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# *skn-1*, a Maternally Expressed Gene Required to Specify the Fate of Ventral Blastomeres in the Early C. elegans Embryo

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## Summary

**By the 4-cell stage of C. elegans embryogenesis, a ventral blastomere, called EMS, is already committed to producing pharyngeal and intestinal cell types. Recessive, maternal-effect mutations in the gene *skn-1* prevent EMS from producing both pharyngeal and intestinal cells. In *skn-1* mutant embryos, EMS instead produces hypodermal cells and body wall muscle cells, much like its sister blastomere. Genetic analysis suggests that the *skn-1* gene product is also required postembryonically for development of the intestine. We have cloned and sequenced the *skn-1* gene and describe sequence similarities to the basic regions of bZIP transcription factors. We propose that the maternally expressed *skn-1* gene product acts to specify the fate of the EMS blastomere.**

## Introduction

Embryonic cells, called blastomeres, become committed to distinct pathways of differentiation very early in the development of many animals. Experimental studies have shown that some blastomeres appear to choose their fate on the basis of cell–cell interactions with surrounding blastomeres. In contrast, the fates of other blastomeres appear to be influenced by an unequal distribution of morphogenetic factors in the oocyte or early embryo; because these blastomeres appear to adopt their normal pattern of development without requiring cell–cell interactions, they are considered to develop autonomously. Despite numerous examples in animal embryos of cell fates that appear to be determined by one of these mechanisms (for reviews, see Davidson, 1990, 1991; Melton, 1991), little is known about the molecular basis for these early events except in the Drosophila embryo (for a review, see Nüsslein-Volhard, 1991). Because of the potential for combining experimental embryology with genetic and molecular studies, we are interested in analyzing embryogenesis in the nematode Caenorhabditis elegans.

Early blastomeres in the C. elegans embryo appear to adopt their fates on the basis of both cell–cell interactions and an unequal distribution of morphogenetic factors in the early embryo. In a 4-cell embryo, two sister blastomeres called ABa and ABp normally have very different patterns of development (see Figure 1 for a brief description of the early embryo). However, the fates of these blastomeres can be interchanged completely simply by switching their positions in the embryo (Priess and Thomson, 1987). Thus, ABa and ABp appear to have equivalent developmental potentials at the 4-cell stage, suggesting that subsequent cell–cell interactions must be responsible for the extensive differences in their fates. At least some of these interactions occur within the next few cell divisions, because when specific descendants of ABa or ABp are killed at the 48-cell stage, the resulting animals lack precisely the cell types that normally would have been made by the killed blastomeres (Sulston et al., 1983).

One difference between the sister blastomeres ABa and ABp is that descendants of ABa are induced to produce pharyngeal cells through interactions with descendants of the EMS blastomere, which also produces pharyngeal cells (Priess and Thomson, 1987). If EMS is removed or destroyed, ABa is unable to produce pharyngeal cells and instead appears to develop much like its sister, ABp. The cell–cell interactions that commit ABa descendants to producing pharyngeal cells require maternal expression of the *glp-1* gene (Priess et al., 1987; Austin and Kimble, 1987). In embryos from homozygous *glp-1* mutant mothers, EMS produces pharyngeal cells but ABa does not. Although the nature of this interaction is not understood, the *glp-1* gene encodes a transmembrane protein (Yochem and Greenwald, 1989) that shares strong sequence similarity to the proteins encoded by the C. elegans gene *lin-12* (Greenwald, 1985; Yochem et al., 1988) and the Drosophila gene *Notch* (Artavanis-Tsakonas et al., 1983), which are also known to participate in cell–cell interactions (Greenwald et al., 1983; Ferguson and Horvitz, 1985; Xu et al., 1990).

Although in normal development many of the pharyngeal cells produced by EMS appear identical to those produced by ABa and express some of the same pharyngeal-specific genes, the effect of *glp-1* mutations only on ABa indicates that there are different genetic mechanisms responsible for the specification of the ABa and EMS blastomeres. Moreover, although ABa cannot produce pharyngeal cells when separated from EMS, EMS can produce numerous pharyngeal cells when separated from ABa (Priess and Thomson, 1987). This suggests that while ABa descendants adopt pharyngeal cell fates through cell–cell interactions with EMS, the abililty of EMS to produce pharyngeal cells may be due to more autonomous processes, possibly involving an unequal distribution of morphogenetic factors in the embryo. For these reasons, we believe an analysis of genes required for pharyngeal cell specification may identify candidates for differentially localized or expressed gene products, and in addition may provide insight into the nature of the interaction between the descendants of EMS and ABa. Because it is likely that maternally expressed genes initiate the specification of early blastomeres in C. elegans, we have searched for maternal-effect mutants that lack pharyngeal cells. In this paper we describe recessive, maternal-effect mutations in a gene we call *skn-1*. Homozygous *skn-1* mutant embryos do not produce pharyngeal cells and also fail to produce intestinal cells. Phenotypic analysis of these embryos suggests that the *skn-1* gene is required to specify the fate of

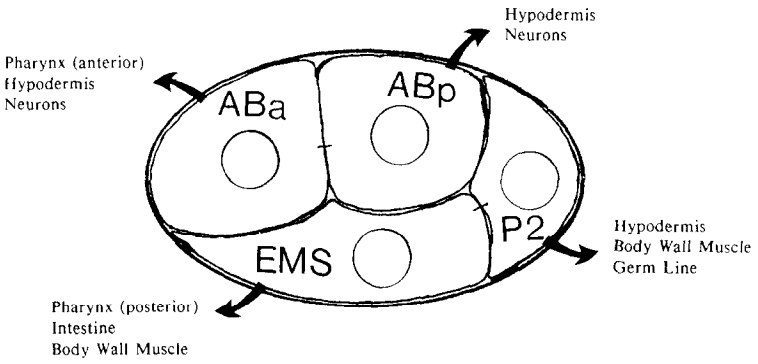



Figure 1. Summary of the Cell Types Produced by Each of the Blastomeres in a 4-Cell Wild-Type Embryo

The drawing depicts a C. elegans embryo at the 4-cell stage. ABa and ABp are sister blastomeres, as are EMS and P2. The major cell types produced by each blastomere during wild-type development are indicated by arrows (for a complete description of the embryonic lineages, see Sulston et al., 1983). The blastomere EMS later divides into daughters called E and MS. E produces a clone of intestinal cells (which form the entire intestine), and MS produces posterior pharyngeal cells and body wall muscle cells. C. elegans embryos are about 50 μm in length. In this drawing, anterior is to the left and dorsal at the top; all embryos in subsequent figures are photographed in this same orientation at approximately 1000×.

EMS, which normally produces pharyngeal and intestinal cells. We discuss models for the function of the *skn-1* gene product, including the possibility that its activity might be localized in the early C. elegans embryo.

## Results

### The *skn-1* Locus

We searched for C. elegans embryonic mutants that lack pharyngeal cells by using a general screen for maternal-effect lethals described previously (Priess et al., 1987; Kemphues et al., 1988). Because the pharynx differentiates relatively late in embryogenesis, we discarded any mutant strains in which embryos failed to produce fully differentiated cells, thereby avoiding mutants defective in general metabolic functions that might indirectly prevent the pharynx from developing. We have found a total of four mutants that produce embryos which lack a pharynx but contain other, fully differentiated cell types. The first mutant was identified in an initial screen of 10,000 mutagenized haploid genomes. Three additional mutants lacking pharyngeal cells were isolated in a subsequent screen of 30,000 mutagenized haploid genomes (C. Mello, M. Krause, B. Draper, and J. R. P., unpublished data). By genetic mapping and complementation tests, we found that all four mutant strains are defective in the same gene, which we call *skn-1* (see Experimental Procedures for details). Most of the results described here are based on an analysis of the strongest allele, *skn-1(zu67)*.

The *skn-1* gene maps to chromosome IV, between the genes *fem-1* and *unc-44* (see Figure 6). The four alleles of *skn-1* each result in a similar recessive, maternal-effect, embryonic-lethal phenotype. The frequency at which we have obtained these alleles (1 mutation in 10,000 mutagenized haploid genomes), their recessive nature, and the complete rescue of homozygous *skn-1* mutant animals by a chromosomal duplication (see Figure 6 and Experimental Procedures) all suggest that these are reduction-of-function mutations (Brenner, 1974; Meneely and Herman, 1979; Greenwald and Horvitz, 1980). Wild-type male sperm cannot rescue embryos from mothers that are homozygous for any of the four mutant alleles, indicating that maternal expression of the *skn-1* gene is necessary for normal embryogenesis. In addition, all progeny are viable when heterozygous *skn-1*/+ mothers mate with homozygous *skn-1* mutant males, showing that, for the alleles described here, maternal expression of the wild-type gene is sufficient for normal embryogenesis.

### *skn-1* Embryos Are Defective in Pharyngeal and Intestinal Development

It is convenient to describe our phenotypic analysis of *skn-1* mutants by reference to a wild-type C. elegans embryo at the 4-cell stage of embryogenesis. Figure 1 provides a brief description of a 4-cell embryo and the cell types that each of the four early blastomeres produce during development. The blastomeres ABa and EMS both produce cells that form the pharynx, a muscular organ used for feeding. The anterior pharyngeal cells are derived from ABa, and the posterior ones from EMS. The pharynx consists of four distinct cell types: pharyngeal muscle cells, gland cells, neurons, and structural cells called marginal cells (Albertson and Thomson, 1976). The intestine is derived entirely from one daughter of EMS, called E, which produces only intestinal cells. All blastomeres in a 4-cell embryo except for EMS produce hypodermal cells, which are epithelial cells that cover the surface of the embryo. Almost all of the body wall muscle cells, which form the locomotory system of the worm, are made by P2 and EMS. Body wall and pharyngeal muscle cells can be distinguished on the basis of morphology and by the immunologically distinct muscle proteins they express (Epstein et al., 1982; Miller et al., 1983; Waterston, 1988).

