rectal cells. This hypothesis is consistent with our observation that midgut development is not inhibited by ectopic *pha-4.*

Exogenous *pha-4* is sufficient to down-regulate LIN-26 expression. During normal pharynx development, PHA-4 and LIN-26 are not found in the same cell or cell lineage (Labouesse et al. 1996; this work). Therefore, it is unlikely that PHA-4 initiates pharynx formation by repressing *lin-26,* which is essential for epidermal and glial cell development (Labouesse et al. 1994, 1996). To test this idea, we examined the phenotype of embryos lacking both *pha-4* and *lin-26.* Pharynx development was not restored in mutants carrying null mutations in both genes, suggesting that repression of *lin-26* by PHA-4 is not sufficient for pharynx development (see Materials and Methods). One facet of maintaining pharynx identity, however, may be the continual repression of *lin-26.* Because *pha-4* encodes a DNA-binding protein, it is possible that PHA-4 represses *lin-26* transcription directly. Interestingly, there are two consensus binding sites for

HNF-3 within the *lin-26* promoter, and these sites are conserved in *lin-26* from *Caenorhabditis briggsae* (P. Chanal and M. Labouesse, unpubl.).

How might *pha-4* govern pharynx fate? One idea is that *pha-4* regulates the proper positioning and migration of cells, and the new location of cells in *pha-4* mutants interferes with normal pharyngeal development. Alternatively, *pha-4* might specify pharynx fate directly, and the loss of pharyngeal identity in *pha-4* mutants lead to altered behaviors. Two lines of evidence support the latter hypothesis. First, most embryos with severe defects in morphogenesis and cell placement still form a pharynx (e.g., Ahnn and Fire 1994; Labouesse 1997; Schnabel and Priess 1997). Second, the ectopic pharyngeal cells seen in our heat shock experiments are often located in regions of the embryo that do not normally produce pharyngeal cells. Thus, normal patterns of cell migration, adhesion, and ingression depend on correct pharynx fate specification by *pha-4.*

### Compartmentalization of the gut during development

Our studies suggest that the subdivision of the digestive tract into foregut, midgut, and hindgut is evolutionarily ancient and relies on conserved molecular mechanisms. We have shown that *pha-4* encodes an *HNF-3* homolog necessary to specify pharyngeal and rectal precursors. In other organisms, HNF-3 proteins have been implicated in foregut and hindgut development, the equivalent of the nematode pharynx and rectum. In *Drosophila fork head* mutants, for example, foregut and hindgut cells are transformed into head sclerite structures, whereas in vertebrates, the absence of *HNF-3β* leads to severe abnormalities in the foregut (for review, see Kaufmann and Knochel 1996). Moreover, the nematode *nkx2.5* homolog *ceh-22* is expressed in foregut muscle, similar to its murine and *Drosophila* counterparts (Okkema and Fire 1994; Harvey 1996 and references therein; Okkema et al. 1997), and *end-1,* a GATA factor involved in nematode midgut development, resembles the *Drosophila* midgut regulator *serpent* (Reuter 1994; Rehorn et al. 1996; Zhu et al. 1997). The conserved patterns of expression and phenotype for these three genes strongly support the notion that the mechanisms that compartmentalize the digestive tract have been phylogenetically conserved.

The anatomy of the foregut, midgut, and hindgut differs substantially from animal to animal. This observation implies that homologs of *pha-4* and *end-1* regulate different target genes in different animals, either directly or indirectly. Few targets have been identified, so it is unclear at what point the pathways diverge. Genes such as *ceh-22* (Mango et al. 1994a; J. Kalb, K. Lau, B. Goszczynski, T. Fukushige, D. Moons, P. Okkema, and J. McGhee, pers. comm.) and *K07C11.10,* a *paired-box* homolog (A. Chisolm, pers. comm.; M. Domeier, and S. Mango, unpubl.), are good candidates to be regulated by *pha-4/HNF-3* proteins in many animals. On the other hand, no obvious match to *lin-26* has been identified in another species, nor to *pha-1,* another gene required for *C. elegans* pharynx formation and potential PHA-4 target (Schnabel and Schnabel 1990). In *Drosophila,* the

