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Socket Cells Mediate Spicule Morphogenesis in *Caenorhabditis elegans* Males

Lily I. Jiang and Paul W. Sternberg[1]

Howard Hughes Medical Institute and Division of Biology, California Institute of Technology, Mail Code 156-29, Pasadena, California 91125

Caenorhabditis elegans **male spicule morphogenesis requires the coordinated cellular behaviors of several types of cells. We found that the spicule neurons and sheath cells, although important for spicule function, are dispensable for spicule morphology. In contrast, the spicule socket cells are essential for both spicule elongation and formation of spicule cuticle. The socket cells are not only necessary but also sufficient to produce spicule cuticle. This functional aspect of socket cells is genetically separable from their function in mediating spicule elongation: elongated spicules with defective spicule cuticle can be formed. During spicule morphogenesis, the expression of an *egl-17::GFP* reporter gene is found in the spicule socket cells and its expression appears to be regulated in the socket cells. Mutants defective in TGF-β signaling display a crumpled spicules phenotype as a result of failure of socket cell movement during spicule morphogenesis. These observations suggest that both the FGF and the TGF-β signaling pathways might be involved in spicule elongation.** © 1999 Academic Press

Key Words: **spicule morphogenesis; socket cell; *C. elegans;* FGF; TGF-β.**

INTRODUCTION

Organogenesis requires coordination of a population of cells. To form a certain structure, specialized cells have to be generated at the correct positions; possess specific morphogenetic properties, including cell migration, contact guidance, and cell adhesion; and exert specific functions. This process involves the specification of terminal cell fates, which in turn regulates cellular behaviors and cell functions. Although cell fate specification is the initial and the most important aspect of organogenesis, the subsequent cellular behaviors and cell functions are also essential for morphogenic events and organogenesis.

Morphogenesis of *Caenorhabditis elegans* male spicules provides a good opportunity to study organogenesis. Spicules are specialized copulatory structures in nematode male tails. In *C. elegans* each male has a pair of spicules, which are used during the mating process to protrude into the hermaphrodite vulva, anchor the male tail to the vulva, and facilitate sperm transfer. Each spicule is composed of two neurons, two sheath cells, and four socket cells (Sulston *et al.,* 1980; Fig. 1A). The two neurons, SPD and SPV, send their processes through the spicule to a pore at the tip of the spicule, which may allow the ciliated sensory endings of the two neurons to sense the environment (Sulston *et al.,* 1980). Therefore, they are thought to be chemosensory neurons. The SPD and SPV neurons are involved in different steps in mating behavior: SPD is required for spicule insertion into the vulva while SPV is responsible for proper sperm transfer (Liu and Sternberg, 1995). The two sheath cells fuse with each other and enwrap the processes of the spicule neurons. The four socket cells also fuse together and form the outer layer of the spicule. A layer of sclerotized refractile cuticle material, spicule cuticle, is laid down outside of each spicule. Posterior–dorsal to the spicules lies another structure covered with the sclerotized refractile cuticle, called the gubernaculum. This structure presumably serves to guide the tips of the spicules ventrally through the cloaca during male mating (Sulston *et al.,* 1980).

The whole spicule structure and the gubernaculum are generated from a single male-specific blast cell, the B cell. At the end of the second larval (L2) stage, B.a generates eight progeny which assume six distinct fates, designated α, β, γ, δ, ϵ (2 each), and ζ (2 each) (Sulston *et al.,* 1980; Chamberlin and Sternberg, 1993; Fig. 1B). During the L3 stage, these cells continue to divide to generate spicule neurons, sheath

[1] To whom correspondence should be addressed. Fax: (626) 568-8012. E-mail: PWS@cco.caltech.edu.

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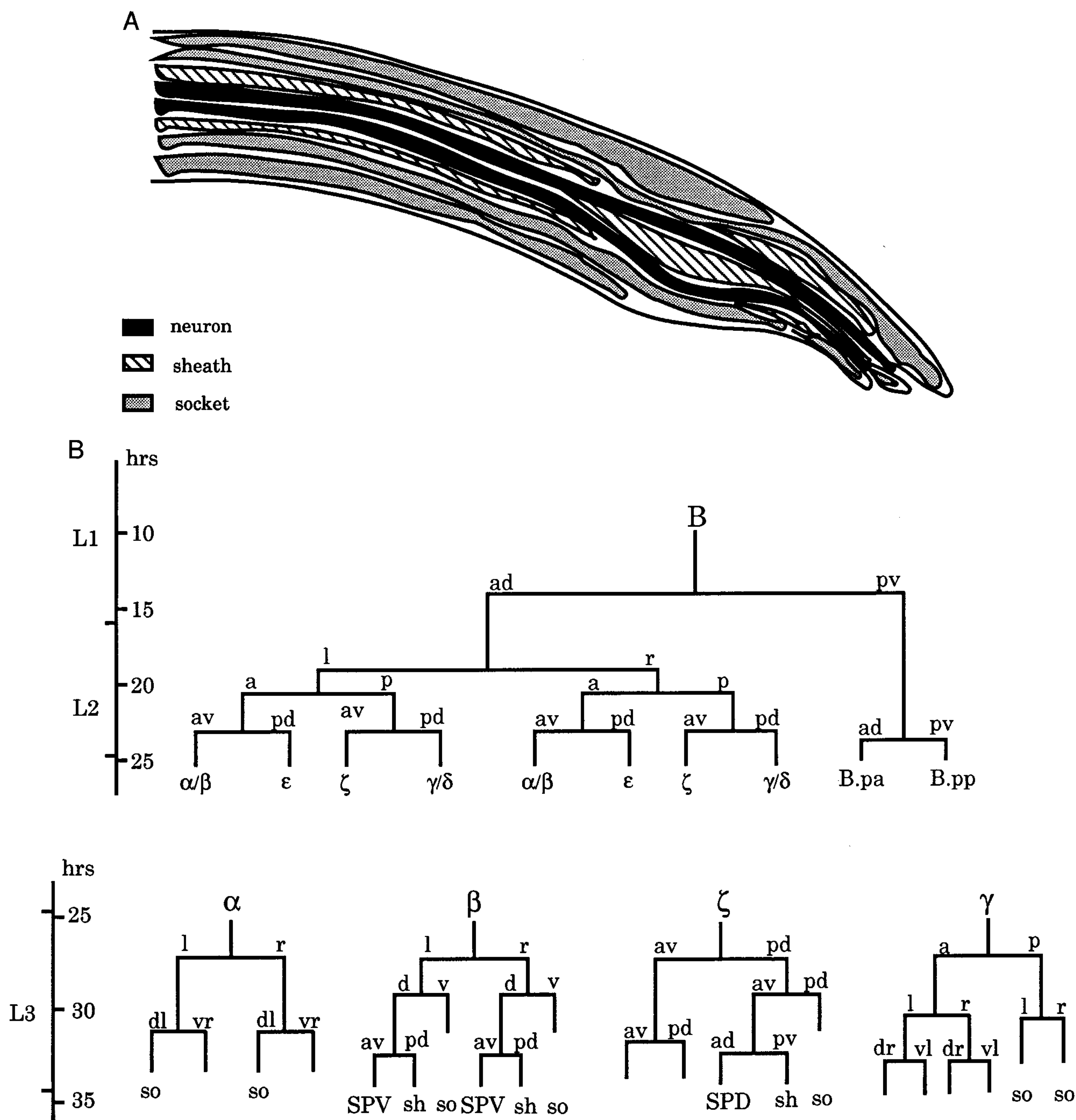