*skn-1* mutant embryos do not contain any structures that morphologically resemble a pharynx in the light microscope, and the majority of *skn-1* embryos also appear to lack an intestine. We have used immunofluorescence microscopy to confirm that specific pharyngeal and intestinal cell types are absent in *skn-1* embryos (Figure 2). Almost all *skn-1* mutant embryos fail to stain with antibodies that recognize three different pharyngeal cell types: pharyngeal muscle cells, gland cells, and marginal cells. For the two strongest alleles, *skn-1(zu67)* and *skn-1(zu138)*, 99% of the embryos do not stain with a pharyngeal muscle–

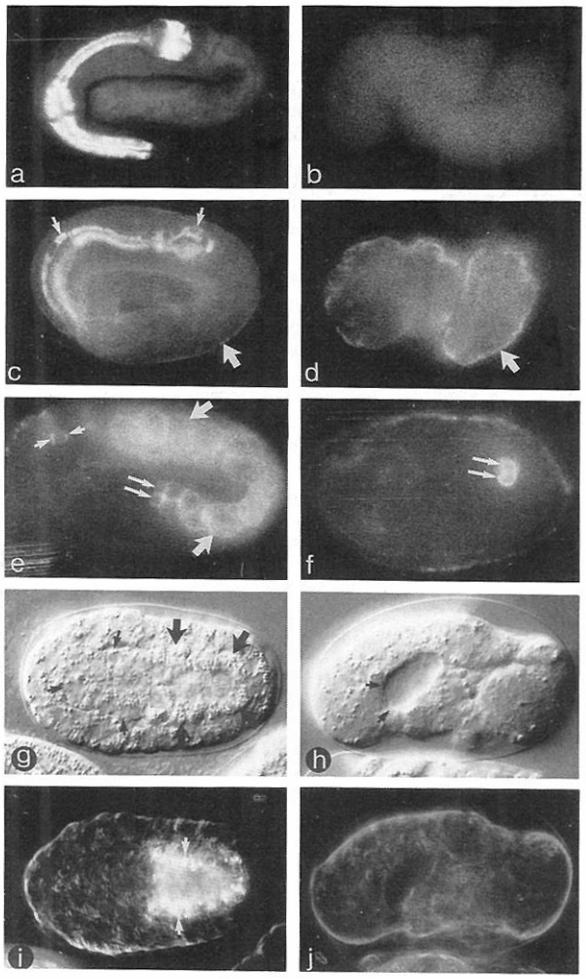



Figure 2. Pharyngeal and Intestinal Cells in Wild-Type and *skn-1* Embryos

Immunfluorescence and light photographs of wild-type (left column) and *skn-1* (right column) embryos. Photographs were made after the embryos had developed for 12 hr at 25°C (a–f, h, and j) or for 6 hr at 25°C (g and i).

(a and b) Wild-type and *skn-1* embryos stained with 9.2.1, a monoclonal antibody (MAb) that recognizes pharynx-specific muscle cells (Miller et al., 1983).

(c and d) Embryos stained with anti-IFA (Pruss et al., 1981; Priess and Thomson, 1987), a MAb that recognizes intermediate filaments in hypodermal cells (large arrows) and in marginal cells in the pharynx (c, small arrows).

(e and f) Embryos stained with 2CB7, a MAb that recognizes pharyngeal gland cells (e, small arrows), intestinal cells (e, large arrows), and the two intestinal valve cells (e and f, thin arrows) normally present at the posterior end of the intestine (see Experimental Procedures). The pharyngeal and intestinal cells are absent in *skn-1* embryos (f), but the two intestinal valve cells are present (f, thin arrows). The intestinal valve cells are produced by ABp (Sulston et al., 1983), which is not affected by mutations in *skn-1* (see text).

(g–j) Living wild-type and *skn-1* embryos viewed with Nomarski optics (g and h) or polarization optics (i and j). Intestine-specific birefringent gut-granules are visible in the wild-type embryo (i, arrows), but not in the *skn-1* mutant. Note the internal cavity in the *skn-1* embryo, which is lined by a hypodermis-specific cuticle (h, arrows; also see Figure 3). Small arrows outline the early pharynx, large arrows the early intestine in the wild-type embryo (g).

Table 1. Differentiation of Pharyngeal and Intestinal Cells in *skn-1* Embryos

| | Embryos with Pharyngeal Cells (%) | | Embryos with Intestinal Cells (%) | |
|---|---|---|---|---|
| | 15°C | 25°C | 15°C | 25°C |
| **Wild type** | 100 | 100 | 100 | 100 |
| ***skn-1* allele** | | | | |
| *zu67* | 8 | 1 | 46 | 19 |
| *zu129* | 16 | 4 | 86 | 54 |
| *zu135* | 21 | 6 | 47 | 21 |
| *zu138* | 11 | 2 | 50 | 20 |
| *zu67/nDf41* | ND | ND | ND | 10 |
| *zu67/zu129* | ND | ND | ND | 38 |

Embryos (300–1000 of each type) were collected from homozygous *skn-1* animals and allowed to develop for either 20 hr at 15°C or 15 hr at 25°C. Embryos with pharyngeal cells were scored by staining with the antibody 9.2.1, which recognizes pharyngeal muscle cells. Embryos with intestinal cells were scored in the light microscope using polarizing optics to detect intestine-specific gut granules. Similar results were obtained by staining with the antibody 2CB7, which recognizes intestinal cells (data not shown). For an example of a wild-type embryo, see Figure 2a. For an example of an *skn-1(zu67)* embryo, see Figure 2b. *skn-1(zu67)/nDf41* embryos were obtained from *zu67/Deficiency* heterozygotes that survived to adulthood. ND: not determined.

specific antibody, compared with about 95% for the two weaker alleles, *skn-1(zu129)* and *skn-1(zu135)* (Table 1). The few embryos that stain positively usually make only 1–5 pharyngeal cells, compared with 33 pharyngeal muscle cells made by wild-type embryos. Most *skn-1* embryos also fail to stain with 2CB7, an antibody that recognizes intestinal cells, and they do not produce birefringent gut granules, which are characteristic of intestinal cells (Laufer et al., 1980). The intestinal phenotype is less penetrant than the pharyngeal phenotype for all four alleles. For example, 80% of *skn-1(zu67)* embryos and 50% of *skn-1(zu129)* embryos lack intestinal cells (Table 1). The mutant embryos that contain intestinal cells typically have 5–10 cells, compared with the 20 intestinal cells in wild-type embryos. Finally, the penetrance of both the pharyngeal and intestinal phenotypes shows some temperature dependence in all four *skn-1* alleles, with a higher percentage of the mutant embryos producing pharyngeal and intestinal cells at lower temperatures (Table 1).

### *skn-1* Embryos Contain Extra Hypodermal Cells

An invariant number and pattern of hypodermal cells normally cover the surface of a C. elegans embryo, eventually secreting the cuticle that surrounds the animal at hatching. *skn-1* embryos appear to have a nearly normal number and pattern of hypodermal cells on their dorsal and lateral surfaces, but in addition have extra hypodermal cells on their ventral surface and inside their bodies, where the pharynx and intestine would form in wild-type embryos. Hypodermal cells can be identified by their morphology in the light microscope, by an antibody that stains hypodermal cell nuclei (Figure 3), and by their association with a hypodermis-specific cuticle (Singh and Sulston, 1978; Priess and Hirsh, 1986). Internal hypodermal cells in *skn-1*