*HNF-3* homolog *fork head* represses *trachealess* to promote salivary gland development (Kuo et al. 1996). At least two *trachealess* homologs exist in *C. elegans,* one of which we have inactivated by RNA interference. No pharyngeal phenotype was observed (M. Domeier and S. Mango, unpubl.). From this short list of genes it is impossible to generalize about how *HNF-3* proteins produce different specialized structures in different animals. However, it is interesting to note that both *trachealess* and *lin-26* appear to be repressed by *HNF-3* homologs. In rats, HNF-3β blocks activation of *aldolase B* transcription directly, by binding to the promoter (Gregori et al. 1993, 1994). These data suggest that PHA-4 and its homologs may function as transcriptional repressors, as well as activators, to control developmental fates.

Experiments performed half a century ago described the ability of dissociated organ rudiments to selectively recognize and adhere to cognate organ cells (Townes and Holtfreter 1955; Moscona 1957). This behavior constitutes a cellular description of organ identity. However, the underlying molecular mechanisms were unknown. We propose that *pha-4* is an essential component of a transcription-based process that endows pharyngeal cells with organ identity. Regulators of organ identity may act combinatorially with factors that control positional, blastomere, or cell type identity to build complex organs and tissues.

## Materials and methods

### Genetics

*C. elegans* strains were maintained as described (Brenner 1974). Mutations and chromosomal balancers used were *dpy-2(e489) lin-26(mc1), unc-4(e120), mnC1* LGII, *unc-101(sy108)* LGIV, *fog-2(q71), stu-3(q265), rol-9(sc148), pha-4(q490), pha-4(q487), pha-4(q500),* and *pha-4(n2498)* LGV.

For the *lin-26;pha-4* double mutant analysis, embryos were collected from *dpy-2 lin-26 unc-4/mnC1; fog-2 pha-4(q490)/stu-3 rol-9* mothers, aged, and stained for LIN-26, PHA-4, P granules, and 3NB12. About 15% of embryos produce one to three extra 3NB12⁺ cells in single *pha-4* mutants ($n = 120$) as well as double *pha-4;lin-26* embryos ($n = 22$).

### Molecular biology

RNA interference was performed as described (Guo and Kemphues 1995) using pE38, which is a nearly full-length *pha-4* cDNA. Fifty percent of injected mothers ($n = 56$) gave rise to arrested progeny, all of which lacked a pharynx by visual inspection. Fewer 3NB12⁺ pharynx muscles and excess LIN-26⁺ ectoderm were made by the progeny.

Microinjection rescue (Mello et al. 1991) was performed using YAC Y79B4, cosmids, or a PCR fragment amplified from genomic DNA and containing the equivalent of nucleotides 30–16,703 of F38A6.

To sequence *pha-4* mutations, exons and flanking splice sites were amplified from genomic DNA obtained from unhatched *pha-4* embryos. Purified PCR products were sequenced by the ABI method. To confirm the original mutation, the relevant fragment was reamplified and sequenced on both strands. Primer sequences are available upon request.

For ectopic PHA-4 expression, the cDNA corresponding to the longest *pha-4* mRNA (pJM33; Azzaria et al. 1996) was inserted into pPD49.78 to generate plasmid pML422. The control plasmid pML423 was obtained by deleting the putative PHA-4 DNA-binding domain (DBD) from pML422. The resulting protein has the sequence IRRHGKVKEREPS across the DNA-binding region. N2 animals were injected (Mello et al. 1991), and integrated lines established. Embryos were placed at 33°C for 30 min and returned to normal growth conditions. The age of the embryos was determined by visual inspection. More than 95% of embryos carrying plasmid pML423 developed normally after heat shock, whereas >80% of the embryos carrying pML422 failed to undergo morphogenesis and displayed the phenotypes discussed in the text. Antibody staining confirmed that PHA-4 was expressed throughout the embryo for at least 10 hr after heat shock.