FIG. 1. The composition of *C. elegans* male spicule and the spicule lineage. (A) Diagram of longitudinal section of a male spicule (adapted from Sulston *et al.,* 1980). Each spicule is composed of two neurons, two sheath cells, and four socket cells. Only cell processes are shown in this view. The sheath cells and the socket cells have begun to fuse. Solid line indicates neurons, slashed area indicates sheath cells, gray area indicates socket cells. (B) The male B lineage (Sulston *et al.,* 1977; Chamberlin and Sternberg, 1993). The B cell generates 10 progeny at the end of the L2 stage. The 10 cells continue to divide to generate spicule neurons, sheath cells, socket cells, and proctodeal cells. The spicule neurons and sheath cells are derived from the β and ζ lineages; the socket cells are generated by α, β, γ, and ζ lineages. sh denotes sheath cell, so denotes socket cell.

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cells, socket cells, and connective tissues. All the spicule neurons and sheath cells are derived from the β and ζ sublineages. The socket cells are generated by α, β, ζ, and γ sublineages, one socket cell from each lineage. Other sublineages give rise to part of the proctodeum, the junction between the spicules and the other structures in the tail region (i.e., alimentary tract, genital tract, sex muscles, etc.). The B.p cell generates the gubernaculum structure.

All the cells are born by the end of L3 stage, yet male tail morphogenesis does not start until mid-L4 stage. During mid-L4 stage to L4 molt, the male tail undergoes extensive reshaping and morphological changes. The tip of the tail retracts, cells in the tail region move anterodorsally, and connections between the alimentary tract, the gonad, and the spicules are made. Spicule morphogenesis occurs as spicule retractor and protractor muscles connect to each spicule and a layer of sclerotized refractile spicule cuticle is laid down outside of each spicule. Gubernaculum morphogenesis presumably occurs in a similar way.

Studies by Sulston *et al.* (1980) have suggested that the connection between male sex muscles and the proctodeal cells is essential for the anterodorsal elongation of the proctodeum during male tail reshaping at the late L4 stage. Ablation of muscle precursor cells results in a compressed proctodeum containing crumpled spicules and gubernaculum (Sulston *et al.,* 1980). Electron micrographs reveal that the proctodeal cells B.a(l/r)appv form specialized contacts with the dorsal spicule retractors and protractors (Sulston *et al.,* 1980). If these contacts are significant, the ϵ(B.a(l/r)ap) progeny B.a(l/r)appv may contribute to proctodeum reshaping. However, it is not clear which spicule cells are important for spicule morphogenesis.

To study spicule morphogenesis, we first dissected the contributions of each type of spicule cell to spicule morphogenesis. We found that the spicule neurons and sheath cells are dispensable for spicule elongation, but the spicule socket cells are essential for spicule morphogenesis. Ablation of all four socket cells in each spicule completely removes the spicule structure. The socket cells are required for two aspects of spicule morphogenesis: spicule elongation and formation of spicule cuticle. The four socket cells of each spicule are partially redundant for these two functions. Seeking a socket cell marker, we found that *egl-17::GFP* is expressed in the spicule socket cells specifically during spicule morphogenesis and its expression pattern appears to be regulated between the socket cells. Using this marker we analyzed three types of mutants. Our results show that the socket cells are not only necessary but also sufficient to secrete spicule cuticle. Spicules specifically missing the spicule cuticle but otherwise wild type can be formed. Thus, the two functions of spicule socket cells are genetically separable. Furthermore, failure of socket cell movement results in crumpled spicules in mutants defective in TGF-β signaling. Thus socket cell movement during spicule morphogenesis requires the TGF-β signaling pathway.

MATERIAL AND METHODS

Strains and Alleles

Routine culturing, maintenance, and genetic manipulations of *C. elegans* strains were performed according to standard procedures (Brenner, 1974). N2 (Bristol) strain was used as wild type (Brenner, 1974). The following strains were used and are referenced in Riddle *et al.* (1997) or as indicated. LG I: *ayIs4[egl-17::GFP+dpy-20(+)]* (Burdine *et al.,* 1998). LG II: *sma-6(e1482).* LG III: *lin-48(sy548)* (this study), *sma-2(e502), sma-3(e491), sma-4(e729), dpy-17(e164).* LG IV: *dpy-20(e1282), dpy-20(e1362).* LG V: *him-5(e1490), syIs20[gpa-1::lacZ+dpy-20(+)]* (J. Mendel and P.W.S., unpublished). LG X: *sy170* (K. Liu, Y. Hajdu-Cronin, H. M. Chamberlin, W. Boorstein, and P.W.S., unpublished).

Anatomy and Cell Ablation

Animal anatomy and cell division patterns were examined under Nomarski optics at 20°C as described by Sulston and Horvitz (1977). For laser ablation, L3 males were mounted on a 5% agar pad and anesthetized with 1–3% sodium azide. Laser killing of cells was performed as described (Sulston and White, 1980; Avery and Horvitz, 1987, 1989). Worms were then recovered from the agar pad and allowed to grow to adulthood.

GFP Expression and Photographs

GFP expression was examined using Chroma High Q GFP LP filter set (450-nm excitation/505-nm emission). Photographs were taken with Fuji Provia ASA 400 film.