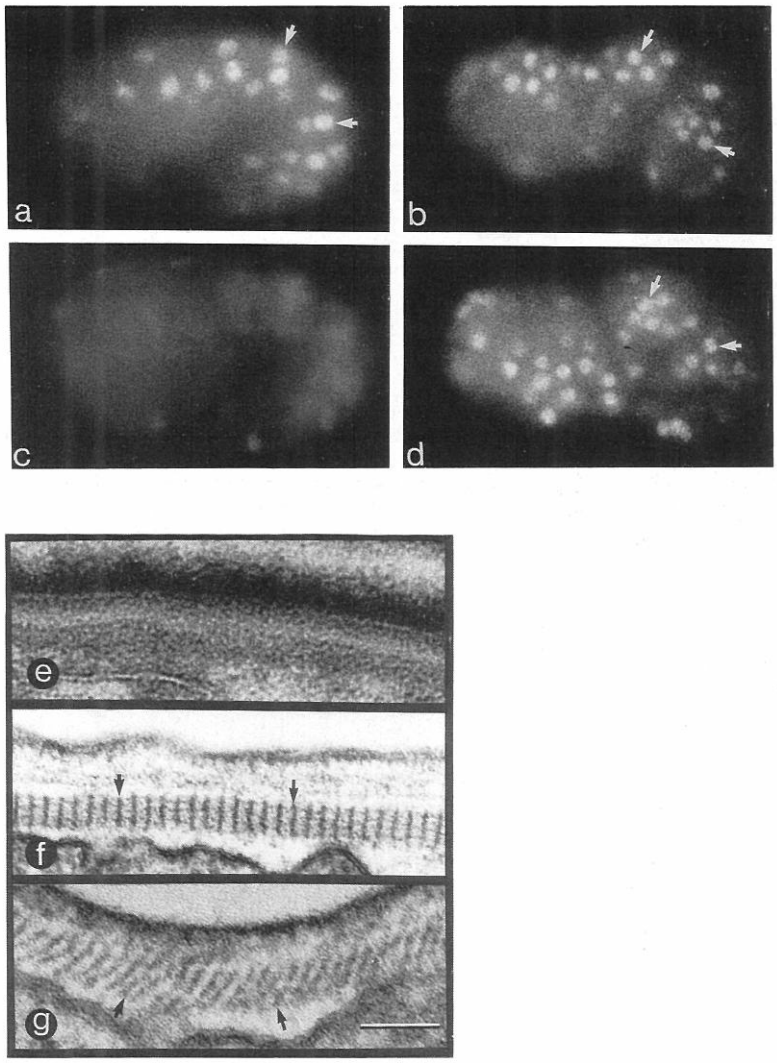



Figure 3. Hypodermal Cells in Wild-Type and *skn-1* Embryos

(a–d) Immunofluorescence photographs of wild-type (a and c) and *skn-1* (b and d) embryos stained with 4D9 (Patel et al., 1989), a MAb that stains hypodermal cell nuclei (arrows) at early stages of morphogenesis in C. elegans embryos (J. Rothman, personal communication; see Experimental Procedures). The left column shows surface (a) and internal (c) focal planes through a single wild-type embryo, with hypodermal cells present only on the embryo's surface. The right column shows surface (b) and internal (d) focal planes through a single *skn-1* embryo, with hypodermal cells present internally.

(e–g) Micrographs of newly hatched wild-type larvae (e and f) and terminally differentiated *skn-1* embryos (g) that were fixed and sectioned for transmission electron microscopy. (e) Extracellular cuticle associated with pharyngeal cells. (f) Extracellular cuticle associated with hypodermal cells in a wild-type larva contains a prominent striated layer (arrows). (g) Cuticle associated with an internal cavity in an *skn-1* embryo (see Figure 2h) contains a striated layer (arrows). Bar: 0.2 μm.

embryos have the same morphology as surface hypodermal cells (see Experimental Procedures) and stain positively with the hypodermal antibody (Figure 3). Two additional antibodies (see Experimental Procedures) also recognize hypodermal cells in C. elegans and stain internal hypodermal cells in *skn-1* embryos (data not shown). However, because none of these antibodies are completely specific, we have examined *skn-1* embryos using electron microscopy to confirm the identity of these presumptive internal hypodermal cells. The hypodermal cells inside *skn-1* embryos often surround internal cavities that, in the light microscope, appear to be lined by a cuticle (Figure 2h). We sectioned *skn-1* embryos for electron microscopy to determine whether this cuticle has the ultrastructure characteristic of hypodermal cuticle or of a different type of cuticle normally associated with the pharynx (Figure 3). The cuticle lining the cavities inside *skn-1* embryos clearly resembles hypodermal cuticle, supporting the view that the internal cells surrounding these cavities are hypodermal cells.

### Mutations in *skn-1* Transform Pharyngeal and Intestinal Precursors into Hypodermal and Body Wall Muscle Precursors

Because *skn-1* embryos lack a pharynx and an intestine and instead produce extra hypodermal cells, we asked if blastomeres that normally produce the pharynx and intestine instead produce hypodermal cells. We tested this hypothesis using a laser ablation strategy. When a laser beam is used to kill all the blastomeres except for EMS in a wild-type embryo, EMS produces pharyngeal, intestinal, and body wall muscle cells, but no hypodermal cells. When all the blastomeres except EMS are killed in an *skn-1* embryo, EMS instead produces hypodermal and body wall muscle cells, but no pharyngeal or intestinal cells (Figure 4). We further examined the effect of *skn-1* mutations on EMS by following the development of the E blastomere, a daughter of EMS that normally produces only intestinal cells. In a wild-type embryo, after ablation of all other blastomeres, E produces only intestinal cells. In *skn-1* embryos in which all blastomeres except E are ablated, E usually

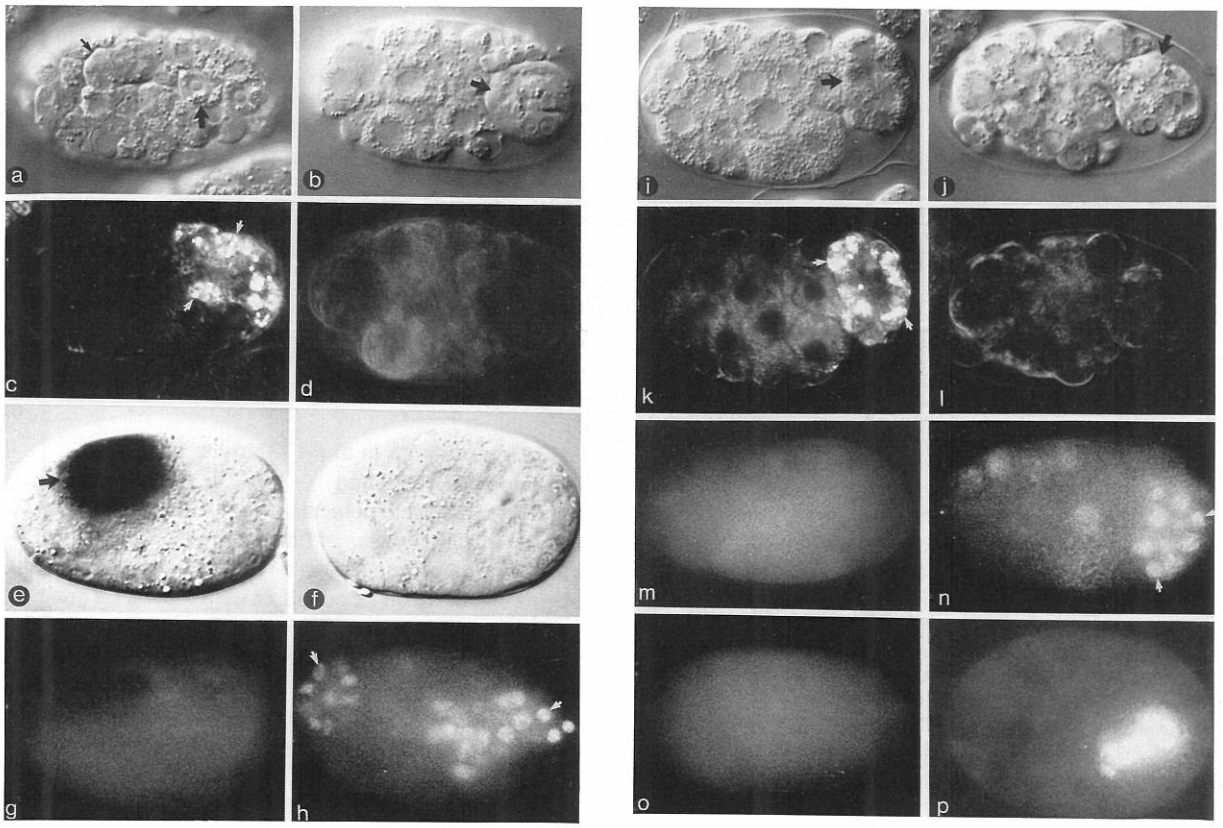



Figure 4. Fate of the Blastomeres EMS and E in Wild-Type and *skn-1* Embryos

(a–h) Light and immunofluorescence photographs of wild-type (left column) and *skn-1* (right column) partial embryos, in which all of the blastomeres except for EMS were killed by laser ablation at the 4-cell stage of embryogenesis. (a and b) Nomarski photographs of operated embryos, showing intestinal cells (a, large arrow) and a small cluster of pharyngeal cells (a, small arrow) only in the wild-type embryo, and a distinct cluster of hypodermal cells (b, arrow) only in the *skn-1* embryo. (c and d) Embryos viewed with polarization optics showing intestine-specific birefringent gut-granules (c, arrows) only in the wild-type embryo. (e and f) Embryos stained for pharyngeal-specific β-galactosidase activity (e, arrow) visible only in the wild-type embryo. (g and h) Immunofluoresence photographs of embryos stained with 4D9, a hypodermal cell antibody (see Figure 3), showing the presence of hypodermal cells (h, arrows) visible only in the *skn-1* embryo. Photographs were made after embryos developed for 12 hr at 25°C (a–f) or 6 hr at 25°C (g and h).

(i–p) Light and immunofluorescence photographs of wild-type (left column) and *skn-1* (right column) partial embryos in which all of the blastomeres except for E (a daughter of EMS) were killed by laser ablation. (i and j) Nomarski photographs of operated embryos, showing intestinal cells (i, arrow) only in the wild-type embryo, and a cluster of hypodermal and body wall muscle cells (j, arrow) only in the *skn-1* embryo. (k and l) Embryos viewed with polarization optics to show the intestine-specific birefringent gut-granules (k, arrows) present only in the wild-type embryo. (m and n) Embryos stained with 4D9, showing the presence of hypodermal cells (n, arrows) only in the *skn-1* embryo. (o and p) Embryos stained with the MAb 5.6.1, which recognizes body wall muscles (Miller et al., 1983), show the presence of body wall muscle cells only in the *skn-1* embryo. Photographs were made after embryos developed for 12 hr at 25°C (i–l, o, and p) or 6 hr at 25°C (m and n).

produces hypodermal and body wall muscle cells instead of intestinal cells (Figure 4).