The average numbers of 3NB12⁺ pharyngeal muscles were $40 \pm 16$ after ectopic PHA-4 expression ($n = 76$), $18.3 \pm 1.7$ in wild-type heat shock-treated embryos ($n = 16$), and $18.2 \pm 1.6$ when the DBD was deleted ($n = 21$). There were $29 \pm 10$ pharyngeal marginal cells in the strain carrying *HS::pha-4* ($n = 21$) compared to $9.4 \pm 0.9$ for the DBD mutant ($n = 27$). The numbers of LIN-26⁺ cells were $45 \pm 42.0$ after ectopic PHA-4 expression ($n = 81$), $131.2 \pm 6.3$ in wild-type heat shock-treated embryos ($n = 16$), and $126.4 \pm 5.0$ with the DBD deleted ($n = 21$). We obtained $28 \pm 14$ ($n = 29$) body wall muscles using *HS::pha-4* as compared to $81 \pm 6$ ($n = 14$) with the DBD deleted control.

### Antibody production and staining

A peptide consisting of the 18 carboxy-terminal amino acids [(C)VYQNTLYSSTNPNSAANL] was conjugated to KLH and used to generate affinity-purified rabbit polyclonal antibodies by Quality Controlled Biochemicals. In situ antibody staining was performed as described (Mango et al. 1994a,b). Antibodies used were α-LIN-26; pharyngeal muscle antibodies 3NB12 and 9.2.1; the intermediate filament antibody αIF, which recognizes pharyngeal marginal cells; the adherens junction antibody MH27; the body wall muscle antibody NE8-3C6; and J126, which detects pharyngeal glands, midgut, and rectal–intestinal valve cells (Mango et al. 1994b; Miller and Shakes 1995; Labouesse et al. 1996; S. Strome, pers. comm.).

### Embryo manipulations

Time-lapse recordings were carried out as described (Schnabel et al. 1997). Wild-type or *fog-2 pha-4(q490)/stu-3 rol-9* embryos, were recorded until the 1.5-fold stage. We followed all cells to the 350-cell stage in two N2 embryos, three Pha-4 embryos, and one non-Pha-4 embryo (i.e., *pha-4/+* or +/+). For two Pha-4 embryos, we observed the complete cell lineage pattern for cells that would normally follow a pharyngeal fate. Pha-4 embryos were identified by the absence of a pharynx, which is easily scored by late embryogenesis. To generate the images in Figure 2, G and H, we noted the positions of all cells every 15–20 min using SIMI BioCell software, which can reconstitute the tridimensional aspect of the embryo (Schnabel et al. 1997).

Individual cells were destroyed with a laser microbeam as described (Sulston and White 1980; Avery and Horvitz 1987; Mango et al. 1991) except that the Micropoint laser system (Photonic Instruments) was used. After ablation, embryos were allowed to mature and then stained. The genotype of individual operated embryos was determined by staining for pharyngeal muscle (ABa or MS; 3NB12) or for rectal valve cells (ABp; J126). The genotype of P2-derived cells could not be determined; however, 25% of these embryos are predicted to be *pha-4*.

## References

Ahnn, J. and A. Fire. 1994. A screen for genetic loci required for body-wall

muscle development during embryogenesis in *Caenorhabditis elegans. Genetics* **137:** 483–498.

Albertson, D.G. and J.N. Thomson. 1976. The pharynx of *Caenorhabditis elegans. Philos. Trans. R. Soc. Lond. B Biol. Sci.* **275:** 299–325.