RESULTS

Socket Cells Are Essential for Spicule Morphogenesis

Each *C. elegans* male has a pair of spicules; each spicule is composed of two neurons, two sheath cells, and four

FIG. 2. The socket cells and the B.paa cell are responsible for spicule morphology and gubernaculum morphology, respectively. (A) Wild-type male tail, dorsal view. (B) Elimination of all spicule neurons and sheath cells does not affect spicule morphology. All the spicule neurons and sheath cells were ablated in this animal, lateral view showing the left spicule. (C) Ablation of socket cells eliminates spicule structure. All four socket cells on the right side were ablated at early L4 stage, and the right spicule structure is not formed. The left side remained intact, and a wild-type spicule structure is formed (subdorsal view). (D) Ablation of the B.paa cell eliminates the gubernaculum while the spicules remain intact (dorsal view, the left spicule is intact and is out of focus). Arrow indicates spicules, arrowhead indicates the gubernaculum structure. Scale bar indicates 20 μm.

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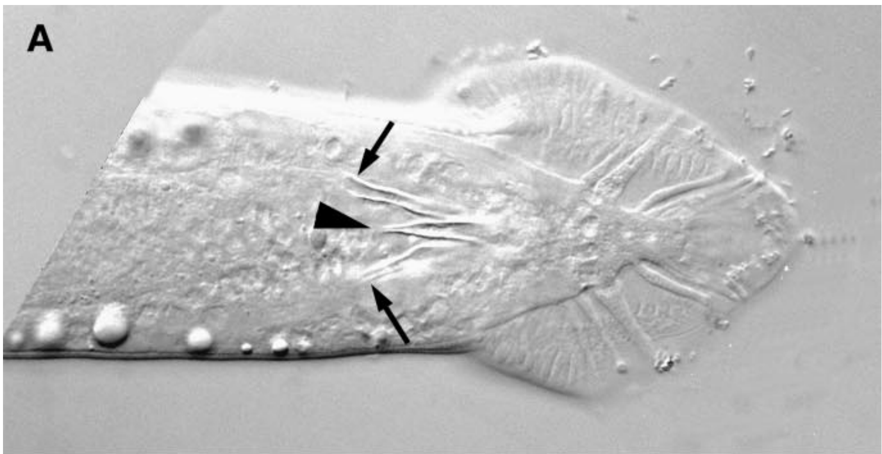
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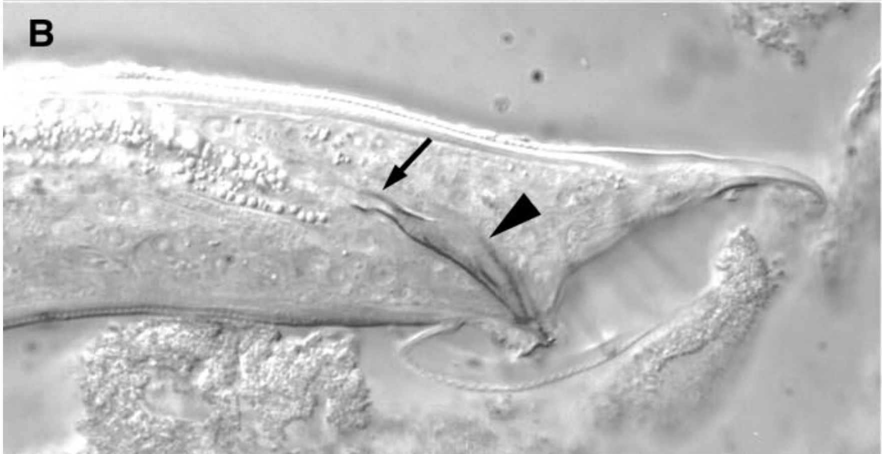
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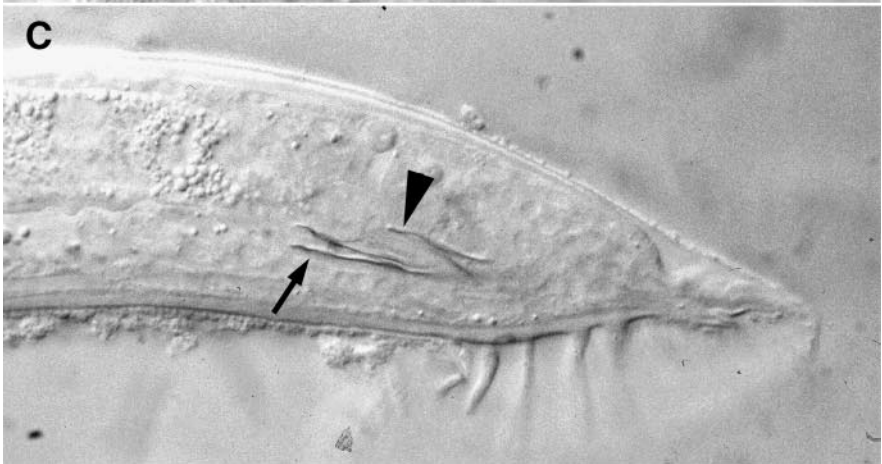
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socket cells (Fig. 2A). Previous studies have shown that each spicule neuron has a specific function during male mating behavior (Liu and Sternberg, 1995). However, we found that elimination of all spicule neurons and spicule sheath cells by cell ablation does not affect spicule morphology (Fig. 2B). Since the male sex muscles have to attach to spicules to mediate spicule protraction and retraction during male mating behavior, and the socket cells form the outer layer of the spicules, we hypothesized that the socket cells might be essential for spicule morphology. We tested this hypothesis by a series of cell ablation experiments.

We ablated the four socket cells on one side at the end of L3 lethargus while leaving the other side intact. When the males grew to adulthood, we found that only one elongated spicule is formed on the intact side (left) and no spicule structure or spicule cuticle material is formed on the socket cell-ablated side (right) ($n = 4$, Fig. 2C). Therefore, the socket cells are essential for spicule morphology including both formation of spicule cuticle and spicule elongation. We noticed that on the socket cell-ablated side the proctodeal cells are apparently posterior to their normal positions. Elimination of the socket cells thus also affects the overall proctodeum elongation to some extent. Sulston *et al.* (1980) suggested that the contact between the B.a(l/r)appv cells and sex muscles might be important for spicule elongation. When we ablated the precursors of B.a(l/r)appv, the B.a(l/r)ap cells, in the early L3 stage, we found that the spicule structure formed might be slightly shorter than wild type but is otherwise wild type ($n = 5$). Ablation of the δ cells does not affect the spicule morphology ($n = 5$).

Each of the four socket cells is derived from one of four different sublineages: α, β, γ, and ζ. Elimination of a single precursor at the late 10-cell stage does not affect overall spicule morphology. We conclude that the four socket cells have redundant functions. To further address this question, we ablated two or three socket cells for each spicule. Elimination of two or more socket cells results in short and crumpled spicules. However, as long as a single socket cell is present, the sclerotized refractile cuticle material can be generated. Therefore, the four socket cells of each spicule appear to have overlapping and partially redundant functions for spicule elongation and formation of spicule cuticle. In intact animals, all socket cells fuse together and thus all contribute to spicule morphology.