In wild-type embryos, EMS produces pharyngeal cells and also induces certain ABa descendants to produce pharyngeal cells (Priess and Thomson, 1987). Because in *skn-1* embryos both EMS- and ABa-derived pharyngeal cells are missing, we have analyzed the fate of these ABa-derived cells by observing their cell division patterns using the light microscope. In three embryos examined, descendants of ABa that normally produce pharyngeal cells had wild-type patterns of cell division initially but stopped dividing one division earlier than in wild-type embryos (see Experimental Procedures for details). At present we do not know the identity of these cells, but two observations suggest they may be hypodermal cells. The only AB descendants that normally stop dividing at this time in wild-type embryos are hypodermal cells, and the ABa-derived cells in *skn-1* embryos have a nuclear morphology similar to that of some of the more anterior hypodermal cells produced by ABa in wild-type embryos.

### Developmental Defects in *skn-1* Embryos Are Limited to Blastomeres That Produce Pharyngeal and Intestinal Cells

Mutations in *skn-1* appear to affect the development of only EMS and the ABa descendants that become pharyngeal cells. By observation in the light microscope, we have not observed any differences between *skn-1* and wild-type embryos in the development of ABarpp, a close relative of ABa pharyngeal precursors, or in the development of AB-

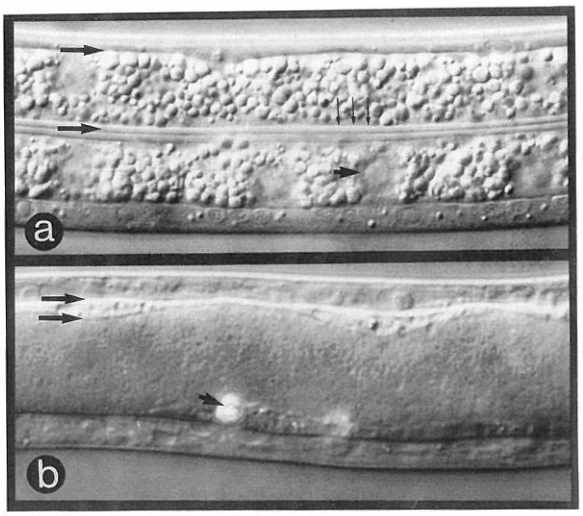



Figure 5. *skn-1(zu67)/Deficiency* Larvae Contain Abnormal Intestinal Cells

Nomarski photographs of the intestine in wild-type (a) and *skn-1(zu67)/Deficiency* (b) early-stage larvae. The width of the intestinal cells is indicated by a pair of large arrows in each panel, and the brush border lining the lumen of the intestine in the wild-type larva is indicated by thin arrows. The wild-type intestinal cells are filled with large droplets, and some of the intestinal cell nuclei are visible (a, small arrow). Larvae in which the allele *skn-1(zu67)* is heterozygous to the chromosomal deficiency nDf41 (kindly provided by Saechim Kim and H. Robert Horvitz), which deletes the *skn-1* gene, have abnormal intestinal cells (b). In *skn-1(zu67)/Deficiency* larvae, the intestinal cells are extremely thin (b, large arrows), lack a brush border, contain very few droplets, and have abnormal aggregates of cytoplasmic granules (b, small arrow). The severity of the defect in the intestinal cells shows some variability in these deficiency heterozygotes; the example shown represents a severely affected animal. *+/Deficiency* larvae appear normal (data not shown).

plap. These two blastomeres are descendants of ABa and ABp that produce hypodermal and neuronal cells. The development of the C blastomere, which produces much of the dorsal hypodermis, also appears normal in *skn-1* embryos. As an alternative to directly following lineages, one can easily recognize stage-specific patterns of hypodermal and neuronal cells on the dorsal and lateral surfaces of C. elegans embryos. These surface patterns appear almost normal in most *skn-1* embryos (see Experimental Procedures), suggesting that the corresponding lineages are not affected. We occasionally observe cells in abnormal positions in *skn-1* embryos, when blastomeres that normally remain on the surface instead move into the interior of the body. These pattern abnormalities probably result from defects in the migration of other blastomeres (see below). We have also compared the development of ABa, ABp, P2, and D (a descendant of P2) in *skn-1* and wild-type embryos after laser ablation of all other blastomeres, and find no apparent differences in the types of differentiated cells produced (data not shown).

Development of the germline in *skn-1* embryos also appears to be normal. P2, the sister of EMS, normally produces two cells that give rise to the germline during larval development. These two germline precursors can be recognized by their morphology and by the presence in their cytoplasm of structures called P-granules. The function of P-granules is not known, but they are initially present in the oocyte cytoplasm and become segregated specifically to the germline precursors during early embryonic cleavages (Strome and Wood, 1982, 1983). In *skn-1* embryos, the germline precursors appear normal in both the light and electron microscopes (except that they usually do not migrate properly during gastrulation; see below), and P-granules are properly segregated into these precursors (data not shown). Finally, the cleavage patterns of the early blastomeres in *skn-1* embryos appear normal up to the 28-cell stage of embryogenesis, in terms of the orientation of the division axes, the asymmetries in cell size, and the timing of cell divisions.

### EMS Descendants in *skn-1* Embryos Show Defects at Gastrulation

The first observable defects in *skn-1* embryos are abnormalities in the migration of EMS descendants at gastrulation. At the 28-cell stage in wild-type embryos, gastrulation begins when two descendants of EMS, called Ea and Ep, move inside the embryo from their initial position at the ventral surface. This migration occurs several minutes before Ea and Ep divide. These cells are followed into the interior by descendants of other early blastomeres. In *skn-1* embryos, Ea and Ep almost always divide before moving into the interior, such that their descendants are found both inside and on the ventral surface of the embryos. The MS descendants of EMS in *skn-1* embryos also exhibit abnormalities at gastrulation; posterior MS cells migrate inside too early, and anterior MS cells usually remain on the surface instead of migrating inside the embryo. Because of these altered cell migrations, the ventral surface of *skn-1* embryos bears little resemblance to that of wild-type embryos after the 28-cell stage of embryogenesis, in contrast to the dorsal and lateral surfaces, which appear nearly normal. These defects at gastrulation probably do not account for the change in fate of EMS descendants in *skn-1* mutants, for gastrulation does not correlate with cell fate in either wild-type or *skn-1* embryos. The ablation experiments described in this paper demonstrate that EMS in wild-type embryos can produce pharyngeal and intestinal cells even if all other early blastomeres have been ablated, thereby preventing gastrulation from occurring. Moreover, descendants of EMS in *skn-1* embryos that do migrate into the interior appear to exhibit the same transformation in cell fate as the descendants that remain on the surface.

### The *skn-1* Gene Functions Postembryonically in the Intestine

The genetic screen we used to isolate the *skn-1* mutants described here requires that homozygous mutant larvae, derived from heterozygous parents, be viable and survive to adulthood. As adults, the homozygous mutant hermaphrodites (which produce embryos by self-fertilization) fail to provide an essential maternal function, and their embryos die. Consequently, if a hermaphrodite has one wild-type copy of the *skn-1* gene, progeny that are homozygous for any of the mutant *skn-1* alleles hatch as larvae and develop

