
Cell fusion*

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Abstract

Selective cell fusion is a natural part of development. It is found in sexually reproducing organisms that require fertilization to propagate and in muscles, placenta, bones, lens of the eye and stem cells. Cell fusion is particularly important in the development of *C. elegans*: in addition to 300 sperm and oocytes that fuse

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during fertilization, 300 of the 1090 somatic cells born, fuse throughout development. Studies of cell fusion in *C. elegans* have shown that although different types of cells fuse, cell membrane merger is initiated through a common mechanism involving the action of one gene, *eff-1*. In worms with mutations that inactivate *eff-1*, almost none of the 300 somatic cells that normally fuse do so, but appear to differentiate, attach and behave in the same way as fusing cells. Such worms develop and survive but have numerous morphological, behavioral and fertility defects associated to cell fusion failure in the epidermis, pharynx, male tail, vulva and uterus. Cell fusion in embryonic dorsal epithelial cells has been analyzed in great detail by confocal microscopy using membrane fluorescent probes, apical junction markers and cytoplasmic aqueous fluorescent probes allowing the direct observation of membrane disappearance, pore expansion and cytoplasmic content mixing. The complete elimination of the membranes between two fusing cells takes about 30 min and involves vesiculation of the fusing membranes. Genetic and cell biological evidence indicates that *eff-1* activity is both necessary and sufficient to fuse epithelial and myoepithelial cells *in vivo*. Based on electron microscopic analyses of intermediates of cell fusion in *eff-1* mutants, it appears that *eff-1* is required for both initiation and expansion of fusion pores, similar to the fusogen of Influenza virus. While only one gene encoding a novel candidate component of the cell membrane fusion machinery has been found, the nematode's cell fusion program is under the control of many cell-specific transcriptional regulators. A large number of these conserved regulators prevent cell fusion by repressing *eff-1* activity. For example, if either *ceh-16/engrailed* or the GATA factor *EGL-18/ELT-5* is inactivated, the lateral epidermal cells that normally do not fuse in the embryo will fuse causing embryonic lethality. And if either the Hox protein *lin-39/Deformed* or its cofactor *ceh-20/Extradenticle* is inactivated, the ventral epidermal vulval precursor cells that normally do not fuse in the larvae will fuse and the hermaphrodite will have no vulva. In addition, there is evidence for coordinated and complex regulation of *lin-39* in the ventral epidermis by Ras, Wnt, Rb/E2F, NuRD and *lin-15* pathways. It appears that in many cells that normally do not fuse, specific transcription complexes repress *eff-1* expression preventing cell fusion. *ref-2* (REgulator of Fusion-2) encodes a Zn-finger protein that is required to generate ventral Pn.p cells and to keep them unfused both in males and hermaphrodites. *ref-2* is necessary, but not sufficient, to maintain Pn.p cells unfused. This review shows that far from cell fusion being an unusual phenomenon, there is the clear prospect that animal cells in all tissues are intrinsically programmed to fuse, and are only prevented from fusing by transcriptional and post-transcriptional control mechanisms. There are three major questions that remain open for future research: (1) How does *eff-1* fuse cells? (2) How do Ras, Wnt, Rb, NuRD, E2F, heterochronic and other pathways control cell fusion? (3) What are the implications of cell fusion beyond worms?

1. Introduction

1.1. Ubiquitous cell fusion

Cell fusion is found in many sexually reproducing organisms that require fertilization or mating to propagate (Heiman and Walter, 2000; Primakoff and Myles, 2002; Shur et al., 2004; Singson et al., 1998; Stein et al., 2004; Trueheart and Fink, 1989; Wasserman et al., 2001; Xu and Sternberg, 2003). Diverse organisms form multinucleate cells in organs such as muscles, placenta, bones, lens of the eye and stem cells, although most cells do not fuse during development. (Baroux et al., 2004; Chen and Olson, 2004 ; Cross et al., 1994; Hickey et al., 2002; Jee and Nolan, 1963; Kuszak et al., 1985; Terada et al., 2002; Xiang et al., 2002; Ying et al., 2002). In addition, cell invasive behaviors involve fusion between cells, such as viral induced cell fusion and anchor cell invasion and fusion in *C. elegans* (Earp et al., 2005; Newman et al., 1996; Sharma-Kishore et al., 1999). Little is known about the mechanisms of cell fusion. In *C. elegans*, some of the components of the machinery that brings about and regulates cell fusion have been identified. While many cell-specific transcriptional regulators of cell fusion have been characterized, to date, only one gene encoding a candidate component of the cell membrane fusion machinery has been identified. This chapter includes sections on transcriptional regulation of cell fusion and on a novel candidate cell fusion machinery gene.

1.2. Cell-to-cell fusion in worms

Over 30% of all somatic nuclei in *C. elegans* are contained within giant cells generated by cell fusion (Hedgecock and White, 1985; Podbilewicz and White, 1994; Sulston and Horvitz, 1977; Sulston et al., 1983; White, 1988; see Figure 1; Wormatlas, Epidermal morphogenesis, Male development and Vulval development). These cells are formed during embryonic and larval development in a genetically programmed process. Syncytogenesis culminates in complex cellular structures, such as, myoepithelial cells in the pharynx and epithelial toroids (ring shape cells) in the uterus, vulva and male tail (Figure 1; Newman et al., 1996; Nguyen et al., 1999; Sharma-Kishore et al., 1999). For reviews on cell fusion in worms see (Podbilewicz, 2000; Podbilewicz and Chernomordik, 2005;

Shemer and Podbilewicz, 2000; Shemer and Podbilewicz, 2003; Witze and Rothman, 2002). For sperm-egg fusion see chapters on Control of oocyte meiotic maturation and fertilization and Spermatogenesis.

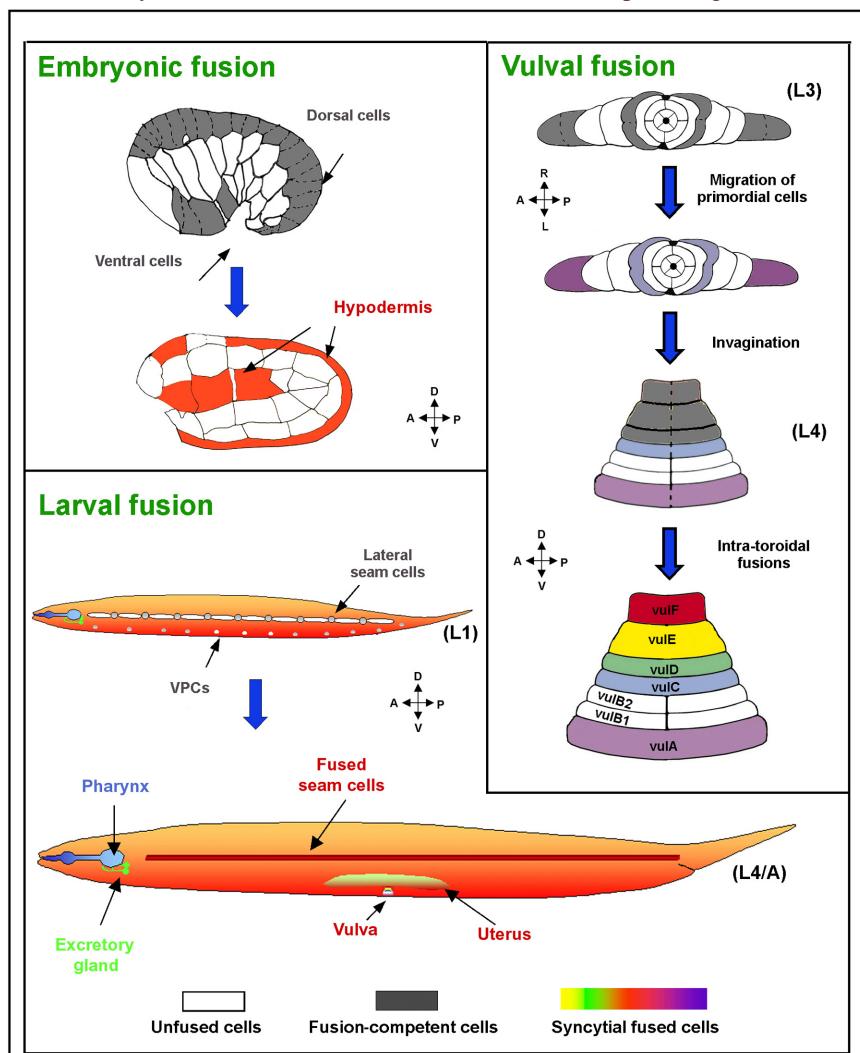


Figure 1. Cell fusion in *C. elegans*. Membranes of cells committed for fusion (gray color and dashed lines) fuse throughout development forming different syncytia (colored). White marks unfused cells. During embryonic elongation most of the dorsal cells, along with several ventral cells fuse to form the hypodermal syncytia (Podbilewicz and White, 1994). More nuclei join these syncytia during larval development as a consequence of fusion of part of the lateral hypodermal seam cells, as well as part of the ventral epidermal cells. The resulting epidermis is comprised of 8 syncytia containing 186 nuclei (not shown) and only 3 unfused cells (for details, see Wormatlas). Other organs, such as the pharynx, excretory gland, uterus and vulva also contain syncytia (Sulston and Horvitz, 1977; Sulston et al., 1983). During vulva organogenesis, cell fusion takes place between migrating cells (precursors of the vulA and vulC rings; violet and light blue, respectively), as well as between cells within the vulval rings (Sharma-Kishore et al., 1999). In total, 300 of 959 somatic nuclei in *C. elegans* are part of syncytia (Shemer and Podbilewicz, 2000). Reproduced from Shemer and Podbilewicz (2003), Copyright (2003), with permission from Wiley.

1.3. Humans and some nematodes have cellular skin but *C. elegans* has syncytia

The largest organ in worms and humans is the epidermis (hypodermis). In some marine nematodes and in vertebrates the skin and most other organs are cellular. However, in *C. elegans* and related nematodes the epidermis is composed of several distinct multinucleate cells (syncytia; Chitwood and Chitwood, 1974; Hedgecock and White, 1985; Podbilewicz and White, 1994; Sulston and Horvitz, 1977). These syncytia are generated by the fusion of mononucleate cells during development. The largest syncytium is hyp7 which is generated in the embryo during morphogenesis (Figure 1; Podbilewicz and White, 1994; Sulston et al., 1983). During larval development additional cells fuse with hyp7 (Podbilewicz and White, 1994; Yochem et al., 1998). Several cells in the ventral and lateral epidermis remain unfused, divide and generate ectodermal organs including sex-specific mating structures and sensory and motor neurons. Thus, in order for all these organs to be formed, it is essential that the pattern of cell fusions be tightly controlled. To define the spatiotemporal constraints of cell fusion in *C. elegans*, the order of

events in which epithelial cells fuse was studied (Figure 1). To study cell boundaries, apical junctions (AJs) were monitored during fusion using the MH27 monoclonal antibody that recognizes the AJM-1 protein (Francis and Waterston, 1991; Koppen et al., 2001). Using confocal microscopy and 3D reconstructions the sequence of fusions was studied (Figure 2; Podbilewicz and White, 1994). Hypodermal cell fusions start after migration of dorsal epithelial cells. Epidermal fusions usually start in the anterior dorsal epidermis and continue posteriorly. Thus, the fusions in the epidermis are not completely invariant. This is in contrast to the sequence of cell fusions in the vulva that are invariant in *C. elegans* (Sharma-Kishore et al., 1999). While in other nematode species the vulval cell fusion sequences are different than in *C. elegans*, they are invariant between animals of the same species (Kolotuev and Podbilewicz, 2004; Louvet-Vallee et al., 2003; See Figure 1 and Figure 2 for more details). The timing and location of postembryonic fusions in the epidermis, vulva, uterus and male tail have been studied in *C. elegans* and in related nematode species (Figure 2; Newman et al., 1996; Nguyen et al., 1999; Sharma-Kishore et al., 1999). These studies complement the observations of cell fusions obtained by electron microscopy and show intermediates in the sequence of cell fusions during development (Albertson and Thomson, 1976; Newman et al., 1996; Nguyen et al., 1999; Sharma-Kishore et al., 1999; Sulston et al., 1983; White, 1988). See Shemer and Podbilewicz (2000) for a detailed review of cell fusion events.