The gubernaculum structure, which lies posterior to the spicules and is thought to play a guidance role for spicule movement during mating behavior, is also covered by a layer of sclerotized cuticle. Transverse section of the male tail region revealed by electron micrographs suggests that the B.paa cell may be responsible for secreting the gubernaculum cuticle (Sulston *et al.,* 1980). We confirmed this by a cell ablation experiment. Ablation of the B.paa cell is able to remove the gubernaculum structure while the spicules remain intact ($n = 5$, Fig. 2D).

egl-17::GFP Is Expressed in Socket Cells

During the mid-L4 to L4 lethargus stage, the male tail undergoes extensive reshaping and cells in the male tail region move extensively and change their morphologies. It is difficult to follow the cell movement and to judge cell fate based on their relative nuclear positions and morphologies of spicule cells. We therefore sought cell fate-specific markers expressed in the male tail region. We have found that *egl-17::GFP* is a marker for the spicule socket cells. *egl-17* encodes a fibroblast growth factor (FGF) and plays an important role for sex myoblast migration in both hermaphrodites and males (Stern and Horvitz, 1991; Burdine *et al.,* 1997, 1998) although no spicule defect is present in *egl-17* mutant males. In males, we found that *egl-17::GFP* expression is turned on in several cells of the B lineage and the Y.p progeny Y.p(l/r)aa during L3 lethargus. The cells from the B lineage appear to be two of the socket cells, one on each side (likely α(l/r)d), B.paa, and B.pppa) (Fig. 3A). These *egl-17::GFP*-expressing cells move anterodorsally during male tail reshaping. The movement of the socket cells is especially dramatic and the distance traveled is about 20–25 μm. At the end of L4 lethargus, *egl-17::GFP* expression can be detected in a cluster of socket cells per spicule (Fig. 3B). At this stage the socket cells have begun to fuse with each other. Shortly after the male reaches adulthood, *egl-17::GFP* expression remains on only in the B.paa cell (Fig. 3C).

To confirm this expression pattern, we performed cell ablation experiments. Ablation of the B.pa or B.paa cell eliminates the expression in the B.paa cell ($n = 5$). When β and ζ cells are ablated in the early L3 stage, which eliminates the inner socket cells, the expression pattern is similar to that of an intact animal ($n = 10$; Fig. 3D). Interestingly, when α and γ cells are ablated, thereby eliminating the outer socket cells, *egl-17::GFP* is still expressed in two cells in the spicule region ($n = 10$; Fig. 3E). The position of the cells is ventral to that of those in intact animals, and we infer that those cells are the socket cells

FIG. 3. *egl-17::GFP* expression pattern in the male tail. (A) At the early L4 stage, *egl-17::GFP* is expressed in α(l/r)d cells, Y.p(l/r)aa cells, B.paa cell, and B.pppa cell. so denotes socket cells; here it points to an α(l/r)d cell. (B) At the end of L4 stage, *egl-17::GFP* is expressed in several socket cells that are clustered together. (C) In adult males, *egl-17::GFP* remains on in the B.paa cell. spic and gub denote spicules and gubernaculum, respectively. These structures show autofluorescence associated with the sclerotized refractile cuticle. (D and E) Socket cell position at early L4 stage. (F and G) Socket cell position at the end of L4 stage. (H) When β and ζ cells were ablated at B lineage 10-cell stage, *egl-17::GFP* expression is similar to that of intact animals. (I) When α and γ cells were ablated, *egl-17::GFP* is expressed in the socket cells derived from β lineage, β(l/r)v. (D–I) Arrowhead indicates socket cell. Scale bar indicates 20 μm.