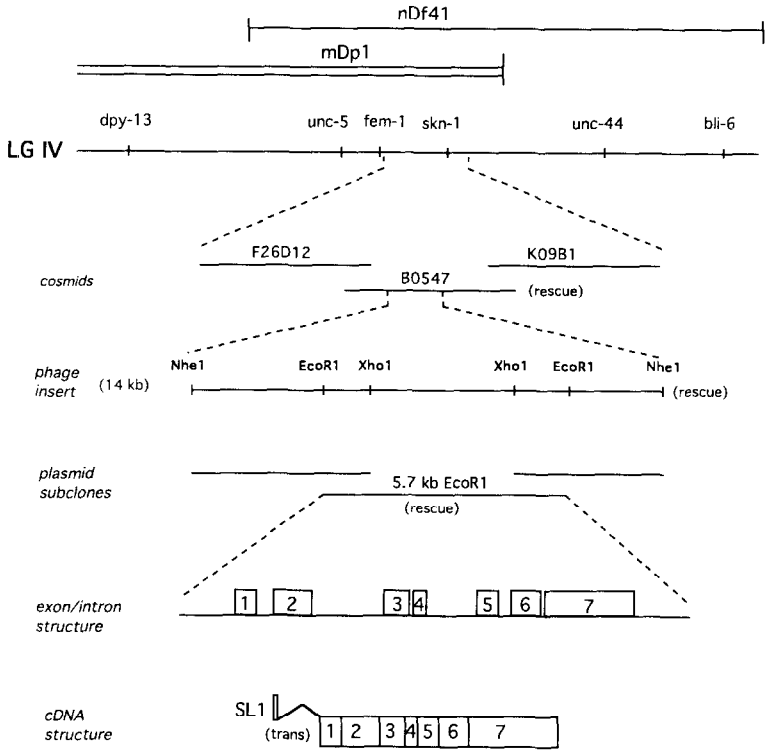



Figure 6. The *skn-1* Gene: Cloning Strategy and Gene Structure

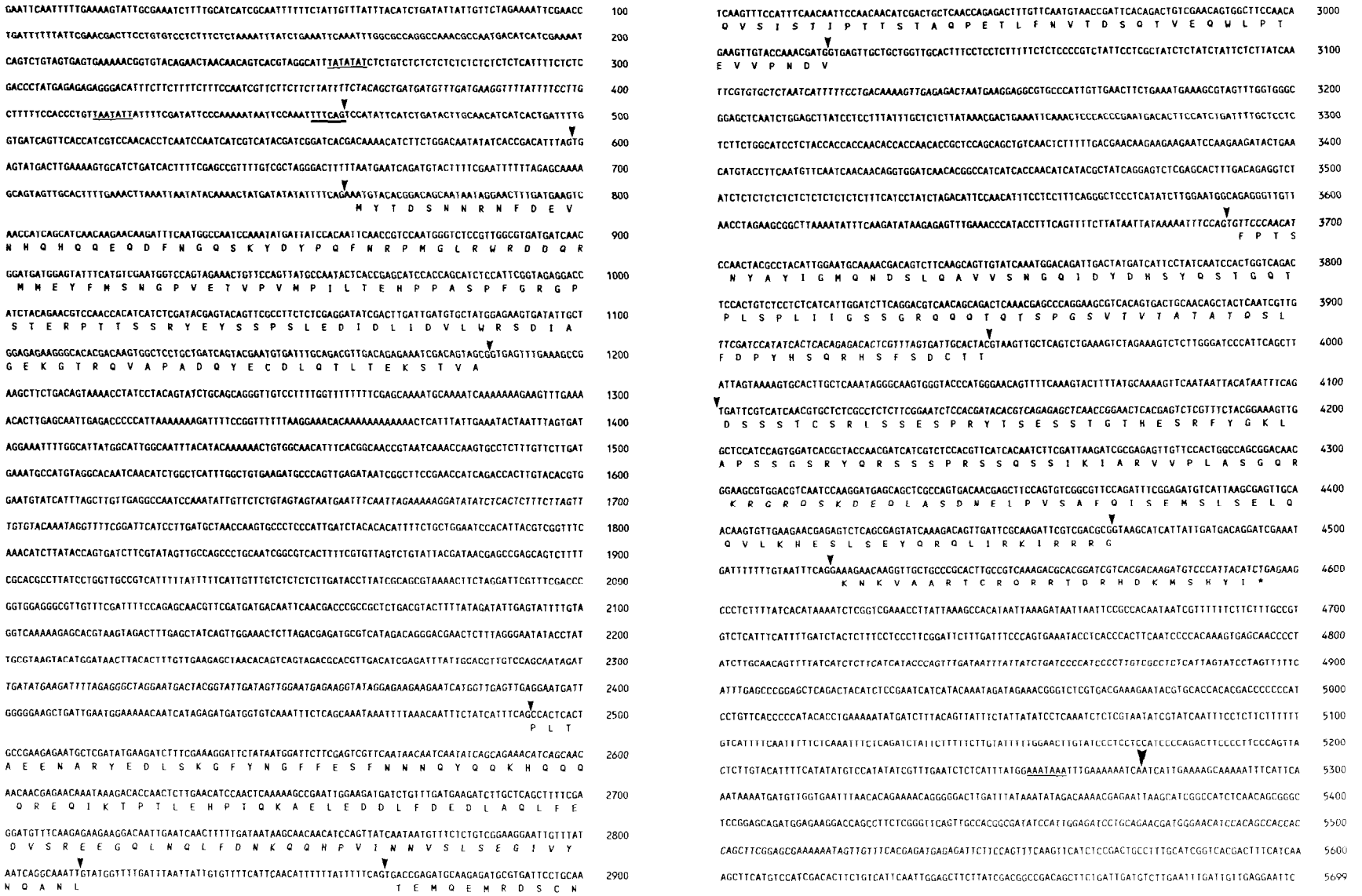
This diagram illustrates the strategy used to clone the *skn-1* gene and summarizes the structure of the gene. *skn-1* maps between the genes *fem-1* and *unc-44* on chromosome IV (LGIV). The free duplication mDp1 rescues *skn-1* mutants, and the deficiency nDf41 uncovers *skn-1*. DNA polymorphism mapping was used to further map *skn-1* to a 60 kb interval of DNA, most of which is contained in the three cosmids depicted. Only the cosmid B0547 contained rescuing activity after microinjection into the gonad of heterozygous *skn-1* animals (microinjection protocol is described in Mello et al., 1991). The cosmid B0547 was used to isolate a λ phage with a 14 kb insert that also rescued. A restriction map of the phage insert is shown. The central 5.7 kb EcoRI fragment and the overlapping NheI–XhoI fragments were subcloned into plasmids; only the 5.7 kb EcoRI fragment contained rescuing activity. Open boxes represent exons as deduced from DNA sequence analysis of the 5.7 kb rescuing genomic fragment and a nearly full-length 2.8 kb cDNA; the 5′-most exon is trans-spliced (see Figure 7).

into adults. However, if the progeny are instead heterozygous for the severe *skn-1* allele, *skn-1(zu67)*, and a chromosomal deficiency that deletes the *skn-1* gene, they hatch but usually die during larval development. These animals appear essentially normal at hatching, but have defects in the morphology of their intestinal cells that become progressively more severe as the larvae grow. The intestinal cells become extremely thin, develop abnormal aggregates of cytoplasmic granules, produce fewer than normal numbers of lipid and yolk droplets, and fail to maintain their microvillar brush border (Figure 5). By late larval stages the intestinal cells are severely atrophied, and the animals die. Other cell types, including pharyngeal cells, appear normal in these larvae. About 15% of *skn-1(zu67)/Deficiency* heterozygotes survive to adulthood and produce embryos, although usually fewer than normal. These embryos phenotypically resemble those produced by *skn-1(zu67)/skn-1(zu67)* mothers except that fewer of them contain intestinal cells (Table 1).

### The *skn-1* Gene

We have cloned the *skn-1* gene as summarized in Figure 6 and described in Experimental Procedures. Briefly, *skn-1* maps between the previously cloned genes *fem-1* and *unc-44*. This genetic interval corresponds to about 300 kb of DNA, all of which has been cloned and ordered into an overlapping set of cosmids and yeast artificial chromosomes as part of the C. elegans genome project (Coulson et al., 1986, 1988). We identified within this interval DNA polymorphisms in wild strains of C. elegans that enabled us to further map *skn-1* to within about 60 kb of DNA. We were able to rescue *skn-1* mutants by transformation with one of three cosmids that together span this 60 kb region. The rescuing cosmid was used to isolate and subclone several smaller genomic fragments that were then tested for rescuing activity. The smallest subclone that rescues contains a 5.7 kb EcoRI fragment, and we used this fragment to isolate *skn-1* cDNAs. The sequences of the 5.7 kb genomic subclone and a nearly full-length 2.8 kb cDNA predict a transcript that includes 7 exons (Figures 6 and 7). Analysis of C. elegans RNA by reverse transcription and the polymerase chain reaction (PCR) shows that the 5′ end of exon 1 is trans-spliced to the SL1 leader RNA (data not shown; trans splicing to SL1 occurs in about 15% of C. elegans messages; Krause and Hirsh, 1986; Bektesh et al., 1988). The single large open reading frame can encode a protein with a predicted molecular weight of 61 kd that is largely hydrophilic, is rich in serine and threonine (20%), and contains no long stretches of hydrophobic amino acids.

From a search of the sequence databases using the PATMAT program (Wallace and Henikoff, 1991), we found a significant similarity between a 25 amino acid region encoded by 3′ sequences in the *skn-1* open reading frame and the DNA binding domains of the "bZIP" family of transcription factor proteins (Figure 8). The basic region of *skn-1* is most closely related to the basic region sequences of *CNC* (for *cap'n'collar*, a segmentally expressed gene from Drosophila of unknown function; see Mohler et al., 1991), the proto-oncogenes *Jun* and *Fos*, and the transcription factor ATF-3. The similarity with *CNC* includes a block in which 18 out of 22 amino acids are identical. The



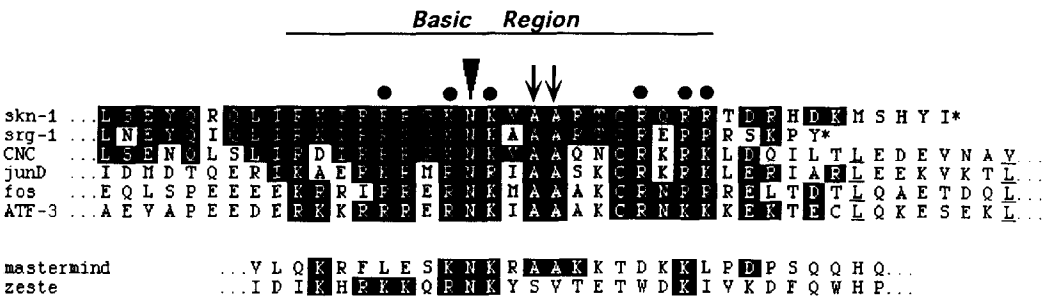



Figure 8. Comparison of the Basic Regions of *skn-1* and a C. elegans Homolog of *skn-1* with the Basic Region Domains of bZIP Transcription Factors, and *mastermind* and *zeste*

The basic region domains of *skn-1* and *srg-1* (an *skn-1* homolog in C. elegans, for "*skn-1*-related gene") are compared with the basic region domains of four bZIP transcription factors. Amino acid identities and similarities (R = K; Q = N; D = E) are indicated by black background, differences with white background. The invariant asparagine of bZIP basic regions (Vinson et al., 1989; Pu and Struhl, 1991) is indicated by a triangle, the most highly conserved basic residues by dots, and the two conserved alanines by arrows. An asterix (*) corresponds to stop codons in *skn-1* and *srg-1*. Leucines in the leucine zippers of the bZIP protein sequences are underlined. Note the presence of a glycine residue near the invariant asparagine in *skn-1*, *srg-1*, and *CNC*. bZIP basic regions form α helices upon binding to specific DNA sequences (O'Neil et al., 1990; Weiss et al., 1990). Because glycines have a strong tendency to break α helices, the presence of the glycine residues is unusual and may be important for the function of these gene products. The more distantly related basic region sequences from *zeste* and *mastermind* are shown at the bottom. Sources for protein sequences are as follows: *CNC*, Mohler et al. (1991); *junD*, Ryder et al. (1989); *fos*, Van Streeten et al. (1983); *ATF-3*, Hari et al. (1989); *mastermind*, Smoller et al. (1990); *zeste*, Mansukhani et al. (1988).

basic region of bZIP transcription factors is characterized by arginines or lysines at conserved positions flanking an invariant asparagine residue, and two conserved alanines (Vinson et al., 1989; Pu and Struhl, 1991). The sequence similarity with *skn-1* includes these characteristic residues.