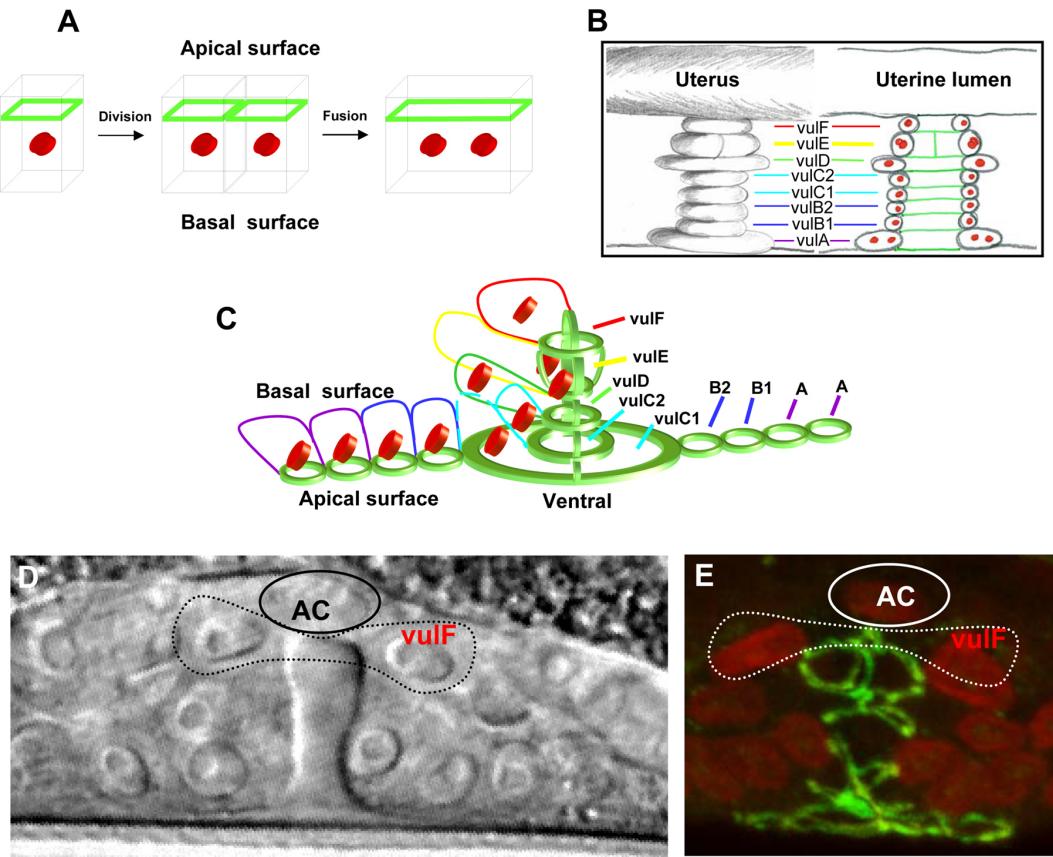
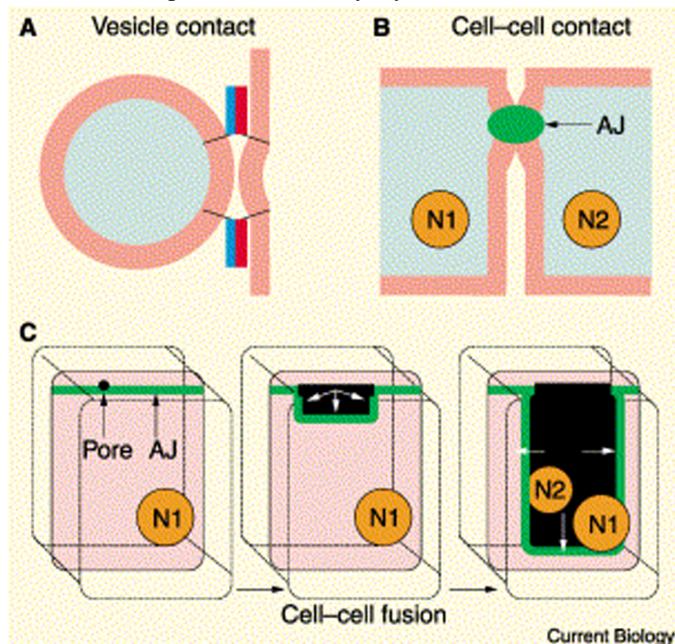


Figure 2. Changes in the morphology of apical junctions (AJ) reveal cell behavior during vulva formation. The monoclonal antibody MH27 stains AJs that represent the apical borders of epithelial cells including all vulval and hypodermal cells in *C. elegans*, *P. pacificus* and other nematodes (Baird et al., 1991; Fitch, 1997; Kolotuev and Podbilewicz, 2004; Louvet-Vallee et al., 2003). Nuclear staining is used to confirm observations concerning the junctions. Shown are negative micrographs of the junctions and schematic drawings based on detailed analysis of rotations and 3D views of the particular image. Note that the apical side of the vulva primordium is facing the ventral region of the animal and during morphogenesis the apical junctions face the lumen of the vulva. (A) Graphical representation of immunofluorescent staining in epithelial cells. Green represents the AJ belt; red cylinders represent nuclei in cells. This is a simplified model of the fusion process at the border between two neighboring cells. The disappearance of the AJ borders represents the fusion process based on EM and other studies (Podbilewicz and White, 1994; Sharma-Kishore et al., 1999) (B) Stack of vulva with 8 toroidal cells (right, VulA-VulF) and the cross section through the vulva (left). The vulva is from a late J3 stage hermaphrodite of *P. pacificus* after completion of invagination and intratoroidal fusions. Green lines represent AJs and red are nuclei. (C) A twenty cells stage vulva primordium after completion of cell proliferation but before initiation of cell fusion events in *P. pacificus*. Four toroids in the center are already formed. In the left side of the picture see the staining of AJs, the nuclei and the basolateral domains based on Nomarski images. Right side shows only the apical AJs without the nuclei and the estimated positions of the basolateral borders. (D) Nomarski image of vulva from middle J3 stage. AC – anchor cell. The dotted line represents the position of the vulF ring containing two nuclei and their nucleoli. The stage of vulva development in this micrograph corresponds almost exactly to the stage of vulva in panel E. Unlike the Christmas tree invagination shape of *C. elegans* vulva, in *P. pacificus* the vulva has a bell shape. (E) Double stained image of vulva middle J3 stage in *P. pacificus*. Green lines represent AJ and red are nuclei. Dotted line sketches estimated position of the vulF toroid based on many observations including Nomarski optics as shown in panel D. AC – anchor cell. Reproduced from Kolotuev and Podbilewicz (2004), Copyright (2004), with permission from Elsevier.

2. Cell biology of plasma membrane fusion

A major advance in the understanding of developmental cell fusion was the development of a system to reproducibly observe ongoing cell fusions within a live organism. Using multiphoton laser scanning microscopy, it is possible to follow the dynamics of cell membranes during cell fusion with four-dimensional reconstruction of live embryos labeled with the vital fluorescent membrane probe FM-4-64 (Mohler et al., 1998). Syncytogenesis is followed as the dynamic disappearance of lateral membranes and AJs labeled with AJM-1::GFP (Figure 2A and Figure 3B). Fusion appears to originate close to the apical junction and to expand along the membranes and basally generating an enlarging fusion wave (Figure 3). The complete elimination of the membranes between two fusing cells takes about 30 min (Mohler et al., 1998). The expansion of the fusion zone is accompanied by the migration of the AJ marker AJM-1::GFP from an apical to a basal position (Figure 3C). Disappearance of the basally located AJM-1::GFP protein occurs only 5–10 minutes after the initiation of membrane fusion (Mohler et al., 1998). However, the loss of AJs is not required for all cell fusions since dorsal and ventral cells fuse in the absence of AJs in the contact zone (Podbilewicz and White, 1994) and myoepithelial cells in the pharynx fuse without disassembling the AJs that remain in the apical side of the syncytia (Shemer et al., 2004).



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Figure 3. Membrane fusion mechanisms. (A) The coiled-coil domains of SNAREs bring vesicle and target membranes within close proximity, facilitating fusion. (B) In epidermal cell fusion in *C. elegans*, the apical junctions (AJ) hold the epidermal cells together where cell fusion is initiated. (C) A fusion pore forms at the apical junction of the membrane barrier and spreads outward behind the migrating junction. The two nuclei of the adjacent cells (N1, N2) share a common cytoplasm after fusion. Reproduced from Witze and Rothman (2002), Copyright (2002), with permission from Elsevier.

TEM on embryos fixed while fusion events were in progress showed vesiculation of membranes at the fusing zone. The irregular vesicles observed (10–50 nm in diameter) were interpreted as part of a cellular fusion machinery that initiates membrane merger by the formation of a single unique pore at the apex of the fusing cells that expands through radial internalization of the cell membranes (Mohler et al., 1998). However, alternative mechanisms could account for the observations (Podbilewicz and Chernomordik, 2005). Fusion pores can be detected by electrophysiology or by following redistribution of cytoplasmic aqueous probes (e.g., GFP; Mohler et al., 2002; Shemer et al., 2004). However, these methods cannot be used to study the expansion of fusion pores between cells from a few nanometers to micrometers in diameter. The mechanisms responsible for late fusion stages and their dependence on the fusogenic proteins that initiated pore formation remain largely unexplored even in well characterized viral-cell fusion systems (Leikina et al., 2004; Podbilewicz and Chernomordik, 2005). In summary, cell fusion in embryonic dorsal epithelial cells has been analyzed in great detail by confocal microscopy using membrane fluorescent probes (FM4-64; Mohler, 1999; Mohler et al., 1998; Mohler and White, 1998), apical junction markers (AJM-1::GFP; Mohler et al., 1998; Rabin and Podbilewicz, 2000) and cytoplasmic aqueous fluorescent probes (Mohler et al., 2002; Shemer et al., 2004) allowing the direct observation of membrane disappearance, pore expansion and cytoplasmic content mixing. TEM studies of the same cells have shown vesiculation as a possible intermediate in pore expansion (Mohler et al., 1998). Other cell fusions during

morphogenesis of the tail showed much larger vesicles but no small irregular vesiculation of the membranes (Nguyen et al., 1999). The vesicles may be recycled to a different domain of the plasma membrane where they can fuse (exocytosis). The overall result of this membrane recycling process may transfer transverse membranes to longitudinal areas allowing efficient elongation of the animal (Podbilewicz, 2000; Shemer and Podbilewicz, 2000). More recently, multiple stable pore intermediates (20–50 nm in diameter) were identified in pharyngeal myoepithelial and epidermal cells in *eff-1* mutants (Figure 4; Gattegno et al., unpublished results; Shemer et al., 2004). The emerging picture suggests tissue-specificity in the mechanisms of initiation and expansion of fusion pores in *C. elegans* offering an amazing system to dissect the stages in the universal and largely unknown process of cell fusion.