Avery, L. and H.R. Horvitz. 1987. A cell that dies during wild-type *C. elegans* development can function as a neuron in a *ced-3* mutant. *Cell* **51:** 1071–1078.

Azzaria, M., B. Goszczynski, M.A. Chung, J.M. Kalb, and J.D. McGhee. 1996. A *fork head/HNF-3* homolog expressed in the pharynx and intestine of the *Caenorhabditis elegans* embryo. *Dev. Biol.* **178:** 289–303.

Brenner, S. 1974. The genetics of *Caenorhabditis elegans. Genetics* **77:** 71–94.

Chanal, P. and M. Labouesse, M. 1997. A screen for genetic loci required for hypodermal cell and glial-like cell development during *Caenorhabditis elegans* embryogenesis. *Genetics* **146:** 207–226.

Desplan, C. 1997. Eye development: Governed by a dictator or a junta? *Cell* **91:** 861–864.

Greenwald, I. 1997. Development of the vulva. In C. elegans *II* (ed. D.L. Riddle et al.), pp. 519–542. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Gregori, C., A. Kahn, and A.L. Pichard. 1993. Competition between transcription factors HNF1 and HNF3, and alternative cell-specific activation by DBP and C/EBP contribute to the regulation of the liver-specific *aldolase B* promoter. *Nucleic Acids Res.* **21:** 897–903.

———. 1994. Activity of the rat liver-specific *aldolase B* promoter is restrained by HNF3. *Nucleic Acids Res.* **22:** 1242–1246.

Guo, S. and K.J. Kemphues. 1995. *par-1,* a gene required for establishing polarity in *C. elegans* embryos, encodes a putative Ser/Thr kinase that is asymmetrically distributed. *Cell* **81:** 611–620.

Han, M. 1997. Gut reaction to *wnt* signaling in worms. *Cell* **90:** 581–584.

Harvey, R.P. 1996. NK-2 homeobox genes and heart development. *Dev. Biol.* **178:** 203–216.

Jones, D., R.H. Russnak, R.J. Kay, and E.P. Candido. 1986. Structure, expression and evolution of a heat shock gene locus in *Caenorhabditis elegans* that is flanked by repetitive elements. *J. Biol. Chem.* **261:** 12006–12015.

Kaufmann, E. and W. Knochel. 1996. Five years on the wings of *fork head. Mech. Dev.* **57:** 3–20.

Kreidberg, J.A., H. Sariola, J.M. Loring, M. Maeda, J. Pelletier, D. Housman, and R. Jaenisch. 1993. WT-1 is required for early kidney development. *Cell* **74:** 679–691.

Kuo, Y.M., N. Jones, B. Zhou, S. Panzer, V. Larson, and S.K. Beckendorf. 1996. Salivary duct determination in *Drosophila:* Roles of the EGF receptor signaling pathway and the transcription factors *Fork head* and *Trachealess. Development* **122:** 1909–1917.

Labouesse, M. 1997. Deficiency screen based on the monoclonal antibody MH27 to identify genetic loci required for morphogenesis of the *Caenorhabditis elegans* embryo. *Dev. Dyn.* **210:** 19–32.

Labouesse, M., S. Sookhareea, and H.R. Horvitz. 1994. The *Caenorhabditis elegans* gene *lin-26* is required to specify the fates of hypodermal cells and encodes a presumptive zinc-finger transcription factor. *Development* **120:** 2359–2368.

Labouesse, M., E. Hartwieg, and H.R. Horvitz. 1996. The *Caenorhabditis elegans* LIN-26 protein is required to specify and/or maintain all non-neuronal ectodermal cell fates. *Development* **122:** 2579–2588.

Mango, S.E., E.M. Maine, and J. Kimble. 1991. Carboxy-terminal truncation activates *glp-1* protein to specify vulval fates in *Caenorhabditis elegans. Nature* **352:** 811–815.