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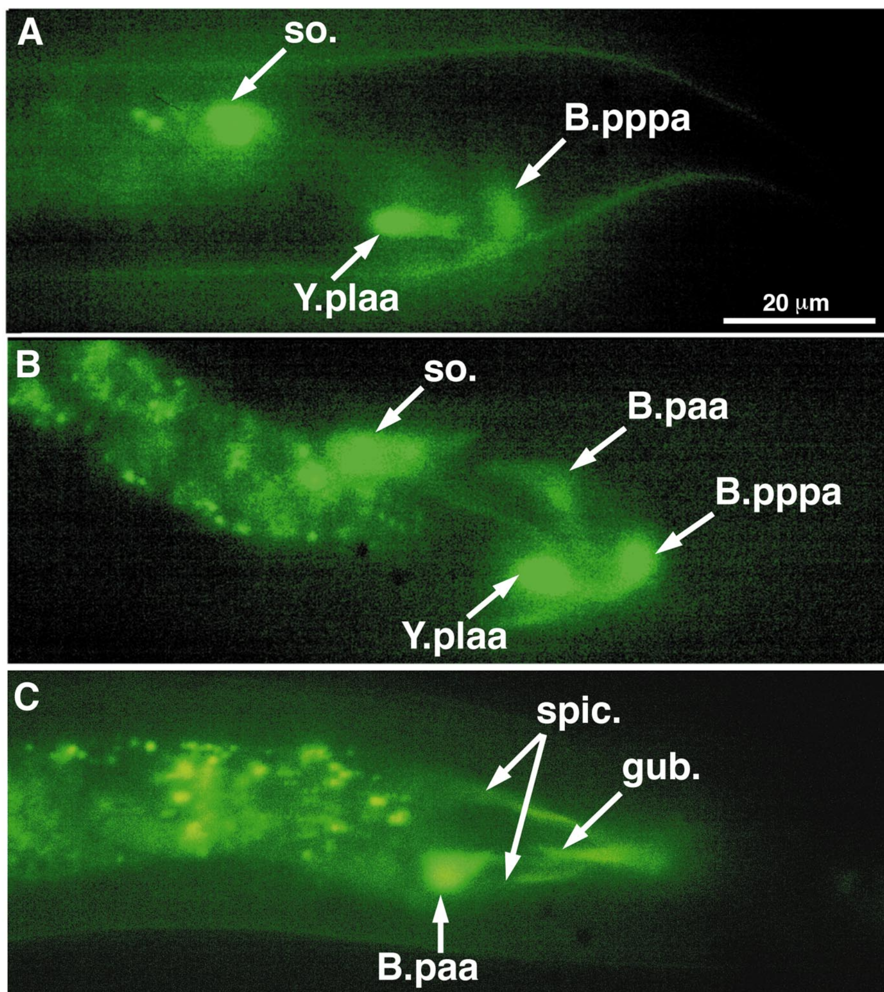
A
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B.pppa
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so.
B.paa
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C
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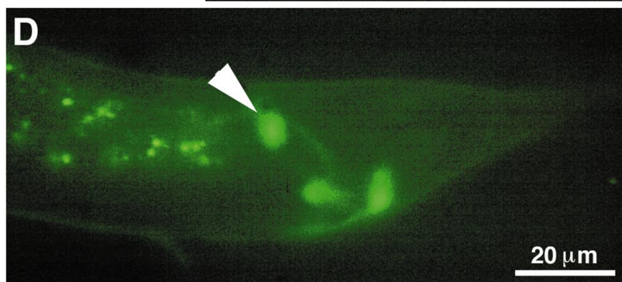
B.paa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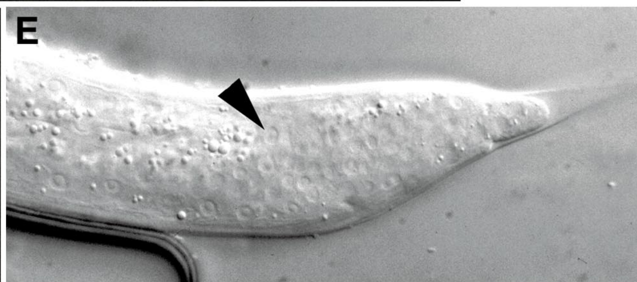
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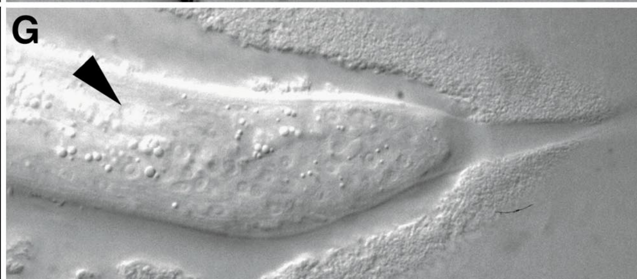
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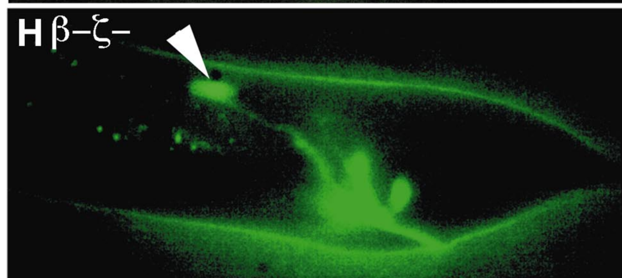
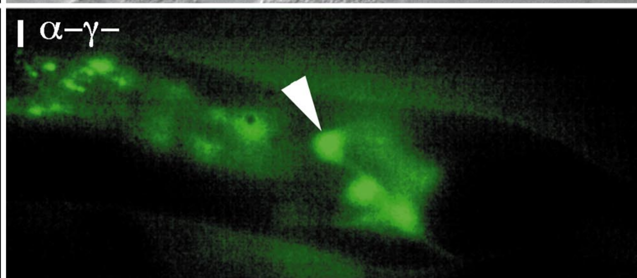

H β–ζ–


I α–γ–

derived from the β lineage. If α, γ, and β cells are ablated in the early L3 stage, two cells in the spicule region are still able to express the *egl-17::GFP* marker although the expression is less intense ($n = 8$). Based on the cell positions and nuclear morphologies, we think those cells are the socket cells derived from the ζ lineages. Only when all the socket cells are ablated is the expression eliminated ($n = 6$). Thus, the socket cells seem to be able to communicate with each other to regulate *egl-17::GFP* expression. *egl-17::GFP* appears to be usually expressed in the most anterior socket cell while inhibited in the more posterior socket cells. When the most anterior socket cell is eliminated, its expression can be turned on in the next most anterior socket cell. The regulation of *egl-17::GFP* expression is consistent with our observation that the socket cells have partially redundant function.

Ectopic Socket Cells Produce Ectopic Spicule Cuticle Material in lin-48 Mutants

In a genetic screen for males with abnormal spicules, we recovered a mutant with an ectopic spicule phenotype. This mutation, *sy548,* is an allele of *lin-48* (Chamberlin, 1994; H. M. Chamberlin, K. B. Brown, P. W. Sternberg, and J. H. Thomas, in preparation). In *lin-48(sy548)* mutant males, a pair of crumpled spicules is formed at the normal position. In addition, an ectopic spicule-like structure is formed anterior–ventral to the crumpled spicules (Fig. 4A). The lineage defect of this mutant will be described elsewhere (H. M. Chamberlin, K. B. Brown, P. W. Sternberg, and J. H. Thomas, in preparation). To determine the composition of this ectopic spicule structure, we examined the expression patterns of markers for two different cell types. We first examined the expression pattern of the socket marker *egl-17::GFP* in *lin-48(sy548)* mutant animals. Ectopic marker-expressing cells are observed in the preanal region (Fig. 4B). Ablation of the left/right pair of Ul/r cells in the early L3 stage is able to eliminate the ectopic marker-expressing cells and the ectopically formed spicule structure ($n = 5$). We also examined the expression pattern of a spicule neuron-specific marker, *gpa-1::lacZ* (J. Mendel and P.W.S., unpublished; Jiang and Sternberg, 1999). The *gpa-1::lacZ* marker is normally expressed in the spicule SPD neurons and phasmid neurons in the tail region. In *lin-48* mutant males, the marker expression is not observed in the ectopic spicule region (Fig. 4A, $n = 20$). The *lin-48* mutant also has defect in B cell progeny. In ~50% of the animals the *gpa-1::lacZ* marker fails to be expressed in SPD of the normally positioned crumpled spicules ($n = 50$). These results suggest that ectopic spicule socket cells are sufficient to produce spicule cuticle and result in a spicule-like structure.