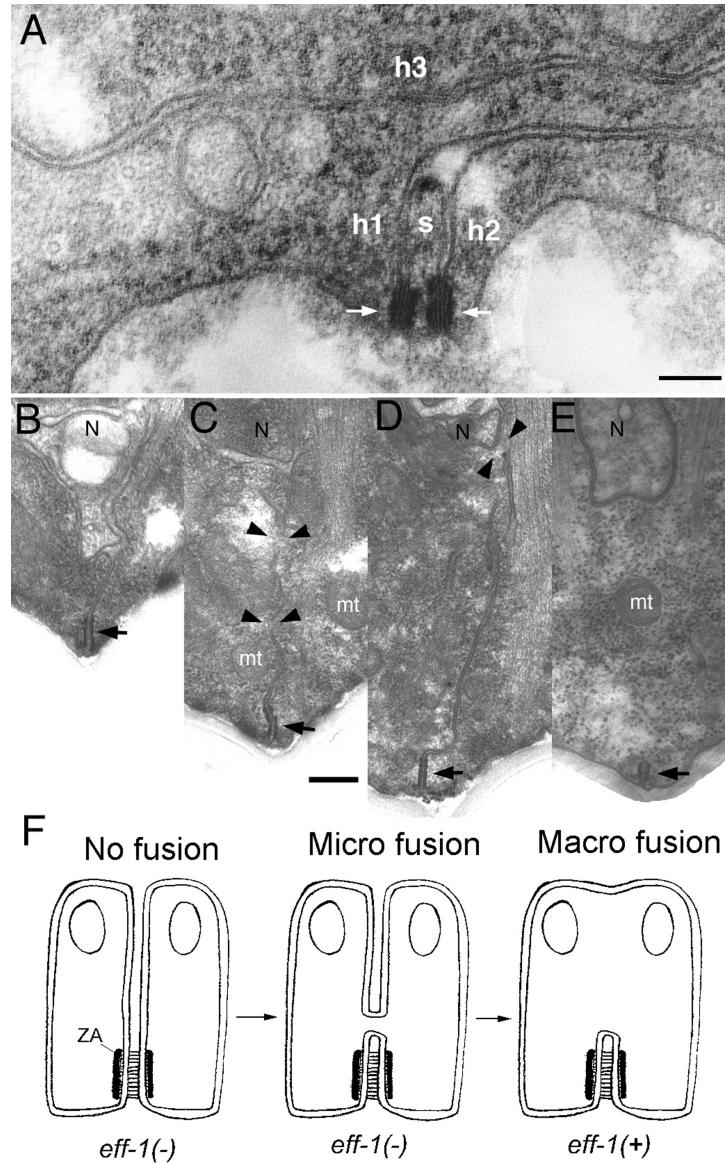


Figure 4. TEM of unfused epidermal and muscle cells. (A) Transverse thin section of mutant *eff-1(hy21)* L4 grown at the restrictive temperature shows normal ultrastructure of the hypodermal membranes, where the separation between apposing plasma membranes that failed to fuse is generally between 9 to 13 nm as in wild-type. The ultrastructure of the AJs (arrows) and membranes appears normal. Here, a seam cell process (s) narrows and three neighboring hypodermal cells (h1, h2 and h3) remain unfused along the lateral body wall. Lateral cuticle lies along bottom edge of panel. (B–E) TEM of pharyngeal muscle pairs shows variable fusion failure in a mutant *eff-1(hy21)* adult grown at the restrictive temperature or successful fusion in wild-type cells. (B) Two cells have failed to fuse, leaving a persistent cell border running from the neuron process (N) to the apical junction (AJ indicated by arrow). (C–D) Above the AJ, two pairs of *eff-1(hy21)* mutant cells have formed microfusions (arrowheads), which are so small that no mitochondria (mt) could pass. (E) Two wild-type cells have fully fused below the neuron process (N), leaving behind a complete AJ on the plasma membrane of the fused cell pair. (F) Model showing a pathway for pharyngeal muscle homotypic fusion based on TEM intermediates. Zonula adherens (ZA) is a component of the AJ (Knust and Bossinger, 2002) (B–E). The scale bars represent 100 nm. Reproduced from Shemer et al. (2004), Copyright (2004), with permission from Elsevier.

3. Genetics of cell fusion

Genes encoding fusogenic proteins required for the fusion of enveloped viruses (Earp et al., 2005) and intracellular membrane traffic (See Figure 3A; Jahn et al., 2003) have been identified and characterized. However, developmental cell fusion proteins are responsible for cell attachment before fusion or regulation of cell fusion (Chen and Olson, 2004; Chen and Olson, 2005; Kontani and Rothman, 2005; Shemer and Podbilewicz, 2003; Stein et al., 2004). Over the last decade a few attempts have been made to isolate mutations that specifically block or affect cell fusion in worms (Alper and Kenyon, 2001; Gattegno, 2003; Labouesse, 1997; Mohler et al., 2002; Podbilewicz, 2000; Podbilewicz and Chernomordik, 2005; Shemer and Podbilewicz, 2000; Shemer and Podbilewicz, 2003; Terns et al., 1997). Many successful screens have been designed to isolate spermatogenesis and oogenesis defective mutants that block fertilization (Singson et al., 1998; Xu and Sternberg, 2003). Still, very few mutants are candidates to affect stages after attachment of the two plasma membranes (see Control of oocyte meiotic maturation and fertilization and L'Hernault, 1997; Singson, 2001). Only recently mutations that block most somatic cell fusions in *C. elegans* were obtained, and they identified one gene that appears to be essential for the merger of the plasma membranes without affecting attachment or other pre-fusion processes (Kontani and Rothman, 2005; Mohler et al., 2002; Gattegno, T., Assaf, N. and BP, unpublished results).

4. *eff-1* is necessary for most, but not all, cell fusion events in *C. elegans*

eff-1 encodes a strong candidate developmental fusogen that is expressed in the fusion competent cells prior to cell membrane fusion. Moreover, *eff-1* mutant cells are wild-type in events prior to cell membrane fusion. Still the mutant cells fail to fuse and they develop as unfused epithelia within subviable animals containing morphological, behavioral and fertility defects (Cassata et al., 2005; Gattegno, 2003; Mohler et al., 2002; Shemer, 2002; Shemer and Podbilewicz, 2002; Shemer and Podbilewicz, 2003; Shemer et al., 2004; Figure 5). *eff-1* is the founder of a novel gene family encoding membrane and secreted proteins conserved within nematodes. No homologous genes have been identified in species outside nematodes (Shemer and Podbilewicz, 2003). Genetic and cell biological evidence indicates that *eff-1* activity is both necessary (Mohler et al., 2002) and sufficient (Shemer et al., 2004) to fuse epithelial and myoepithelial cells of the epidermis, vulva, uterus and pharynx *in vivo*. However, *eff-1* is not required in at least one fusion event in worms, the fusion between the anchor cell and the utse cells (Shemer et al., 2004); Anna Newman, personal communication). *eff-1* encodes at least four alternatively-spliced isoforms, two predicted type-I membrane glycoproteins (*eff-1A* and *eff-1B*) and two secreted proteins (*eff-1C* and *eff-1D*; Shemer and Podbilewicz, 2003). *eff-1* contains a putative fusion peptide within the protein similar to some class II viral fusogens (Wormbase; Shemer and Podbilewicz, 2003).

Recently, ectopic expression of genomic *eff-1A::GFP* showed dynamic GFP localization from the cytoplasm to the sites of cell-cell contact, prior to, and during, cell fusion (del Campo et al., 2005). The functions of two extracellular domains of *eff-1* that had been hypothesized to have roles in membrane fusion have been tested (Shemer and Podbilewicz, 2003). While transgenic proteins mutated in a putative phospholipase A2 active site rescued a weak *eff-1* mutant, two independent mutations in the putative hydrophobic fusion peptide, failed to rescue (del Campo et al., 2005). However, the putative fusion peptide mutated proteins were not expressed on the surface. Thus, the putative fusion peptide is required for correct folding, transport or localization to the site of contact and the direct function of this domain on cell fusion has not been determined (del Campo et al., 2005). It was proposed that homotypic interactions of *eff-1A* proteins facilitate cell fusion by bringing the membranes into close contact in analogy to SNARE-mediated intracellular fusion (See Figure 3A; del Campo et al., 2005). Since these experiments were done in the background of weak *eff-1* mutations, it is conceivable that the ectopic expression of *eff-1* protein isoforms can interact and rescue the endogenous partially active *eff-1* mutant proteins. Further structure-function analyses of *eff-1* isoforms, genetic mosaic analyses and *in vitro* reconstitution of membrane fusion will determine whether *eff-1* alone is sufficient to induce membrane fusion. Nevertheless, it is still possible that *eff-1* controls fusion at a post-binding, pre-membrane merger stage. Based on electron microscopic analyses of intermediates of cell fusion in *eff-1* mutants, it appears that *eff-1* is required for both initiation and expansion of fusion pores (Figure 4), similar to the well characterized fusogen of Influenza virus (Gattegno et al., unpublished results; Leikina et al., 2004; Podbilewicz and Chernomordik, 2005; Shemer et al., 2004).