Mango, S.E., E.J. Lambie, and J. Kimble. 1994a. The *pha-4* gene is required to generate the pharyngeal primordium of *Caenorhabditis elegans. Development* **120:** 3019–3031.

Mango, S.E., C.J. Thorpe, P.R. Martin, S.H. Chamberlain, and B. Bowerman. 1994b. Two maternal genes, *apx-1* and *pie-1,* are required to distinguish the fates of equivalent blastomeres in the early *Caenorhabditis* embryo. *Development* **120:** 2305–2315.

Mello, C.C., J.M. Kramer, D. Stinchcomb, and V. Ambros. 1991. Efficient gene transfer in *C. elegans:* Extrachromosomal maintenance and integration of transforming sequences. *EMBO J.* **10:** 3959–3970.

Miller, D.M. and D.C. Shakes. 1995. Immunofluorescence microscopy. In Caenorhabditis elegans: *Modern biological analysis of an organism* (ed. H.F. Epstein and D.C. Shakes), Vol. 48, pp. 365–394. Academic Press, San Diego, CA.

Moscona, A. 1957. The development in vitro of chimeric aggregates of dissociated embryonic chick and mouse cells. *Proc. Natl. Acad. Sci.* **43:** 184–194.

Okkema, P.G. and A. Fire. 1994. The *Caenorhabditis elegans NK-2* class homeoprotein CEH-22 is involved in combinatorial activation of gene expression in pharyngeal muscle. *Development* **120:** 2175–2186.

Okkema, P.G., E. Ha, C. Haun, W. Chen, and A. Fire. 1997. The *Caenorhabditis elegans NK-2* homeobox gene *ceh-22* activates pharyngeal muscle gene expression in combination with *pha-1* and is required for normal pharyngeal development. *Development* **124:** 3965–3973.

Rehorn, K., H. Thelen, A.M. Michelson, and R. Reuter. 1996. A molecular aspect of hematopoiesis and endoderm development common to vertebrates and *Drosophila. Development* **122:** 4023–4031.

Reuter, R. 1994. The gene *serpent* has homeotic properties and specifies endoderm versus ectoderm within the *Drosophila* gut. *Development* **120:** 1123–1135.

Schnabel, H. and R. Schnabel. 1990. An organ-specific differentiation gene, *pha-1,* from *Caenorhabditis elegans. Science* **250:** 686–688.

Schnabel, R. and J. Priess. 1997. Specification of cell fates in the early embryo. In Caenorhabditis elegans *II* (ed. D.L. Riddle et al.), pp. 361–382. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Schnabel, R., H. Hutter, D. Moerman, and H. Schnabel. 1997. Assessing normal embryogenesis in *Caenorhabditis elegans* using a 4D microscope: Variability of development and regional specification. *Dev. Biol.* **184:** 234–265.

Schuchardt, A., A.V. D'Agati, L. Larsson-Blomberg, F. Costantini, and V. Pachnis. 1994. Defects in the kidney and enteric nervous system of mice lacking the tyrosine kinase receptor *Ret. Nature* **367:** 380–383.

Sulston, J.E. and J.G. White. 1980. Regulation and cell autonomy during postembryonic development of *Caenorhabditis elegans. Dev. Biol.* **78:** 577–597.

Sulston, J.E., E. Schierenberg, J.G. White, and J.N. Thomson. 1983. The embryonic cell lineage of the nematode *Caenorhabditis elegans. Dev. Biol.* **100:** 64–119.

Townes, P.L. and J. Holtfreter. 1955. Directed movements and selective adhesion of embryonic amphibian cells. *J. Exp. Zool.* **128:** 53–120.

Zhu, J., R.J. Hill, P.J. Heid, M. Fukuyama, A. Sugimoto, J.R. Priess, and J.H. Rothman. 1997. *end-1* encodes an apparent GATA factor that specifies the endoderm precursors in *Caenorhabditis elegans* embryos. *Genes & Dev.* **11:** 2883–2896.