In bZIP proteins the basic region is adjacent to a "leucine zipper" domain, characterized by the presence of leucine or other hydrophobic amino acids at every seventh position in an amphipathic α helix (Vinson et al., 1989; O'Neil et al., 1990). However, the *skn-1* cDNA does not encode a leucine zipper and instead has multiple stop codons immediately downstream of its basic region. Since dimerization of the basic region, normally mediated by the leucine zipper domain, has been shown to be essential for bZIP proteins to bind DNA (for references, see Talanian et al., 1990), we have used several techniques to examine the 3′ sequences in *skn-1* mRNA for evidence of heterogeneity near the basic region. We have sequenced the 3′ region of four *skn-1* cDNAs from two different libraries, analyzed six other cDNAs using PCR, and used reverse transcriptase–PCR analysis of C. elegans RNA to look for any *skn-1* messages with alternative 3′ sequences. These analyses indicate that the basic region sequences in *skn-1* are followed by a stop codon, with no evidence for alternative processing creating a message that could encode a leucine zipper adjacent to the *skn-1* basic region.

By screening a genomic library at low stringency, we have found a second gene in C. elegans that has a similar structure to the *skn-1* gene. This homolog, which we call *srg-1*, is about 80% identical in sequence to *skn-1* (data not shown) and encodes a similar basic region without an associated leucine zipper (Figure 8). We have searched the database for other proteins with this general structure and have found that two Drosophila genes, *mastermind* and *zeste*, encode somewhat similar basic regions that are not adjacent to leucine zippers (Figure 8).

### The *skn-1* Gene Is Expressed Maternally

To determine if *skn-1* is expressed maternally, we compared the levels of *skn-1* mRNA in two nematode strains, one that makes very few germ cells and one that produces a gonad with many unfertilized oocytes. A strand-specific probe for the *skn-1* gene detects two messages of about 3.0 and 2.9 kb (Figure 9), both of which are enriched in the strain with unfertilized oocytes, consistent with the genetic requirement for maternal expression of *skn-1*. We can also detect a low level of somatic expression of both *skn-1* transcripts (B. B. and J. R. P., unpublished data), consistent with the genetic requirement for postembryonic *skn-1* function in intestinal cells.

## Discussion

### The *skn-1* Gene Is Required to Specify the Fate of the Early Blastomere, EMS

During C. elegans embryogenesis, the ventral-most blastomere in a 4-cell embryo, EMS, produces both pharyn-

Figure 7. Sequence of the *skn-1* Gene and Flanking Regions

Genomic sequence is shown with the amino acid sequence of the encoded protein printed below the line using the one-letter code. Nucleotide positions are indicated by the column of numbers to the right of each line of sequence. The first methionine is at nucleotide position 762, in the second exon. Splice sites are indicated by small arrowheads. Two potential TATA boxes are underlined at the 5′ end of the gene (thin lines). A potential polyadenylation signal is underlined at the 3′ end of the gene, with the poly(A) addition site indicated by the large arrowhead. The putative trans-splice acceptor site is underlined upstream of the first exon (bold line).

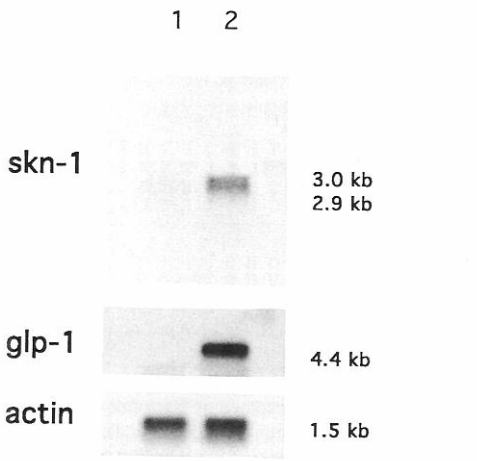



Figure 9. Northern Analysis of *skn-1* Transcripts

Two transcripts are detected with a strand-specific *skn-1* probe (see Experimental Procedures). Poly(A)$^+$ RNA from SS104(bn2), a temperature-sensitive mutant that contains many fewer germ cells than wild-type animals when raised at restrictive temperature (lane 1), and from a temperature-sensitive *fer-1* mutant, which, at the restrictive temperature, is defective in fertilization and contains large numbers of unfertilized oocytes (lane 2). The same filter was subsequently hybridized with a probe for *glp-1*, which is known to be maternally expressed (Austin and Kimble, 1989), and with a probe for *act-1*, an actin gene (Krause and Hirsh, 1987), to control for loading of the RNA.

geal and intestinal cells in an apparently cell-autonomous manner. Blastomere isolation experiments have shown that P1, the parent of EMS, can produce pharyngeal and intestinal cells autonomously (Laufer et al., 1980; Priess and Thomson, 1987), and that EMS can produce pharyngeal and intestinal cells after its sister blastomere has been removed from a 4-cell embryo (Priess and Thomson, 1987). We have shown here that EMS will produce pharyngeal and intestinal cells when all other blastomeres are killed by a laser microbeam at the 4-cell stage of embryogenesis. These results are consistent with the hypothesis that in normal development the EMS blastomere uniquely contains some factor(s) required for the autonomous specification of pharyngeal and intestinal cell fates.

An interpretation of the *skn-1* mutant phenotype is that the *skn-1* gene encodes such a factor. We have shown that mutations in *skn-1* prevent the development of several different cell types, most of which appear to be related only by their common origin in EMS. Except for the altered fate of the ABa-derived blastomeres that normally are induced by EMS to produce pharyngeal lineages, the fates of other blastomeres appear not to be affected in *skn-1* embryos. Although the *skn-1* gene could function late in embryogenesis to specify the fate of several different cell types produced by EMS, we think it likely that the *skn-1* gene product acts at an early step common to these different developmental pathways. The genetic requirement for maternal expression of *skn-1*, the maternal expression of *skn-1* mRNA, and the migration defects of EMS descendants in 28-cell stage *skn-1* embryos are consistent with *skn-1* acting early in embryogenesis.

We have shown that the fate of EMS in *skn-1* embryos is altered such that it produces hypodermal and body wall muscle cells from lineages that normally do not produce these cell types (Figure 4). The fate of EMS in *skn-1* embryos is intriguingly similar to that of its sister cell, P2, which produces hypodermal and body wall muscle cells in both *skn-1* and wild-type embryos. Although EMS and P2 are more similar in *skn-1* than in wild-type embryos, we do not believe that expression of the *skn-1* gene accounts for all their differences. In wild-type embryos, P2 produces the germline and contains structures called P-granules. P-granules are present initially in the cytoplasm of the oocyte and are segregated specifically to P2 during the early embryonic cleavages (Strome and Wood, 1982, 1983). In addition, because of asymmetries in the early embryonic cleavages that produce the posterior-most blastomeres, P2 is smaller in size than EMS. The factor(s) responsible for these properties of P2 appear to be localized at the posterior pole of the fertilized oocyte, in a region that eventually becomes part of P2 (Schierenberg, 1985, 1987). Mutations in *skn-1* do not appear to affect the function of the posterior pole, because P2 but not EMS produces germline progenitors, P-granules are segregated normally, and the asymmetries in early cleavages are still seen in *skn-1* embryos. It is possible that the restriction of *skn-1* activity to EMS and the independent function of a posterior pole in P2 might together account for most of the differences in the fates of these sister blastomeres.

Mutations in *skn-1* result in a profound alteration in the fate of EMS, but have no apparent effect on almost all of the other embryonic cells we have examined. The exception is in the development of ABa-derived blastomeres that normally produce pharyngeal cells. Because ABa descendants in wild-type embryos require *glp-1*-mediated cell–cell interactions with EMS descendants to produce pharyngeal cells (Priess and Thomson, 1987), we do not know at present whether the requirement for *skn-1* function in the ABa descendants is direct or indirect. *skn-1* activity might be required within ABa descendants for the production of pharyngeal cell types, or the ABa descendants may not be able to interact properly with EMS descendants that have been transformed by mutations in *skn-1*. The sequence similarity in the basic regions of *skn-1* and *mastermind* (Figure 8; also see below), though limited, is interesting because genetic data indicate that *mastermind* interacts with *Notch* (de la Concha et al., 1988; Brand and Campos-Ortega, 1990; Xu et al., 1990), the Drosophila homolog of the C. elegans gene *glp-1* (Yochem et al., 1988).