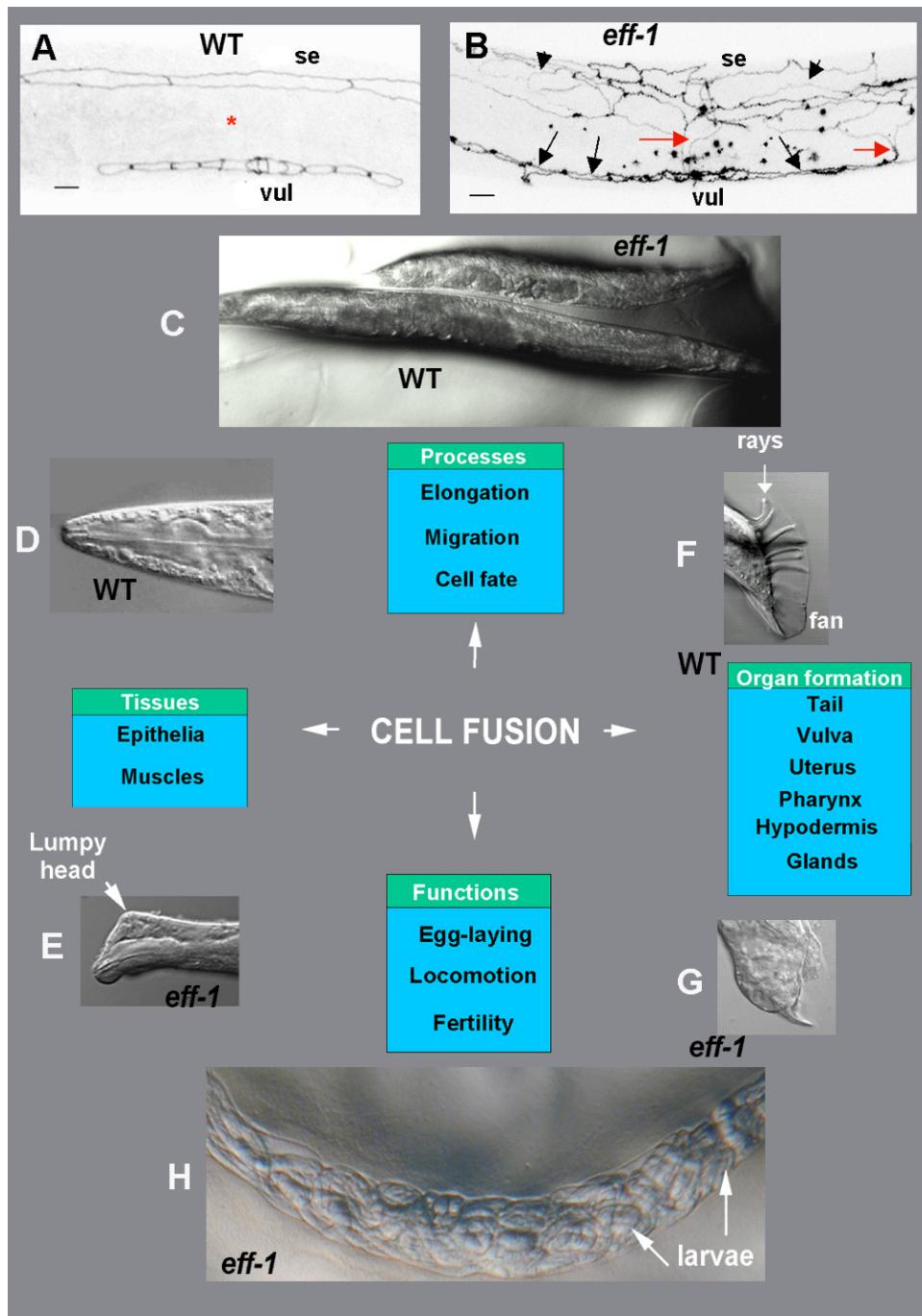


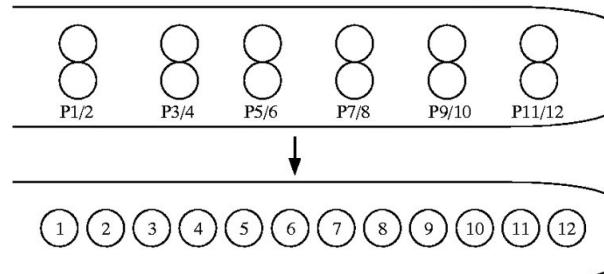
Figure 5. Cell fusion is essential for normal behavior and morphogenesis in *C. elegans*. Fluorescence negative images (A, B) and Nomarski (D-H) micrographs of wild-type and *eff-1* mutant *C. elegans*. In all pictures anterior is to the left and dorsal up. (A, B) Staining of the apical domains of epithelial cells using the anti-ZA MH27 antibody. (A) In wild-type (WT) animals lateral hypodermal seam cells (se) divide with the successive fusion of the non-stem daughter cells to the hypodermal syncytium (red asterisk). The non-fusing seam cells ultimately form two lateral rows along the body (one row is seen in this focal plain). (B) In *eff-1* mutant animals, after failing to fuse with the surrounding epidermis, the daughter cells of the seam cells migrate throughout the epidermis (arrowheads) and form a misarranged net (Mohler et al., 2002). This abnormal migration is also exhibited in vulval ectopically unfused cells (vul) that extend across the ventral surface (black arrows) and meet with the migrating seam cells (red arrows). (C) Cell fusion defects in *eff-1* mutants cause inhibition of elongation and result in short and dumpy animals. (D, E) Details of heads in wild-type (D) and *eff-1* mutant (E), showing a Lumpy phenotype expressed as hypodermal defects throughout the body and especially near the head (arrow). (F) In males, the correct patterning and positioning of special sensilla (rays), enveloped in a cuticular fan, is based on earlier cell fusion between four cells in the tip of the tail (Emmons and Sternberg, 1997). This event, followed by retraction of the newly-formed syncytium and secretion of fluid to the extracellular space, allows essential shape changes in the tip of the tail (Nguyen et al., 1999). (G) When cell fusion is blocked, the cells cannot retract, and the normal formation of the male-specific tip of the tail is disrupted, resulting in altered rays and fan and in mating disability. (H) *eff-1* mutants are egg-laying defective. Embryos that fail to get out of the uterus, develop to L1 larvae, eat their mother's tissues and get out of their body (a "bag of worms" phenotype). Reproduced from Shemer and Podbilewicz (2003), Copyright (2003), with permission from Wiley.

5. Regulation of cell fusion

5.1. Transcriptional regulation of cell fusion

During morphogenesis the main body epidermis is patterned in three distinct regions with different fusogenic activities (Figure 1; Ding et al., 2004; Michaux et al., 2001; Priess and Hirsh, 1986; Simske and Hardin, 2001): dorsal, lateral and ventral. 1) Two rows of dorsal cells interdigitate forming a single row of cells that fuse forming the hyp6 and hyp7 syncytia as the embryo elongates (Podbilewicz and White, 1994; Priess and Hirsh, 1986; Sulston et al., 1983). 2) Two independent rows of lateral seam cells that elongate but do not fuse during embryogenesis, but their descendants fuse with hyp7, generate neurons or maintain the seam cells during postembryonic development. 3) Two rows of ventral P blast cells that do not fuse in the embryo, but their descendants either migrate and fuse with hyp7 or divide and differentiate into neuronal, vulval, anal or male tail tissues during larval development (Figure 6; Sulston and Horvitz, 1977). Different transcription factors have been identified as regulators of the fusion activities that pattern the epidermis and associated sexual and neuronal organs (Table 1; see also Transcriptional regulation and Transcription mechanisms).

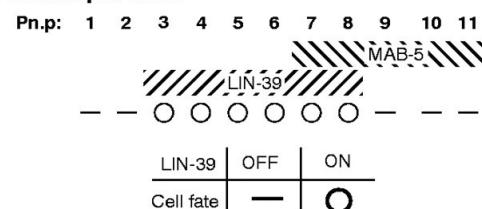
A. P cells migrate into the ventral cord (ventral view) - early L1



B. 12 Pn.p cells line the ventral surface (lateral view) - late L1



C. Hermaphrodite



D. Male

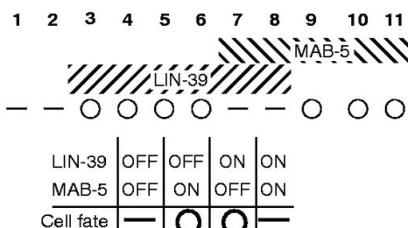


Figure 6. The Pn.p cell fusion pattern is regulated by the Hox genes *lin-39* and *mab-5*. (A) Ventral views of early-L1 (top) and mid-L1 (bottom) larvae. Six pairs of epidermal P blast cells initially lie along the ventral surface (top). During L1, the P cells migrate around each other so that a row of 12 P cells lines the ventral surface (bottom). Shortly after they migrate into the ventral cord, the P cells divide. The anterior (Pn.a) daughters become neuroblasts, while the posterior (Pn.p) daughters remain epidermal. Anterior is towards the left. (B) Lateral view of a late L1 larva with the 12 ventrally located Pn.p cells shown. Some of the Pn.p cells fuse with hyp7, which is located more dorsally along most of the mid-body region. (C, D) Pn.p cell fusion in wild-type hermaphrodite (C) and male (D). White circles indicate Pn.p cells that remain unfused, short horizontal lines indicate cells that fuse with the hyp7 syncytium. Hox gene expression domains are similar in both sexes, as indicated in hatched regions. The tables underneath show how Hox gene expression information is interpreted in the cell fusion decision. Reproduced from Alper and Kenyon (2002), Copyright (2002), with permission from The Company of Biologists.

Table 1. Regulators of developmental cell fusion in *C. elegans*

Gene	Protein structure and proposed function	References
Ventral cell fusions		
<i>lin-39</i>	Hox (<i>Dfd/Scr</i>)-represents <i>eff-1</i> expression in hermaphrodites	Clark et al., 1993; Maloof and Kenyon, 1998; Shemer and Podbilewicz, 2002; Wang et al., 1993
<i>lin-39</i>	Hox (<i>Dfd/Scr</i>)-inhibits fusion in males when <i>mab-5</i> is OFF	Clark et al., 1993; Maloof and Kenyon, 1998; Wang et al., 1993
<i>lin-39 + mab-5</i>	Hox (<i>Dfd/Scr + Antp</i>) promote fusion in males	Clark et al., 1993; Salser and Kenyon, 1994; Wang et al., 1993
<i>mab-5</i>	Hox (<i>Antp</i>) inhibits fusion in males when <i>lin-39</i> is OFF	Alper and Kenyon, 2001; Kenyon, 1986
<i>ceh-20</i>	Hox co-factor (<i>exd/pbx</i>)-represents <i>eff-1</i> expression	Shemer and Podbilewicz, 2002
<i>nhr-25</i>	Ftz-F1/SF1 Hox co-factor-represses fusion with <i>lin-39</i> in P(3-8).p	Chen et al., 2004
<i>nhr-25</i>	Ftz-F1/SF1 co-factor-promotes fusion in non-VPCs	Chen et al., 2004
<i>acn-1</i>	Angiotensin-converting enzyme-transcriptional target of <i>nhr-25</i>	Brooks et al., 2003; Chen et al., 2004
<i>ref-1</i>	bHLH TF (hairy)-inhibits <i>mab-5</i> activity and <i>lin-39</i> expression	Alper and Kenyon, 2001
<i>egl-27</i>	Chromatin remodelling-regulates Pn.p cell fusion in both sexes	Ch'ng and Kenyon, 1999
<i>ref-2</i>	Zinc-finger TF-prevents Pn.p fusion in L1	Alper and Kenyon, 2002
<i>egl-18/elt-6</i>	GATA factors-repress cell fusion L2-L4; targets of <i>lin-39/ceh-20</i>	Koh et al., 2004; Koh et al., 2002
<i>sem-4</i>	Zinc-finger TF-positively regulates <i>lin-39</i> , preventing fusion	Grant et al., 2000
<i>bar-1</i>	Wnt pathway (beta-catenin)-positively regulates <i>lin-39</i>	Eisenmann et al., 1998
<i>apr-1</i>	Wnt pathway (APC related factor)-activates <i>bar-1</i>	Hoier et al., 2000
<i>let-60</i>	Ras inhibits fusion L1-L3; promotes fusion in late vulva development	Han et al., 1990; Shemer et al., 2000
SynMuv class A (e.g., <i>lin-15a</i>)	TFs, NURD complex components and chromatin remodeling-work with class B to antagonize Ras pathway signaling	Chen and Han, 2001; Fay and Han, 2000; Herman and Hedgecock, 1990
SynMuv class B (e.g., <i>lin-35/Rb</i>)	TFs, Rb/E2F complex components and chromatin remodeling-work with class A to antagonize Ras pathway signaling promoting fusion	Chen and Han, 2001; Fay and Han, 2000; Herman and Hedgecock, 1990; Myers and Greenwald, 2005
SynMuv class C (e.g., TRRAP)	Tip60/NuA4-like histone	Ceol and Horvitz, 2004