sy170 Mutant Males Lack Spicule Cuticle but Have Elongated Wild-Type Spicules

sy170 was originally recovered from a screen for copulation-defective mutants (K. Liu, Y. Hajdu-Cronin, H. M. Chamberlin, W. Boorstein, and P.W.S., unpublished). This mutant has normally elongated spicules but the sclerotized refractile cuticle material appears to be missing (Fig. 4C). The mutant spicules are transparent and flaccid. Mutant males cannot mate. The gubernaculum structure also lacks the sclerotized cuticle in *sy170* mutant males. But *sy170* mutant animals do have wild-type larval and adult cuticle. All-lineage analysis does not reveal any cell division abnormality (H. M. Chamberlin, L.I.J., and P.W.S., unpublished). We examined both neuronal marker and socket marker expression in this mutant. The neuronal marker, *gpa-1::lacZ,* is detected in both the cell bodies and the neuronal dendrites through the spicules, indicating that the spicule neurons are fully differentiated (Fig. 4C). Thus, the lack of spicule cuticle in *sy170* mutants is not due to the cells lacking adulthood characteristics. Expression of the socket marker *egl-17::GFP* is normal in *sy170* mutant males (Fig. 4D). The presence of socket cells would explain the wild-type-shaped spicule structure. Taken together, we conclude that the *sy170* mutant is specifically defective in making the sclerotized refractile cuticle material, including both the spicule cuticle and the gubernaculum cuticle. As socket cells are responsible for formation of spicule cuticle, and *sy170* mutant males do have socket cells and elongated spicules, the socket cell function for formation of spicule cuticle is uncoupled from socket cell fate specification as well as from its function for mediating spicule elongation.

Socket Cells Fail to Move Anterodorsally in Mutants Defective in TGF-β Signaling

The *sma* class mutants were isolated based on their small body size (Brenner, 1974). This class of mutants also has defects in patterning of the rays of the male tail (Baird *et al.,* 1991). Molecular and genetic studies have shown that several *sma* mutants define components of the TGF-β signaling pathway. *sma-2, sma-3,* and *sma-4* encode SMAD family proteins (Savage *et al.,* 1996). *sma-6* encodes a type I TGF-β receptor (Krishna *et al.,* 1999). In addition to the small body size and ray defects, these *sma* mutants also share a crumpled spicule phenotype (Savage *et al.,* 1996; Krishna *et al.,* 1999). We wondered if the crumpled spicule phenotype is a result of failure of socket cell movement during male tail reshaping.

Using the *egl-17::GFP* marker we examined socket cell positions in several *sma* mutants. As a control, we first examined marker expression in *dpy-17(e164)* mutant males. *dpy-17* mutants have a small body size that is comparable to that of the *sma* mutants. However, in contrast to the *sma* mutants, *dpy-17* mutant males have wild-type spicules. As shown in Figs. 5A–5D, socket cells in *dpy-17* mutant males travel anterodorsally through a substantial distance during male tail reshaping at the end of L4 stage ($\Delta \approx 20$–25 μm, comparable to wild type). In *sma-3* and *sma-4* mutants, the socket cells fail to move anterodorsally even though they still express the *egl-17::GFP* marker (Figs. 5E–5L). At the end of L4 stage, the socket cells still

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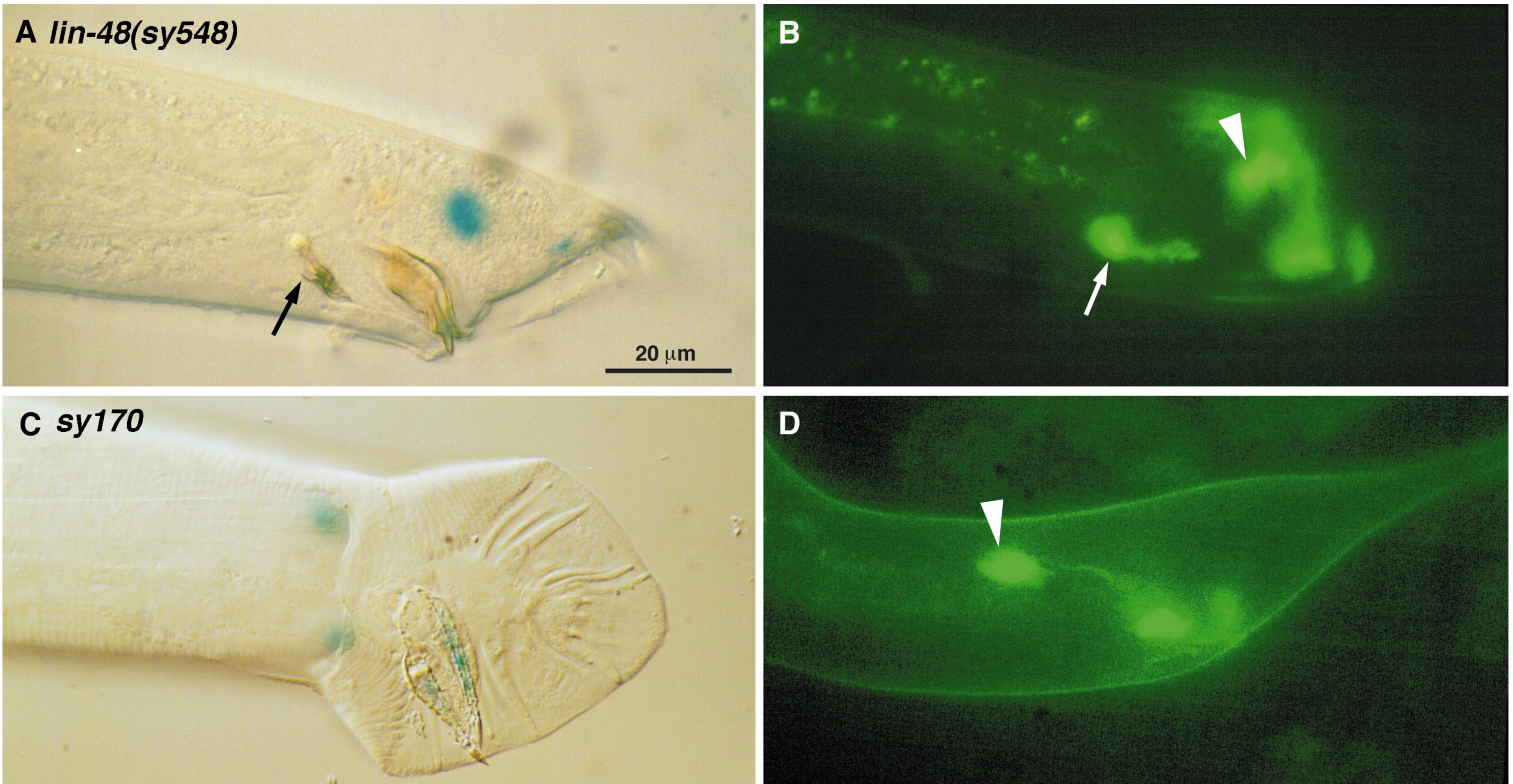
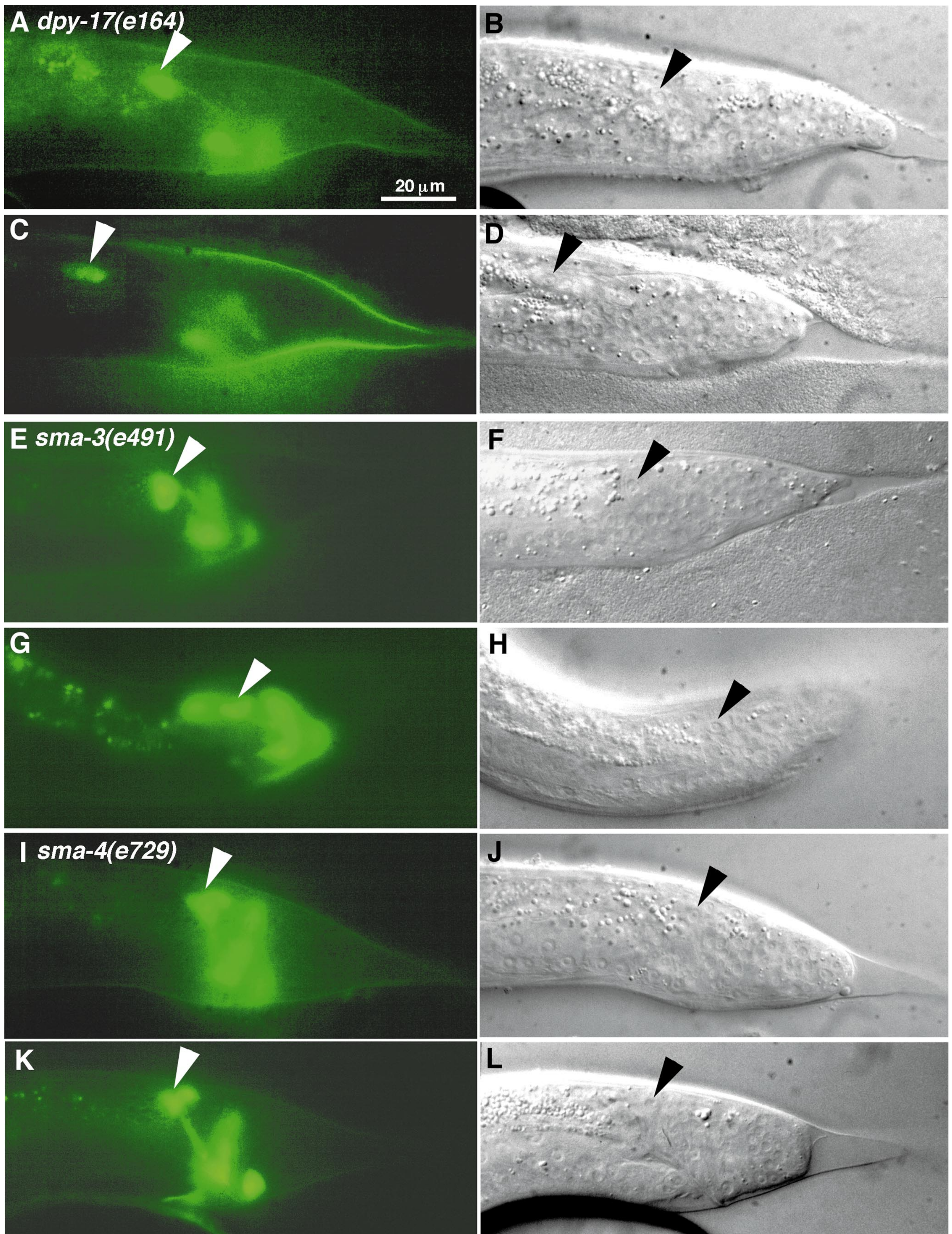