Although the *skn-1* mutations described here appear to reduce the function of *skn-1*, they do not represent null alleles. All four *skn-1* alleles show incomplete penetrance (Table 1), and larvae in which a strong allele of *skn-1* is heterozygous to a chromosomal deficiency usually die before becoming adults. The appearance of the intestinal cells in *skn-1(zu67)/Deficiency* larvae (Figure 5) suggests that the *skn-1* gene may also function postembryonically to maintain the differentiated state of intestinal cells. Although in *skn-1* mutant embryos the pharyngeal phenotype is more penetrant than the intestinal phenotype, the pharyngeal cells in *skn-1(zu67)/Deficiency* animals are

normal. It is possible that *skn-1* is not required postembryonically in pharyngeal cells, or that other gene products are functionally redundant with *skn-1* in these cells. *srg-1*, the C. elegans homolog of *skn-1* that we have identified, does not appear to be expressed maternally but is expressed postembryonically (data not shown), raising the possibility that *srg-1*, or both *skn-1* and *srg-1*, could function in pharyngeal cells during larval development. Such a partial redundancy in function has been observed in C. elegans for the closely related genes *lin-12* and *glp-1*; animals with mutations in both genes exhibit defects in developmental events that are not affected by mutations in either gene alone (Lambie and Kimble, 1991).

### The *skn-1* Gene May Encode a Transcription Factor with an Unusual Structure

The similarity in the predicted *skn-1* protein to the basic region, or DNA binding domain, of bZIP transcription factors (Figure 8) suggests that the *skn-1* gene product might regulate transcription during early development. However, the *skn-1* gene encodes a basic region that does not appear to be associated with a leucine zipper. *srg-1*, a C. elegans homolog of *skn-1*, also appears to encode a bZIP basic region but no leucine zipper (Figure 8). Moreover, we have found sequence similarities between *skn-1* and two genes from Drosophila, *mastermind* and *zeste*, that both encode basic regions lacking adjacent leucine zipper domains (Mansukhani et al., 1988; Smoller et al., 1990). *mastermind* encodes a nuclear protein, although it is not known if this protein binds DNA (Smoller et al., 1990), and the *zeste* protein has been shown to bind specific DNA sequences (Benson and Pirotta, 1987). Perhaps these genes encode transcription factors that function as monomers or form dimers using novel mechanisms. If *skn-1* encodes a transcription factor, it could function either by activating genes required for development of the pharynx and the intestine, or instead by inhibiting the production of hypodermis, with other factors then acting to specify pharyngeal and intestinal lineages.

### Does *skn-1* Encode a Localized Determinant?

The possibility that *skn-1* encodes a transcription factor is intriguing in terms of the possibility that localization of the *skn-1* gene product might explain some of the different properties of early blastomeres in the C. elegans embryo. The three systems that define the anterior–posterior and dorsal–ventral coordinates of the Drosophila embryo all appear to function by causing an unequal distribution of a maternal gene product that functions as a transcription factor (Nüsslein-Volhard, 1991): the *bicoid* gene product becomes trapped at the anterior pole of the oocyte (Berleth et al., 1988); *nanos* becomes localized to the posterior pole of the oocyte, where it acts to specify posterior patterns by inhibiting the maternally-encoded transcription factor *hunchback* (Irish et al., 1989; Struhl, 1989; Lehmann and Nüsslein-Volhard, 1991; Wang and Lehmann, 1991); and *dorsal* encodes a transcription factor that is evenly distributed throughout the early embryo but becomes localized to the nuclei of ventral cells, forming a dorsal–ventral gradient of nuclear concentration (Rushlow et al., 1989; Steward, 1989; Roth et al., 1989), with the *cactus* gene product inhibiting the nuclear localization of *dorsal* (Roth et al., 1991).

At least two different models can account for the observation that mutations in *skn-1* prevent the normal development of EMS, but do not appear to affect the development of most other blastomeres: the *skn-1* gene product could program the autonomous production of pharyngeal and intestinal cells by being specifically localized to EMS during early embryonic cleavages (much as *bicoid* becomes localized to the anterior pole in Drosophila), or the *skn-1* gene product might be present throughout the embryo but function in EMS because of interactions with other factors, some of which might be localized (for example, gene products analogous to *nanos* or *cactus* could inhibit *skn-1* function in blastomeres other than EMS).

Finally, we are interested in the possibility that localization of the *skn-1* gene product or regulation of its activity might be important for establishing the dorsal–ventral axis in the early C. elegans embryo. Establishment of the dorsal–ventral axis in Drosophila appears to depend on a localized signal from the extra-embryonic follicle cells that surround the early embryo (Stein et al., 1991). In contrast, dorsal–ventral axis determination in C. elegans can occur when no extra-embryonic cells are present (Priess and Thomson, 1987). The ventral surface of the C. elegans embryo is defined by the position of the blastomere EMS, and the polarity of the dorsal–ventral axis can be reversed by altering the position of EMS in a 4-cell embryo. Since we have shown that the *skn-1* gene product appears to act early in embryogenesis to specify the fate of EMS, determining how *skn-1* activity is regulated may be helpful in understanding how the C. elegans embryo establishes a dorsal–ventral axis. By extending our molecular and genetic analysis of the *skn-1* gene, we hope to improve our understanding of how early blastomeres become different during C. elegans embryogenesis.

## Experimental Procedures

### Strains and Alleles

N2 Bristol strain was used as the standard wild-type strain. The following mutant alleles were used in the genetic analysis of the *skn-1* locus (*bli*, Blister; *dpy*, Dumpy; *fem*, Feminized; *lon*, Long; *unc*, Uncoordinated): linkage group I, *dpy-5(e61)*; linkage group II, *rol-6(e187)*; linkage group III, *unc-32(e189)*; linkage group IV, *bli-6(sc16)*, *dpy-13(e184sd)*, *fem-1(e1965)*, *unc-5(e53)*, *unc-8(n491sd)*, *unc-44(e362)*; linkage group V, *dpy-11(e224)*; linkage group X, *lin-2(e1309)*, *lon-2(e678)*. The translocations nT1 and DnT1 (in both of which the left arm of linkage group V and the right arm of linkage group IV are joined; DnT1 also has a dominant Unc mutation linked to the translocation) and the free duplication mDp1 (which includes all of the right arm of linkage group IV) were also used. These mutations are listed in Brenner (1974) and O'Brien (1990). The strain PD192 contains a stably integrated β-galactosidase gene driven by the C. elegans pharyngeal myosin promoter (Fire, 1986; Fire et al., 1989). The deficiency *nDf41* uncovers the markers *unc-5* and *bli-6*, which flank the *skn-1* gene (S. A. Kim and H. R. Horvitz, personal communication). The free duplication *mDp1* covers the *skn-1* gene and completely rescues the maternal-effect lethality of *skn-1* mutants. mDp1 extends to the left end of linkage group IV and its right end breaks between *skn-1* and *unc-44*, the next genetic marker to the right of *skn-1* on the genetic map.

### Genetic Analysis

All four alleles of *skn-1* were isolated in screens for recessive, noncon-

ditional, maternal-effect, embryonic-lethal mutants. The screening procedure has been described previously (Priess et al., 1987; Kemphues et al., 1988). We mutagenized with ethylmethane sulfonate as described in Brenner (1974). The basic methods of C. elegans culture, genetics, and nomenclature were as described in Brenner (1974).

The original *skn-1(zu67)* allele was found to be linked to chromosome IV by standard linkage tests using the visible markers *dpy-5*, *rol-6*, *unc-32*, *unc-5*, *dpy-11*, and *lon-2* (see Brenner, 1974). The other three alleles, *zu129*, *zu135*, and *zu138*, were shown to be alleles of *skn-1* by complementation tests and three-factor mapping using genetic markers. Dpy, non-Unc and Unc, non-Dpy recombinants were picked from an *skn-1(zu67)/dpy-13 unc-8* strain. Seven of 12 Dpy, non-Unc recombinants were *skn-1(zu67)* and 5 of 15 Unc, non-Dpy recombinants were *skn-1(zu67)*, placing *skn-1* about two-thirds of the distance between *dpy-13* and *unc-8*. Similar three-factor data were obtained for the other three *skn-1* alleles. Unc, non-Fem recombinants were picked from an *skn-1(zu67)/fem-1 unc-44* strain. Twelve of 18 Unc, non-Fem recombinants were *skn-1(zu67)*, placing *skn-1* about 0.2 map units to the right of *fem-1*. All four alleles were outcrossed eight times, and both chromosome arms crossed off of each allele to ensure that no other mutations were responsible for the phenotypes we describe.

The genomic DNA between *fem-1* and *unc-44* has been cloned and ordered into an overlapping set of cosmids and yeast artificial chromosomes (Coulson et al., 1986, 1988). We identified restriction fragment length DNA polymorphisms in the wild C. elegans strain, DH424, using the cosmids K02B2, C55C3, and F26D12 as probes on Southern blots of genomic DNA from DH424 worms. A strain was constructed in which an outcrossed, N2-derived chromosome with *skn-1(zu67)* linked to *unc-44* was made heterozygous to the DH424 polymorphic chromosome IV. Seven recombinants were isolated in which crossovers to the polymorphic chromosome occurred between *unc-44* and *skn-1*. Analysis of these recombinant chromosomes by Southern blots probed with radioactively labeled DNA from the three cosmids showed that in 2 out of 7 cases the crossovers occurred to the left of the cosmid C55C3 but to the right of F26D12, which narrowed the region containing the *skn-1* gene to about 60 kb of DNA to the right of *fem-1*.

### Microscopy

Animals homozygous for *skn-1* mutations were obtained by picking non-Dpy, non-Unc progeny from *skn-1(zuX)/dpy-13(e184semidominant) unc-8(n491semidominant)* hermaphrodites. Embryos were obtained by allowing homozygous *skn-1* adults to lay eggs for 3–5 hr, removing the adults from the plates, and allowing the eggs to develop for an interval of time equivalent to normal embryogenesis (12 hr at 25°C or 20 hr at 15°C).