Gene	Protein structure and proposed function	References
	acetyltransferase complex and P400 work with class A and B to antagonize Ras pathway signaling	
<i>fus-1</i>	E subunit of V-ATPase-represses <i>eff-1</i> activity; late embryogenesis	Kontani et al., 2005
Lateral cell fusions		
<i>nhr-25</i>	Ftz-F1/SF1 TF-promotes cell fusion and/or differentiation	Chen et al., 2004
<i>acn-1</i>	Angiotensin-converting enzyme-Molting and morphogenesis	Brooks et al., 2003
<i>lin-29</i>	Zinc-finger TF-promotes fusion in L4 molt; heterochronic pathway	Ambros and Moss, 1994; Rougvie and Ambros, 1995
<i>egl-18/elt-6</i>	GATA factors-repress cell fusion	Koh and Rothman, 2001
<i>ceh-16</i>	Homeobox TF (<i>engrailed</i>)-represses <i>eff-1</i> expression	Cassata et al., 2005
<i>lin-25</i>	Novel protein TF?-overexpression disrupts cell fusion	Nilsson et al., 1998
Dorsal cell fusions		
<i>ceh-13</i>	Hox (<i>labial</i>)-promotes some anterior cell fusions	Brunschwig et al., 1999
<i>idf-1</i>	Nd-promotes embryonic cell fusion	Gattegno, 1998; Gattegno, 2003
<i>fus-1</i>	E subunit of V-ATPase-represses <i>eff-1</i> activity, late embryogenesis	Kontani et al., 2005
Male tip of the tail cell fusions		
<i>tlp-1</i>	Zinc-finger TF-required for polarity of T lineage and hyp(8-10) fusions	Zhao et al., 2002
<i>lep-1</i>	Nd-Required for correct timing of cell fusions and morphogenesis	Nguyen et al., 1999; Zhao et al., 2002
Anchor cell-uterine seam cell fusion (AC-utse; vulval-uterine connection)		
<i>lin-11</i>	LIM -homeobox TF-differentiation of cells that fuse with AC	Newman et al., 1999
<i>cog-2/egl-13</i>	Sox-domain TF-maintains fates of cells that fuse with AC	Cinar et al., 2003; Hanna-Rose and Han, 1999
<i>smo-1</i>	SUMO-1-Small ubiquitin-like modifier-Modifies <i>lin-11</i> activity	Broday et al., 2004
ty10	sec-18/nsf homolog-intracellular vesicular fusion, promotes AC fusion	A. Newman, personal communication
Epithelial and pharyngeal cell fusions but not AC-utse cell fusion		
<i>eff-1</i>	Novel transmembrane and secreted isoforms-fusion machinery	Mohler et al., 2002; Shemer et al., 2004
Identity and putative functions (if mentioned) of genes that control cell fusion during <i>C. elegans</i> development. Class A of the SynMuv genes works redundantly with class B genes to promote fusion. Class C works with classes A and B. Each one of these classes contains several genes. nd- not determined TF-transcription factor. Modified from Shemer and Podbilewicz (2003), Copyright (2003), with permission from Wiley.		

5.2. Ventral cell fusions

During morphogenesis six ventral cells fuse to 17 dorsal cells to form the large syncytium hyp7 in the embryo. However, 12 P cells covering most of the ventral epidermis do not fuse in the embryo. In the L1, six posterior descendants of the P cells (P(3-8).p; the ventral precursor cells, VPCs) are each competent to generate vulval cells in the hermaphrodite. In the wild-type L3, only the nearest VPCs to the uterine anchor cell (AC) generate the 22 nuclei of the vulva, while the three others fuse to hyp7. The vulval primordial cells divide, migrate and fuse in a stereotyped manner to form an epidermal tube during the L3 and L4 stages (Greenwald, 1997; Kornfeld, 1997; Michaux et al., 2001; Podbilewicz and White, 1994; Sharma-Kishore et al., 1999; Sulston and Horvitz, 1977; Sulston et al., 1983; Wang and Sternberg, 2001). For an overview of ventral cell biology in hermaphrodites and males (see Figure 1 and Figure 6 and the chapters: Epidermal morphogenesis, Hermaphrodite cell-fate specification, Male development, and Vulval development).

5.2.1. Hox genes protect specific cells from fusion allowing organ formation

The six Hox genes in *C. elegans* are expressed and determine cell fates along the antero-posterior body axis (Brunschwig et al., 1999; Chisholm, 1991; Clark et al., 1993; Van Auken et al., 2000; Wang et al., 1993; See chapter on Hox genes). In the L1, the Hox genes *lin-39/Sex combs reduced/Deformed* and *mab-5/Antennapedia* regulate cell fusion of the Pn.p cells to hyp7 (Figure 6). P(3-8).p cells express *lin-39* and P(7-11).p express *mab-5* in both sexes (Maloof and Kenyon, 1998; Salser and Kenyon, 1994; Wang et al., 1993). *lin-39* prevents fusion of P(3-8).p (vulva precursor cells; VPCs) in the hermaphrodites (Clark et al., 1993; Wang et al., 1993) by repression of *eff-1* expression (Shemer and Podbilewicz, 2002). The *lin-39* prevention of cell fusion of P(3-8).p is maintained until P(5-7).p cells are induced in the L3 (Maloof and Kenyon, 1998; Shemer and Podbilewicz, 2002). Wnt and Ras signaling control *lin-39* activity in the VPCs (Chen and Han, 2001; Eisenmann et al., 1998; Maloof and Kenyon, 1998; see Wnt signaling).

In hermaphrodites *mab-5* appears to be largely inactive in P(7-11).p during the L1 cell fusion fate decision (Figure 6C; see below). However, in *mab-5* mutant worms, P11.p is sometimes transformed into P12.p (Kenyon, 1986) and in 15% of the *mab-5* (lf) hermaphrodites P11.p fails to fuse in the L1 (Alper and Kenyon, 2001). It has been shown that *mab-5* has a dynamic and fine control over diverse cellular and morphogenetic activities (Salser et al., 1993). It is possible that *mab-5* may also have a complex and dynamic activity in the posterior Pn.p cells during the L3 since some vulval phenotypes have been observed in *mab-5* hermaphrodites (Clandinin et al., 1997) and *mab-5* mutations in *Pristionchus pacificus* strongly affect vulva formation (Gutierrez et al., 2003; Jungblut et al., 2001; Jungblut and Sommer, 1998; Ira Kolotuev and BP, unpublished results; see Evolution of development in nematodes related to *C. elegans*).

5.2.2. Hox cofactors

eff-1 is also epistatic to *ceh-20*, encoding a known cofactor of *lin-39* (Koh et al., 2002; Liu and Fire, 2000). *ceh-20*/Extradenticle may act in a complex with *lin-39* and other cofactors to repress *eff-1* transcription (Shemer and Podbilewicz, 2002). *nhr-25/Ftz-F1/SF1* may also act as a cofactor of *lin-39* during regulation of fusion in the Pn.p cells (Asahina et al., 2000; Chen et al., 2004; Gissendanner and Sluder, 2000). *nhr-25* may act with *lin-39* to repress fusion of P(3-8).p while acting with another transcription factor in neighboring cells to promote cell fusion and cell shape dynamic changes (Chen et al., 2004; Silhankova et al., 2005). A potential transcriptional target of *nhr-25* is *acn-1*, a gene encoding a member of the angiotensin-converting enzyme family (Brooks et al., 2003; Chen et al., 2004).

The following working model is emerging for the regulation of cell cycle and cell fusion effectors by Hox proteins and cofactors in the ventral epidermis. First, a complex of *lin-39/ceh-20* (Koh et al., 2002; Liu and Fire, 2000) together with other transcription factors and cofactors represses *eff-1*-mediated plasma membrane fusion during competence and induction in the L1 and L3 respectively. Second, *lin-39* also stimulates cell division. Third, VPCs that failed to fuse and to divide in *lin-39;eff-1* double mutants can perform other cellular behaviors characteristic of their normal great-granddaughters, such as formation of vulval rings (Shemer and Podbilewicz, 2002) and partial Mid-L4 expression of vulval markers like *egl-17::GFP* (Burdine et al., 1998; Cui and Han, 2003). Thus, in the absence of *lin-39* activity, induction of the VPCs is independent of their patterns of cell division (sublineages) and a double block in cell fusion and cell cycle can allow cell differentiation (Shemer and Podbilewicz, 2002). Cell fusion inhibition is not sufficient for the VPCs to form a vulva because *lin-39* is also required for these cells to proliferate. Although undivided VPCs do form vulval rings, these are non-functional

(Shemer and Podbilewicz, 2002). Finally, during late stages in vulva morphogenesis, Ras appears to have a cell fusion promoting function (Shemer et al., 2000).

5.2.3. L1-specific regulation of cell fusion by Hox, *ref-1*, *ref-2* and *egl-27*

Two transcription factors, *egl-27* and *ref-1*, maintain *mab-5* inactive in the hermaphrodite. In a screen for mutations that modify the pattern of Pn.p cell fusion, *ref-1* (*mu220*; REgulator of Fusion-1) was isolated. *ref-1* mutation affects Pn.p cell fusion early but not later during larval development (Figure 7). *ref-1*, a protein with two bHLH domains, allows fusion of posterior Pn.p cells in the hermaphrodites by inhibiting the sex-specific *mab-5* activity (Alper and Kenyon, 2001). In addition, *ref-1* functions together with *mab-5* to prevent *lin-39* expression in the posterior region allowing fusion of posterior Pn.p cells. The third action of *ref-1* is independent of *mab-5*, *ref-1* can influence the Hox gene *egl-5*'s ability to regulate Pn.p cell fusion (Alper and Kenyon, 2001). *egl-27*, a chromatin regulatory factor (Ch'ng and Kenyon, 1999; Herman et al., 1999; Solari et al., 1999), regulates Pn.p cell fusion in both sexes. In *egl-27* mutant hermaphrodites, posterior Pn.p cells fail to fuse. *egl-27* regulates expression of *lin-39*, *mab-5* and *egl-5* controlling fusion of specific ventral cells. In males, *mab-5* and *lin-39* inhibit each other's activity when expressed in the same Pn.p cell, resulting in fusion of these cells (See Figure 6D). In males, *egl-27* permits the combinatorial interaction between *lin-39* and *mab-5*. Thus, *egl-27* activation appears to provide cell- and sex-specific regulation of Hox function that creates antero-posterior patterns of cell fusion (Ch'ng and Kenyon, 1999). *egl-27* and *ref-1* could directly, or indirectly through Hox and GATA factors and other cofactors, control transcription of *eff-1* along the antero-posterior axis to generate a complex pattern of Pn.p cell fusion (see below).