FIG. 4. Phenotypes and marker expression patterns in lin-48(sy548) and sy170 mutants. (A) gpa-1::lacZ expression in lin-48(sy548) mutant males. Arrow indicates ectopic spicule-like structure. (B) egl-17::GFP expression in lin-48(sy548) mutant males. Arrow indicates ectopic socket cell, arrowhead indicates the normal socket cell. (C) gpa-1::lacZ expression in sy170 mutant. Adult males have transparent spicules that lack spicule cuticle. Marker expression is detected in both neuron cell bodies and dendrites through the spicules. (D) egl-17::GFP expression in sy170 mutant. Arrowhead indicates socket cells. Scale bar indicates 20 μm.


A dpy-17(e164)
20 μm
B
C
D
E sma-3(e491)
F
G
H
I sma-4(e729)
J
K
L

remain at the same positions as at early L4 stage ($\Delta < 5$ μm). *sma-2* and *sma-6* mutants display a similar defect. We believe that the failure of socket cell movement is the cause of the crumpled spicule phenotype observed in the *sma* mutants. As the *sma* genes represent components of a TGF-β signaling pathway, we speculate that the TGF-β signaling pathway is involved in spicule elongation during spicule morphogenesis.

DISCUSSION

The Socket Cells Are Essential for Formation of Spicule Cuticle and Spicule Elongation during Spicule Morphogenesis

The male tail reshaping at the L4 stage is one of the most complex morphogenic events that occurs during *C. elegans* development. Similar to morphogenesis and organogenesis in other organisms, extensive cellular behaviors occur, including cell movement, cell contact, cell fusion, and cell reshaping (Sulston *et al.,* 1980; Baird *et al.,* 1991). Using cell ablation techniques, molecular markers specific for cell fates, and genetic mutants, we have begun to elucidate the process of spicule morphogenesis in *C. elegans* males. We found that the socket cells are essential for two aspects of spicule morphogenesis: formation of spicule cuticle and spicule elongation. The four socket cells of each spicule are partially redundant for these functions. In contrast, the spicule neurons and sheath cells are dispensable for spicule morphogenesis. Similar results have been obtained for the generation of other sensory structures. In *Drosophila* bristle development, the shaft cell is both necessary and sufficient to generate the bristle structure (reviewed in Jan and Jan, 1994). Loss of shaft cells results in loss of bristles phenotype, while excessive shaft cells at the expense of neurons are able to generate more bristles. In *C. elegans* male sensory ray development, elimination of a ray structural cell eliminates the corresponding ray structure (Sulston and Horvitz, 1977). Therefore, generation of a sensory structure requires the structural cells rather than the neurons.