Light microscopy was performed with a Zeiss Axioplan microscope equipped for epifluorescence, polarizing optics, and differential interference contrast. Photographs were taken on Kodak Technical Pan film and Kodak T-Max 400 film and developed in HC110 and T-Max 400 developer, respectively. Embryos were processed for light microscopy following the procedures of Sulston et al. (1983). Embryos carrying the β-galactosidase–pharyngeal myosin promoter were stained for light microscopy following the procedures of Fire (1986) and Fire et al. (1989).

Embryos were processed for immunofluorescence microscopy following the fixation procedures of Albertson (1984) but rehydrated through a graded series of acetone (1 min each in 90%, 60%, 30%, and 10% acetone–$dH_2O$). The fixed embryos were preincubated at room temperature in a solution of 1% bovine serum albumin and 1% ovalbumin in Tris–Tween (100 mM Tris–HCl [pH 7.5], 200 mM NaCl, 0.1% Tween 20) for 30 min, incubated with primary antibody in Tris–Tween for 1 hr, and incubated with a rhodamine-conjugated goat anti-mouse secondary antibody (Tago) in Tris–Tween for 1 hr. The fixed embryos were washed three times for 5 min after each antibody incubation and were finally mounted in phosphate-buffered saline (150 mM NaCl, 3 mM KCl, 8 mM $Na_2HPO_4$, 1.5 mM $NaH_2PO_4$, 1 mM $MgCl_2$), overlaid with a coverslip, and sealed with fingernail polish for viewing with epifluorescence.

The intestinal valve cells that stain in *skn-1* embryos with the antibody 2CB7 (see Figure 2) can be distinguished from pharyngeal gland cells and intestinal cells by their morphology. The identity of the intestinal valve cells in *skn-1* embryos has been confirmed by showing that, as in wild-type embryos, they stain earlier in embryogenesis than the pharyngeal gland cells (B. B. and J. R. P., unpublished data). The lack of intestinal valve cells in *par-1* embryos has been confirmed by determining that the cells in *par-1* embryos that stain with 2CB7 are present only at late stages in embryogenesis, consistent with their identity as pharyngeal gland cells. The antibody 2CB7 was isolated in a general screen for antibodies that stain specific cell types in C. elegans (H. Okamoto and J. N. Thomson, unpublished data). The antibody 4D9 stains hypodermal cell nuclei in C. elegans (J. Rothman, personal communication). As shown in Figure 3, the staining at early stages (bean to 1.5-fold stage) is specific for the characteristic rows of dorsal, lateral, and ventral hypodermal cells. At later stages of embryogenesis, near hatching, we see lighter staining of other internal cell nuclei, but the hypodermal cell nuclei stain more brightly with a characteristic punctate pattern. The wild-type and *skn-1* embryos shown in Figure 3 all were stained at approximately bean stage, or about 6 hr after fertilization at 25°C. The staining is most specific when the embryos are incubated with antibody and washed at 25°C. When the antibody incubations are done at 15°C, lighter nuclear staining of other cell types is seen even in bean-stage embryos. We have examined nine different subclones of the 4D9 hybridoma cell line (kindly provided by N. Patel). Some of these lines are less specific for hypodermal cells; the lines 4D9-B and 4D9-G are two that specifically stain hypodermal cell nuclei in bean-stage embryos. We have also shown staining of internal hypodermal cells in *skn-1* embryos using additional antibodies that stain hypodermal cells (B. B. and J. R. P., unpublished data), including MH27 (see Priess and Hirsh, 1986) and anti-IFA (which stains intermediate filaments in hypodermal cells; see Pruss et al., 1981). However, both MH27 and anti-IFA stain pharyngeal cells in wild-type embryos and are therefore less specific for hypodermal cells than the 4D9 antibody. Hypodermal cells in C. elegans can be identified at the bean stage by the following criteria: most of them contain large numbers of yolk droplets in their cytoplasm, have large and flattened nuclei with prominent nucleoli, and have cell bodies that are also large and flattened. The internal hypodermal cells in *skn-1* embryos also exhibit this characteristic morphology.

### Laser Ablation Experiments

A VSL-337 laser (Laser Science, Inc.) was attached to a Zeiss Axioplan microscope according to Avery and Horvitz (1989). A graded density filter to control the intensity of the laser beam was constructed by briefly exposing to a point source of light a strip of 35mm film held at about a 45° angle. Early embryos were obtained by cutting open gravid adults and prepared as for light microscopy. The laser beam was focused on the nucleus or spindle poles of selected blastomeres, and blastomeres were hit with approximately 30–60 pulses of laser light until substantial cellular debris accumulated in the cells.

### Lineage Analysis

The complete embryonic lineage of C. elegans is described in Sulston et al. (1983). Because all descendants of early blastomeres cannot be followed simultaneously, lineage analysis was performed piecemeal on selected lineage branches. In this study, development of *skn-1* embryos was followed up to the 28-cell stage with all four alleles, and selected lineages followed further in *skn-1(zu67)* embryos. The ABarpp lineage was examined in four *skn-1(zu67)* embryos, the ABplap lineage in four embryos, the Caa lineage in two embryos, and the Cap lineage in two embryos. For analysis of the ABa lineages that normally produce pharyngeal cells, the ABaraapa lineage was followed in four *skn-1* embryos. When *skn-1* embryos were examined at the bean stage, before the onset of morphogenesis, the characteristic rows of dorsal hypodermal cells (hyp 7 cells), lateral hypodermal cells (V cells), and ventral hypodermal cells (P cells) could be identified in most embryos. The ventral and ventrolateral surfaces at this stage appear highly abnormal, due to the presence of hypodermal cells produced by EMS lineages in *skn-1* embryos. These EMS-derived hypodermal cells on the ventral surface of *skn-1* embryos did not substantially affect the more lateral and dorsal patterns of hypodermal cells until later in morphogenesis.

### Cloning the *skn-1* Gene

As described above, the *skn-1* gene was mapped to a 60 kb interval of DNA, most of which is contained in three overlapping cosmids, F26D12, BO547, and K09B1. These cosmids were individually microin-

jected into the gonad of *skn-1(zu67)/dpy-13 unc-8* hermaphrodites, using the procedures of Mello et al. (1991). Rescue was scored in the F1 of these injected animals, and only the cosmid B0547 was able to rescue *skn-1* mutants. A radiolabeled probe made from this cosmid was used to screen a C. elegans genomic λ library. One of these phage contained a 14 kb insert and was able to rescue *skn-1* mutants. Three smaller overlapping fragments of this phage insert were subcloned into Bluescript plasmids (Stratagene). A central 5.7 kb EcoRI fragment from the phage insert was able to rescue, and the flanking NheI–XhoI fragments (which partially overlap the central 5.7 kb EcoRI fragment; see Figure 6) did not rescue. In these rescue experiments, about 20% of the transformed animals (assessed by expression of a plasmid encoding a dominant allele of *rol-6*—see Mello et al., 1991) showed rescue of the maternal-effect lethality caused by *skn-1(zu67)*. Three of 11 animals transformed with the cosmid B0547 were rescued, 2 of 10 animals transformed with the 14 kb phage subclone were rescued, and 8 of 30 animals transformed with the 5.7 kb plasmid subclone were rescued (see Figure 6). Restriction digests, cloning, plaque screening, and Southern blot analyses were all done using standard methods as described by Sambrook et al. (1989) and Ausubel et al. (1989).

### cDNA Cloning and DNA Sequencing

The 5.7 kb RI genomic fragment that rescues *skn-1* mutants was used to screen two different C. elegans λ cDNA libraries (Barstead and Waterston, 1989; Kim and Horvitz, 1990), from which 10 *skn-1* cDNAs were isolated. Five cDNA inserts were isolated and cloned into Bluescipt vectors (Stratagene). A series of nested deletions of both the 5.7 kb genomic subclone and the 2.8 kb cDNA subclone were made using exonuclease III and mung bean nuclease according to manufacturers' protocols (Promega). These deletions were sequenced using Sequenase (United States Biochemical) by the method of Sanger et al. (1977). Gaps in the sequence from the nested deletion series were filled in using specific oligonucleotides as primers. The entire sequence from one strand of the cDNA was obtained, and the entire sequence of the other strand was obtained from the genomic clone. Partial sequences were also obtained from four other cDNA clones. The nearly full-length 2.8 kb cDNA terminated at nucleotide position 479 near the 5′ end of the apparently noncoding exon 1 in the genomic sequence shown in Figure 7.

### Northern Blot Analysis

Pellets of synchronized cultures of worms were frozen in liquid nitrogen and stored at −70°C. Total RNA was prepared as described by Austin and Kimble (1989) and the concentration was determined spectrophotometrically. Poly(A)$^+$ RNA was prepared by oligo(dT) selection. Northern blots were prepared by electrophoresing 2 μg per lane of each of the RNA samples described in Figure 11 on 1.5% agarose–formaldehyde gels, followed by transfer to a nylon membrane (Hybond-N) and UV crosslinking. A single primer PCR reaction using Amplitaq (Cetus), a specific oligonucleotide primer, and a gel-purified restriction fragment from the 2.8 kb cDNA as a template were used to generate a strand-specific, 120 base DNA probe complementary to the sequences from nucleotide position 820 to 940 in the genomic sequence shown in Figure 7. Hybridization conditions were those of Church and Gilbert (1984). Hybridization and washes were done at 60°C.

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