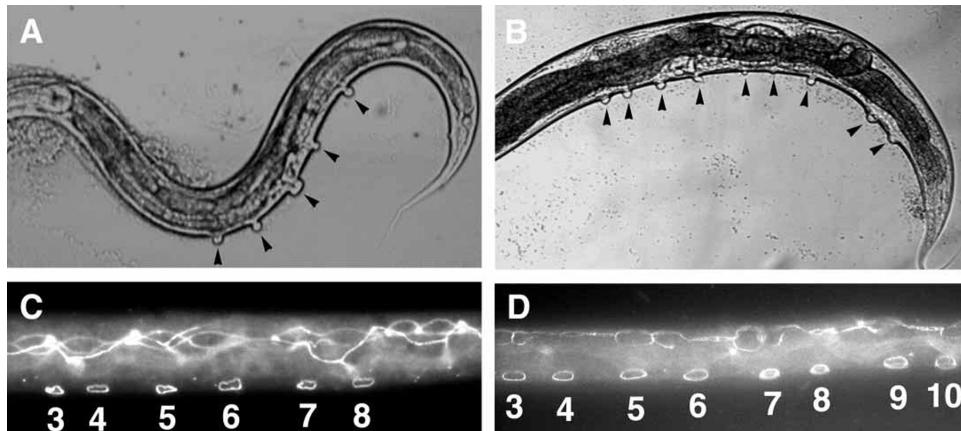


Figure 7. Posterior Pn.p remain unfused inappropriately in *ref-1(mu220)* mutants. (A, B) Unfused Pn.p cells are inferred from the presence of pseudovulval-like structures (indicated by black arrowheads) on the ventral surface of the worm on a *lin-12(n137)* background. *lin-12(n137)* hermaphrodites (A) usually contain five and no more than six pseudovulvae. *ref-1(mu220)*; *lin-12(n137)* hermaphrodites frequently have extra pseudovulvae in the posterior body region (B). (C, D) Unfused Pn.p cells visualized by immunostaining animals with the MH27 monoclonal antibody. Wild-type worms have six unfused cells (P(3-8).p) (C) while *ref-1(mu220)* worms often have extra unfused cells in the posterior (P(3-10).p unfused in this picture; D). Reproduced from Alper and Kenyon (2001), Copyright (2001), with permission from The Company of Biologists.

In males, *lin-39* and *mab-5* proteins inhibit each other even when one of the Hox proteins is ectopically over-expressed relative to the other (Salser et al., 1993). Alper and Kenyon hypothesized that both Hox proteins may bind to regulatory sites in a cell fusion gene encoding a direct effector of cell fusion (Alper and Kenyon, 2002). In their model, the binding of either Hox protein alone would inhibit expression of a fusion gene and expression of both Hox proteins would promote expression of a fusogen resulting in Pn.p cell fusion. Alternatively, the two Hox proteins together could repress a cell fusion inhibitor (*ref-2*; REgulator of Fusion-2) required to prevent expression of a cell fusion gene promoting cell fusion (Figure 8; Alper and Kenyon, 2002). To investigate this complex sex-specific regulation of Pn.p cell fusion, the dominant *ref-2* (*mu218*) mutation was isolated and cloned (Figure 9A; Alper and Kenyon, 2002). *ref-2* encodes a Zn-finger protein of the odd-paired (opa)/Zic family and is required to generate Pn.p cells and to keep them unfused both in males and hermaphrodites. *ref-2* is necessary, but not sufficient, to maintain Pn.p cells unfused. In addition, an active Hox gene is also necessary to prevent fusion (Figure 8–Figure 10).

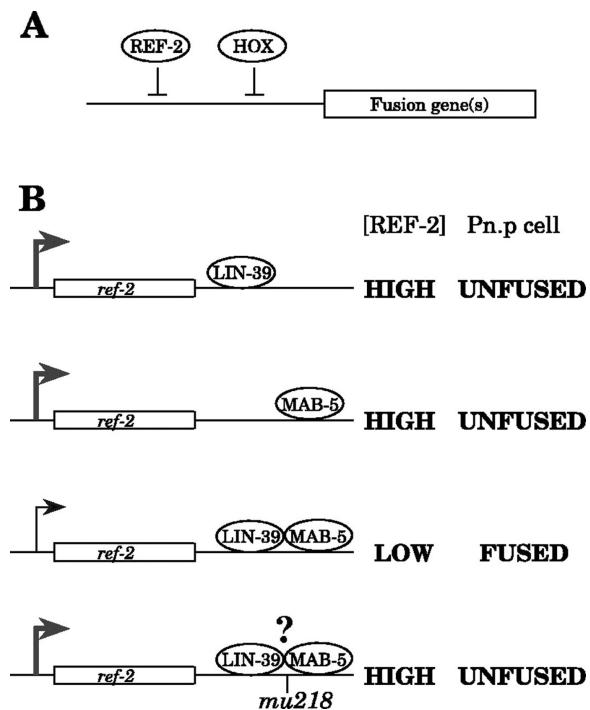


Figure 8. Two stages in *ref-2*-dependent patterning of Pn.p cell fusion. (A) A model for how REF-2 prevents Pn.p cell fusion. REF-2 and a Hox protein (LIN-39 or MAB-5) act by cooperatively inhibiting a common set of fusion gene targets. Alternatively, the two transcription factors could together activate a gene(s) required to prevent cell fusion. (B) A model for how *ref-2* expression might be regulated. In Pn.p cells in males, either Hox protein alone (LIN-39 or MAB-5) can activate *ref-2* transcription and thus can keep the concentration of REF-2 high in the Pn.p cells when the cell fusion decision is made. However, when both Hox proteins are present, *ref-2* expression decreases in the Pn.p cells and those cells fuse with hyp7. The *mu218* mutation allows *ref-2* to be expressed even in cells containing both LIN-39 and MAB-5 (*mu218* could affect the ability of Hox proteins to bind *ref-2* regulatory sites). Reproduced from Alper and Kenyon (2002), Copyright (2002), with permission from The Company of Biologists.

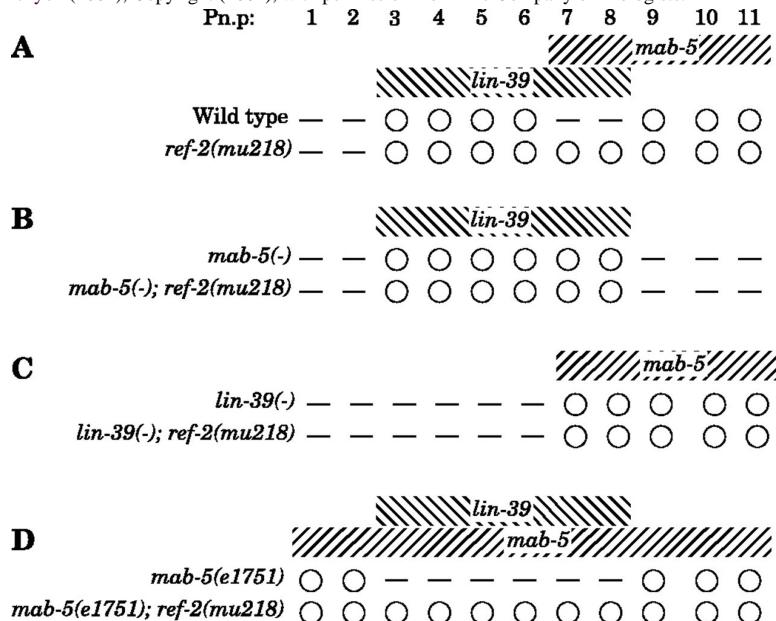


Figure 9. The *ref-2(mu218)* mutation prevents LIN-39 and MAB-5 from canceling each other's activities in males. The indicated mutants were stained with MH27 in early L2 to score Pn.p cell fusion in males. White circles indicate unfused Pn.p cells, short horizontal lines indicate Pn.p cells that have fused with hyp7. Hox gene expression patterns in each set of Hox mutant strains are indicated above each set of genotypes. Alleles used: *lin-39(n1760)* and *mab-5(e2088)*, both null mutations, and *mab-5(e1751)*, in which wild-type *mab-5* is misexpressed in all Pn.p cells. All strains also harbored the *him-5(e1490)* mutation to generate males. At least 50 worms of each genotype were scored. All phenotypes listed are 100% penetrant except for: in *mab-5(e1751)*; *him-5(e1490)* worms, P1.p fused with hyp7 in 1/52 worms and P3.p remained unfused in 1/52 worms, and in *mab-5(e1751)*; *him-5(e1490)*; *ref-2(mu218)* worms, P6.p fused with hyp7 in 1/51 worms. Reproduced from Alper and Kenyon (2002), Copyright (2002), with permission from The Company of Biologists.

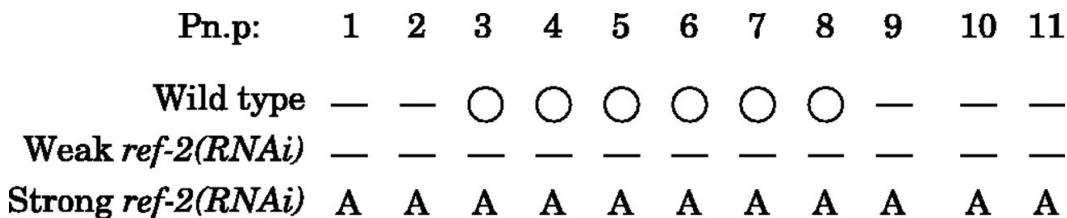


Figure 10. Wild-type *ref-2* is required for Pn.p cells to be generated and to remain unfused. *ref-2* gene function was inhibited by RNAi. A range of phenotypes was observed depending on the concentration of dsRNA injected. Weaker RNAi produced worms in which most of the Pn.p cells had fused (short horizontal lines). Stronger RNAi produced worms in which most of the Pn.p cells were absent from the ventral cord (A). White circles indicate unfused Pn.p cells. Pictured are data for hermaphrodites; similar phenotypes were also observed in males. Reproduced from Alper and Kenyon (2002), Copyright (2002), with permission from The Company of Biologists.

In summary, either *mab-5* or *lin-39* act with *ref-2* to inhibit Pn.p cell fusion in males. When *mab-5* and *lin-39* are both active, fusion of Pn.p cells is not inhibited, perhaps due to low *ref-2* expression. In hermaphrodites, *egl-27* and *ref-1* prevent *mab-5* from affecting Pn.p cell fusion and they may act by preventing *mab-5* activity on *ref-2* expression. It appears that *eff-1* is the ultimate effector of cell fusion in the ventral epidermis whose complex expression must be finely regulated through Hox, *ref-1*, *ref-2* and other interacting genes in both sexes (Figure 8).

5.2.4. L2-L4 cell fusion regulation by GATA factors and interactions with *lin-39*

ELT-5/EGL-18 and ELT-6 GATA factors repress cell fusion in the VPCs (Figure 11; Koh et al., 2004; Koh et al., 2002). The cell fusion repression in the ventral epidermis appears to be controlled by *lin-39/ceh-20* heterodimers binding to two HOX/PBC sites in a vulval enhancer of *egl-18/elt-6*. Thus EGL-18/ELT-5 and ELT-6 are genetically redundant regulators of cell fusion in the vulva primordium and may be direct targets of *lin-39* (Koh et al., 2002). Moreover, reduction of *lin-39* activity resulted in lower *elt-6*::GFP expression and over-expression of *egl-18* is sufficient to partially rescue vulval defects in *lin-39* (RNAi), consistent with the model in which *egl-18/elt-5* and *elt-6* act downstream of *lin-39*. It has been proposed that these GATA factors repress genes that promote Pn.p cell fusion, such as *eff-1* (Koh et al., 2004; Koh et al., 2002; see below) and also promote cell proliferation (Koh et al., 2004). One speculation is that EGL-18 and ELT-5 may also act in desilencing of condensed chromatin in the developing vulva (Koh et al., 2004). In addition, SEM-4 Zn finger transcription factor promotes vulval cell fates through *lin-39* (Eisenmann and Kim, 2000; Grant et al., 2000).