For the formation of spicule cuticle, the socket cells are both necessary and sufficient. We think the socket cells act cell-autonomously to produce spicule cuticle. First, ectopic socket cells result in ectopic deposition of spicule cuticle material in *lin-48* mutants. Second, only ablation of all the spicule socket cells is able to eliminate the spicule cuticle. Ablation of combinations of other cells cannot eliminate the sclerotized refractile cuticle material. Moreover, the existence of the *sy170* mutant suggests that formation of complete spicule cuticle is genetically separable from spicule elongation. Elongated spicules that lack detectable spicule cuticle are formed in *sy170* mutant males. The *sy170* mutant may define a component for spicule cuticle synthesis that is regulated by socket fate-specific genes.

The Dynamic Expression Pattern of egl-17::GFP Implicates the egl-17/FGF Signaling Pathway in Spicule Morphogenesis

Spicule elongation requires coordinated behavior of several cell types. The sex-specific muscles, the spicule socket cells, and the proctodeal cells all contribute to spicule elongation and proctodeum elongation during male tail reshaping. Communication between the sex muscles and the spicule socket cells is important to mediate the connection of these two cell types which leads to spicule elongation.

We observed a dynamic expression pattern of *egl-17::GFP* during male spicule morphogenesis. This dynamic expression pattern might suggest that the *egl-17*-mediated FGF signaling pathway plays a role in mediating sex muscle connection to the spicules. First, *egl-17::GFP* is expressed at the right time during male tail reshaping. Its expression in the male tail region is turned on right before male tail reshaping and turned off shortly after males reach adulthood. Second, *egl-17::GFP* is expressed in the right cells to mediate sex muscle connection to the proctodeum. The socket cells and B.paa cell are essential for the morphogenesis of the spicules and the gubernaculum, respectively. *egl-17::GFP* is expressed in both cell types. As the contact between male sex muscles and socket cells is important for proctodeum morphogenesis, it is possible that the socket cells use the FGF signal EGL-17 to attract male sex muscle positioning and attachment to the proctodeum. However, *egl-17* mutant males have wild-type spicules and can mate well. Therefore redundant signaling pathways must be used to control male tail morphogenesis.

In hermaphrodites, *egl-17::GFP* is found to be expressed in vulval cells and this expression pattern has suggested a role of the *egl-17*-mediated FGF signaling pathway in mediating the precise positioning of sex myoblasts and coordinating sex muscle attachment to vulval cells in the development of a functional egg-laying system (Burdine *et al.,* 1997, 1998). The expression pattern of *egl-17::GFP* in males suggests a similar function of the FGF signaling pathway and supports this hypothesis.

FIG. 5. *egl-17::GFP* expression in *sma* mutants. (A–D) *dpy-17(e164).* (E–H) *sma-3(e491).* (I–L) *sma-4(e729).* Left is GFP expression image, right is Nomarski image of the same animal. For each mutant, top shows the expression pattern at early L4 stage, bottom shows the expression pattern at late L4 stage. The distance traveled by the socket cells was measured as the difference in the distances between the socket cells and the Y.plaa cell at the early and late L4 stages. As the body size of these mutants is comparable, the travel distances of the socket cells are compared directly. Arrowhead indicates socket cells. Scale bar indicates 20 μm.

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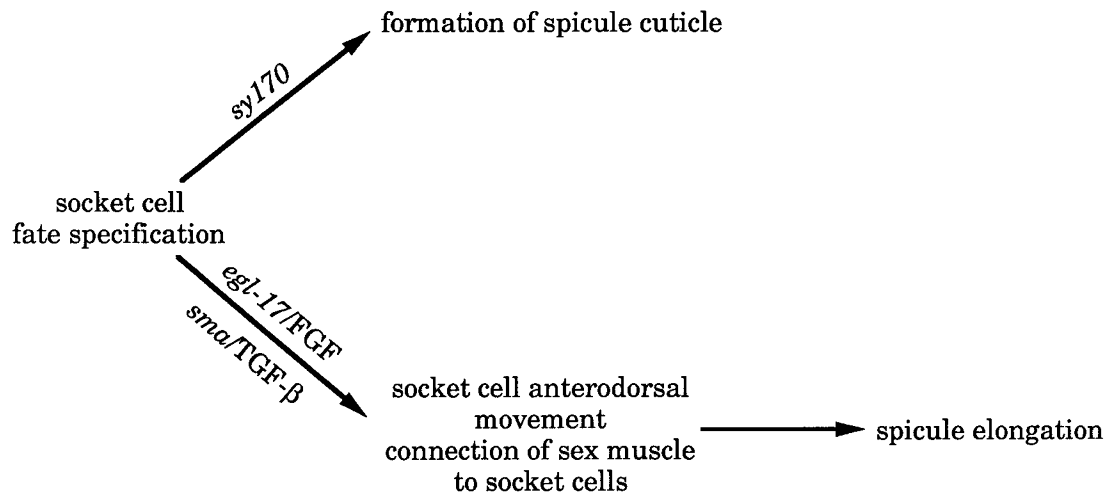

FIG. 6. Developmental pathway of spicule morphogenesis. Specification of the socket cell fate activates two parallel developmental pathways. One pathway controls the formation of spicule cuticle. The other pathway mediates spicule elongation by regulating the anterodorsal movement of the socket cells and the connection between the sex muscles and the socket cells. The gene defined by the *sy170* locus is involved in the former pathway. The FGF and TGF-β signaling pathways are involved in the latter pathway.

The TGF-β Pathway Is Involved in Spicule Elongation

Mutants that disrupt the TGF-β signaling display a crumpled spicule phenotype. Examination of *egl-17::GFP* expression pattern revealed that the spicule socket cells fail to move anterodorsally during male tail reshaping in those mutants. Therefore, the TGF-β signaling pathway may mediate socket cell movement during spicule elongation. Both the sex muscles and the spicule socket cells are required for spicule elongation. However, we do not know whether the connection between the sex muscles and the socket cells occurs before or after the anterodorsal movement of the socket cells. Analyses of the expression patterns of the TGF-β pathway components and mosaic analyses would be necessary to address the site of action of the TGF-β pathway and how it functions in spicule morphogenesis.

Developmental Pathway for Spicule Morphogenesis

We propose the following developmental pathway for spicule morphogenesis in *C. elegans* males (Fig. 6). Specification of socket cell fate turns on socket cell-specific genes. The socket cell-specific genes activate two morphogenetic pathways. One pathway regulates the formation of spicule cuticle. The gene defined by the *sy170* locus is involved in this pathway. The other pathway controls the anterodorsal movement of the socket cells and the connection between sex muscles and the socket cells which leads to spicule elongation. The EGL-17/FGF and SMA/TGF-β signaling pathways may be involved in this process. The same developmental pathway applies to the formation of the gubernaculum structure.

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