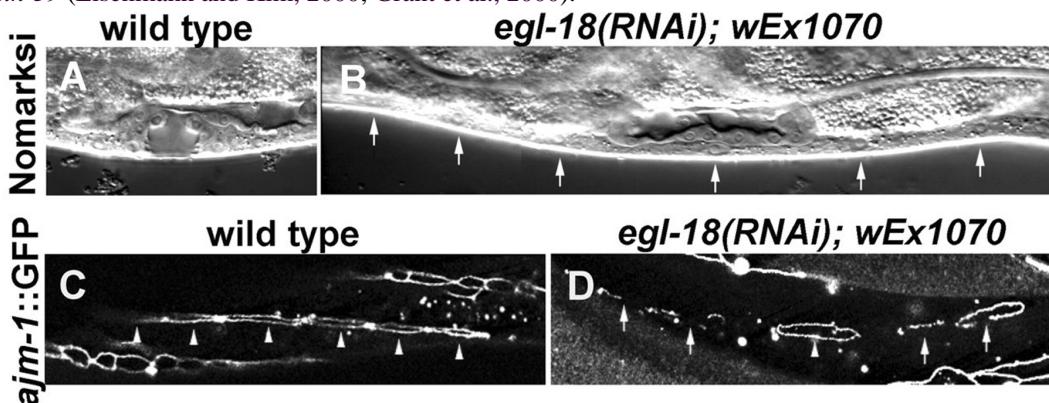


Figure 11. Vulval phenotypes of *egl-18(RNAi)* animals rescued for lethality by *wEx1070*. (A) Nomarski image of a wild-type vulval opening at the 'Christmas tree' (L4) stage. (B) Nomarski image of an *egl-18(RNAi)*; *wEx1070* animal, in which all of the six P3.p-P8.p cells (arrows) did not divide and appear to have fused. (C) *ajm-1::GFP* (adherens junction marker) expression in a wild-type animal at the early L3 stage. All six VPCs (arrowheads) are clearly demarcated by *ajm-1::GFP*. One of the VPCs is partially out of focus in this image. (D) *ajm-1::GFP* expression in an *egl-18(RNAi)*; *wEx1070* animal at a similar stage to the animal shown in C. All but one VPC are in the process of fusion as indicated by fragmented *ajm-1::GFP* expression (arrows). One VPC remains unfused as shown by a complete ring (arrowhead). Reproduced from Koh et al. (2002), Copyright (2002), with permission from The Company of Biologists.

5.2.5. Do *lin-35/Rb*, *lin-15* and other SynMuv genes promote hyp7-VPCs cell fusion?

SynMuv genes and in particular Rb/E2F and NuRD (nucleosome remodeling and histone deacetylation) complexes regulate *lin-39* expression and related activities during Pn.p fusion, competence and inductions (Ch'ng and Kenyon, 1999; Chen and Han, 2001; Chen and Han, 2001; Herman et al., 1999; Solari and Ahringer, 2000; Solari et al., 1999). While the NuRD complex negatively regulates *lin-39* activity, Rb/E2F appears to have a negative role controlling *lin-39* activity and a positive role regulating its expression (Chen and Han, 2001). These

apparently complex genetic interactions may not be as a result of direct transcriptional regulation in all cases because some SynMuv genes do not function in the VPCs where *lin-39* activity is expressed. In particular, mosaic analyses have shown that *lin-15* (Herman and Hedgecock, 1990), *lin-37* (Hedgecock and Herman, 1995) and *lin-35/Rb* (Myers and Greenwald, 2005) act in the hyp7 hypodermal syncytium and not in the VPCs to inhibit vulval fates. Thus, these results are inconsistent with the view that Rb, E2F, histone deacetylases and components of the NURD and other chromatin remodeling complexes (Ceol and Horvitz, 2001; Ceol and Horvitz, 2004; Chen and Han, 2001; Chen and Han, 2001; Lu and Horvitz, 1998; Solari and Ahringer, 2000; Thomas et al., 2003; von Zelewsky et al., 2000) antagonize the inductive signaling pathways only by acting in the VPCs to transcriptionally repress effector genes required for vulval cell fates. *lin-15*, *lin-35*, *lin-37* and probably other SynMuv genes may repress vulval fates by regulating a negative signal from hyp7 to the VPCs or by promoting cell fusion probably through activation of a cell fusion effector gene such as *eff-1* (Herman and Hedgecock, 1990; Myers and Greenwald, 2005).

In summary, there is evidence for coordinated and complex regulation of *lin-39* in the ventral epidermis by Ras, Wnt and SynMuv pathways (Chen and Han, 2001; Eisenmann et al., 1998; Fay and Han, 2000; Gleason et al., 2002; Hoier et al., 2000; Lu and Horvitz, 1998). In addition, the SEM-4 transcription factor (Eisenmann and Kim, 2000; Grant et al., 2000) and GATA factors (Koh et al., 2004; Koh et al., 2002) regulate *lin-39*. Overall, there appears to be several signaling pathways that control the ability of each of the ventral epidermal cells to fuse, or not to fuse, to hyp7.

5.3. Lateral cell fusions

Lateral seam cells actively participate in embryonic elongation but do not fuse in the wild-type embryo. However, during postembryonic development the ectoblastic seams have dynamic cell behaviors essential for the growth of hyp7 by cell fusion, generation of neurons, molting and larval elongation (Hedgecock and White, 1985; Podbilewicz and White, 1994; Singh and Sulston, 1978; Sulston and Horvitz, 1977).

5.3.1. *ceh-16/engrailed* represses lateral cell fusion in the embryo

It has recently been shown that *ceh-16/engrailed* is expressed in the lateral seam cells during embryonic development and induces the expression of seam cell markers including ELT-5 (Cassata et al., 2005). In contrast, *ceh-16* represses *eff-1* expression in the seam cells preventing these cells from fusing (Figure 12).

In *ceh-16* mutants and *ceh-16* (RNAi) worms, *eff-1* is ectopically expressed in the seam cells resulting in cell fusion, morphogenesis block and embryonic lethality (Figure 13). In addition, *ceh-16* mutant seam cells migrate dorsally or ventrally losing their lateral position. Animals expressing *ceh-16* in some but not all seam cells in *eff-1*(-) background had a complete fusion failure suggesting that *eff-1* acts downstream of *ceh-16* (Figure 13). Thus, *ceh-16* appears to maintain embryonic epidermal compartments by preventing cell fusion and maintaining the lateral seam cell borders that separate the dorsal and ventral hypodermal compartments during embryogenesis (Figure 12D; Cassata et al., 2005).

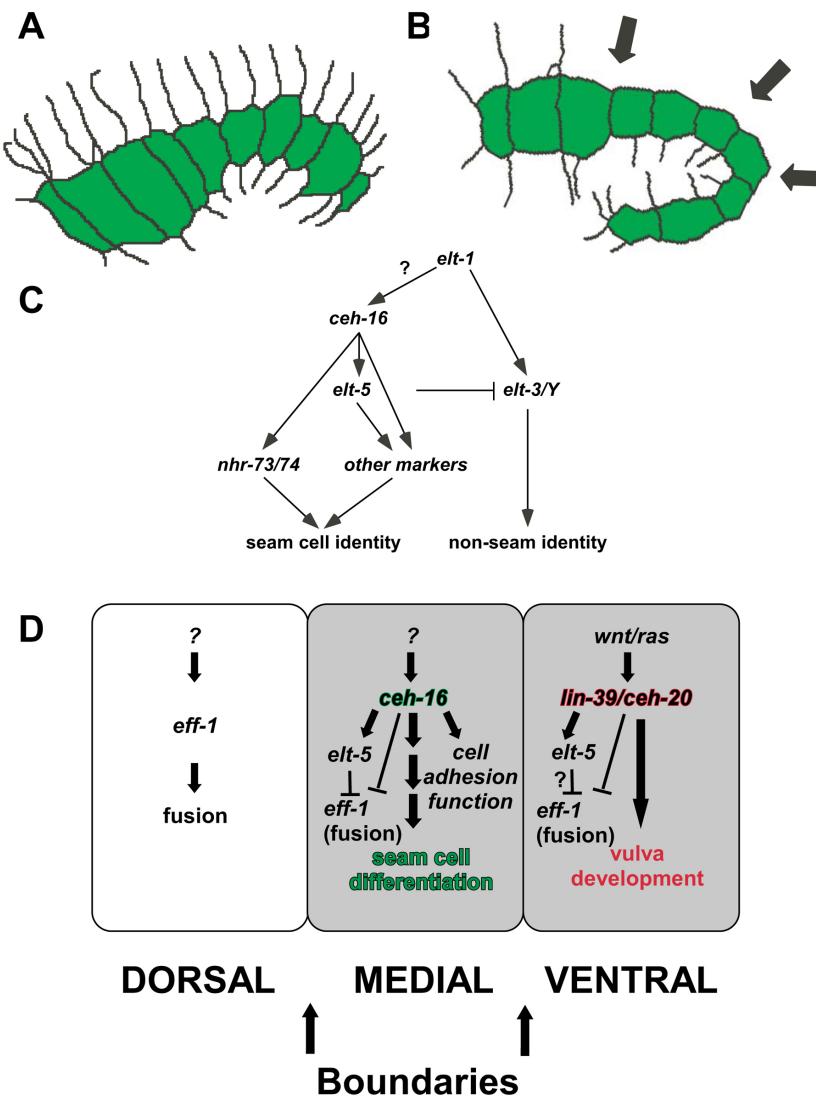


Figure 12. Model for the function of *ceh-16/engrailed* in *C. elegans*. (A) *ceh-16/engrailed* keeps the seam cells in a linear organization (green), due to its repression of seam cell fusion and cell migrations. (B) During elongation, hyp7 (dorsal) start to fuse; *ceh-16/engrailed* acts as a fusion repressor and as a regulator of seam-cell differentiation in order to maintain a straight boundary. At this stage genes important for “seam-cell-ness” are turned on/regulated by *ceh-16/engrailed*. (C) Transcriptional cascade regulating seam cell fate. (D) Summary of genetic regulatory cascades in the three rows of epidermal cells: Dorsal: *eff-1* is expressed and required for the fusion of hyp7 into a syncytium preceding and during 1.5 fold stage. It is not known which genes regulate *eff-1* expression in the dorsal epidermis. Medial or Lateral: in the seam cells *ceh-16/engrailed* represses the expression of *eff-1* (not known if directly) preceding and during 1.5 fold stage. This is necessary to allow *ceh-16/engrailed* to turn on genes required for correct positioning of the seam cells (cell adhesion molecules) and for the differentiation of the seam cells (*elt-5* etc.). Genes that regulate *ceh-16/engrailed* in *C. elegans* are unknown. Ventral: similar to *ceh-16/engrailed* in the seam cells, *lin-39/HoxD4/Dfd* and *ceh-20/Exd* are required for the regulation of *elt-5* and for the repression of *eff-1* to allow vulva formation. During postembryonic development the seam cells act as a belt with two straight boundaries that are lost in the absence of *ceh-16/engrailed*. Reproduced from Cassata et al. (2005), Copyright (2005), with permission from The Company of Biologists.

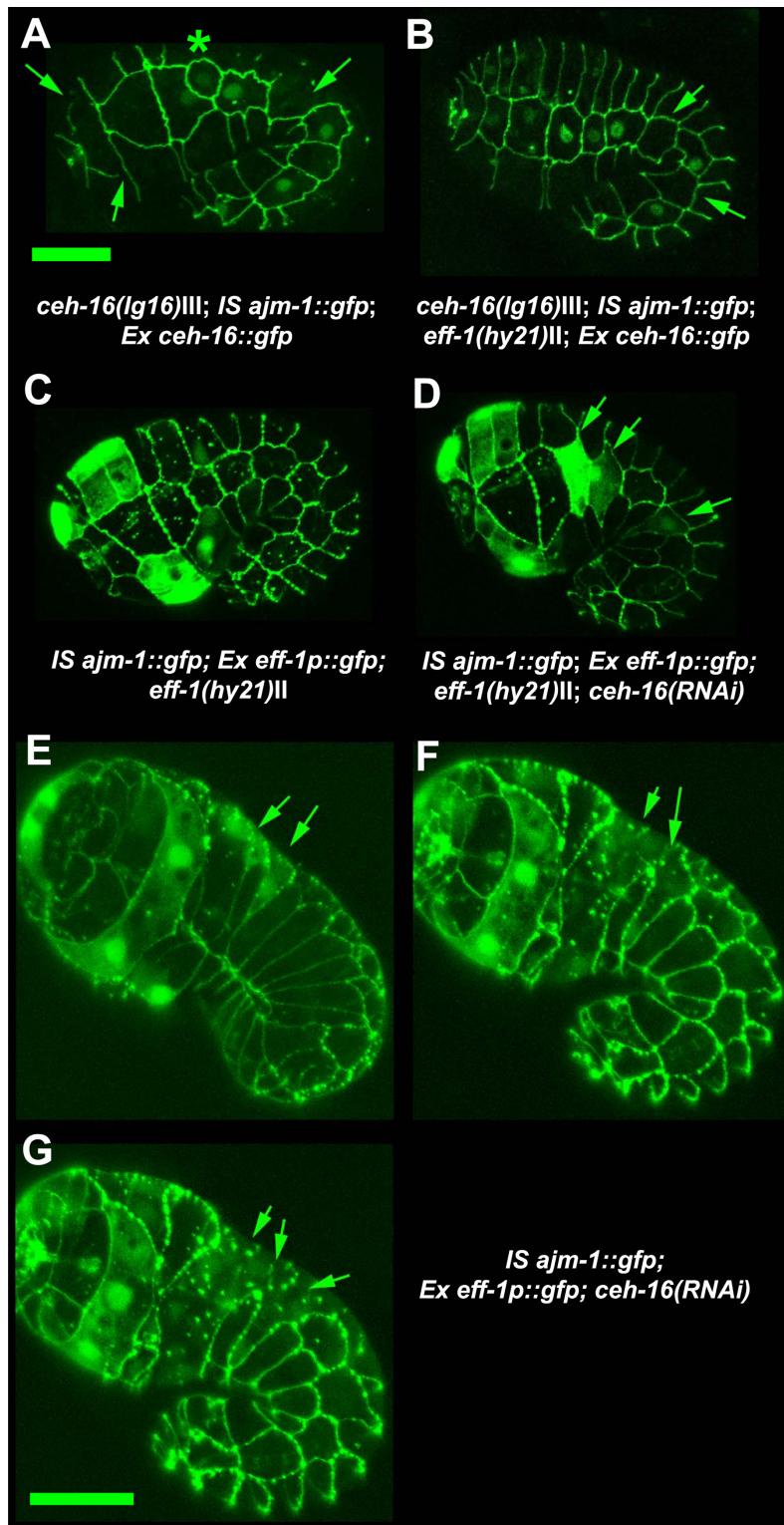


Figure 13. *eff-1* expression is repressed by *ceh-16* in the embryonic seam cells. (A) *ceh-16(lg16)* mosaic for the rescue marker *ceh-16::gfp* shows seam cell fusions (arrows) and cell migrations (asterisk). B) Mosaic animal as in (A) crossed into *eff-1(hy21)*; suppression of fusion (arrows). (C) *eff-1(hy21)* animal transgenic for an *eff-1::gfp* transcriptional construct that is expressed in cells committed to fuse, (D) as in (C) but in addition this animal is *ceh-16(RNAi)*. Ectopic *eff-1::gfp* expression (de-repression) in the seam cells (arrows) as a result of *ceh-16* inactivation and ectopic migration. (E-G) *ceh-16(RNAi)* embryo transgenic for *eff-1::gfp* taken at three time points: de-repression of *eff-1* expression in seam cells. Seam cells in which *eff-1* (expression of *eff-1p::gfp*) is de-repressed fuse (arrows). All embryos are transgenic for *ajm-1::gfp*. All animals are 1.5-fold stage except for (E) = bean stage. Scale bars: 10 μ m. Reproduced from Cassata et al. (2005), Copyright (2005), with permission from The Company of Biologists.

5.3.2. ELT-5 and ELT-6 repress lateral cell fusion in the embryo and larvae

Two GATA factors, ELT-5/EGL-18 and ELT-6 (see above), are critical for the differentiation and repression of cell fusion of the lateral seam cells (Figure 14; Koh and Rothman, 2001). Since in *elt-5 (RNAi)* animals the seam cells migrate and fuse ectopically (Koh and Rothman, 2001), *ceh-16* may act indirectly by activating *elt-5*, which in turn represses *eff-1*. This is supported by inappropriate expression of *eff-1 p::gfp* in *elt-5 (RNAi)* embryos (Figure 15; Cassata et al., 2005). Since *elt-5/elt-6* and *ceh-16* are necessary to maintain the identity of the seam cells, the repression of *eff-1*-mediated cell fusion is probably only one of the functions of these transcription factors (Figure 12).

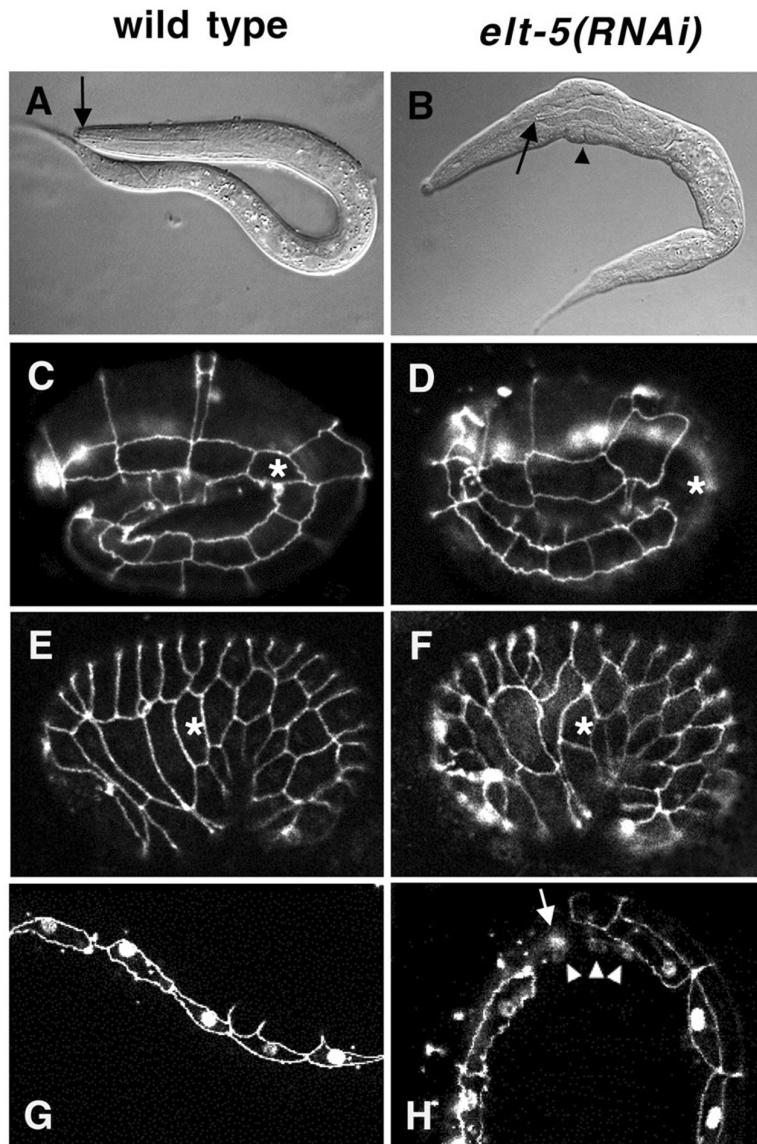


Figure 14. Phenotypes of *elt-5(RNAi)* embryos and larvae. (A,C, E, G) Wild-type; (B, D, F, H) *elt-5(RNAi)* animals. (A) Nomarski image of wild-type L1 larva. Arrow points to the buccal capsule, which is attached to the anterior end of the worm. (B) *elt-5(RNAi)* L1 larva showing lumps (arrowhead) and a detached buccal capsule (arrow) at the anterior end of the pharynx, which has contracted toward the posterior. (C-F) Embryos stained with mAb MH27 to visualize adherens junctions of epidermal cells. The asterisks mark the V1 seam cell. (C) Lateral view of a wild-type embryo at the 2.5-fold stage. The row of 10 lateral seam cells, all completely surrounded by adherens junctions, is prominently visible. (D) Lateral view of an *elt-5(RNAi)* embryo at the 2.5-fold stage. One of the seam cells, V1 (asterisk), does not show adherens junctions, indicating that it has fused with the neighboring hyp7 syncytium on the dorsal and ventral sides. (E) Lateral view of a wild-type embryo slightly past the comma stage. All ten seam cells are visible, although part of the most posterior seam cell, T, is out of focus. (F) Lateral view of an *elt-5(RNAi)* embryo slightly past the comma stage. One of the seam cells, V1 (asterisk), is ventrally misplaced, and its neighbors, H2 and V2, inappropriately contact each other. (G, H) L1-stage larvae expressing the seam cell marker SCM (nuclear signal) and AJM-1::GFP, a marker for adherens junctions. (G) Wild-type larva showing SCM expression in seam cells only. (H) *elt-5(RNAi)* larva showing SCM expression in nuclei of syncytial cells (arrowheads) near a fused seam cell (arrow). Reproduced from Koh and Rothman (2001), Copyright (2002), with permission from The Company of Biologists.

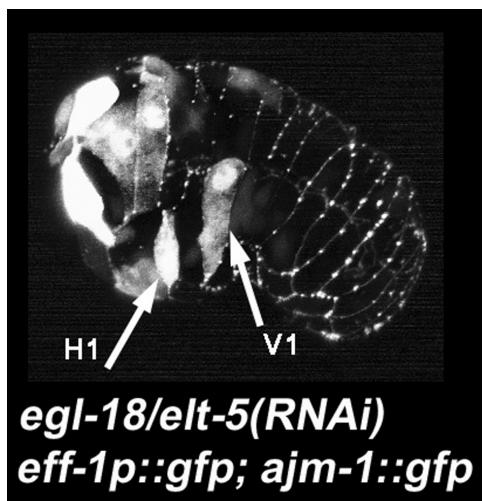


Figure 15. *elt-5/egl-18* represses *eff-1* expression. The arrows show two seam cells (H1 and V1) abnormally expressing *eff-1p::gfp*. Micrograph kindly provided by Gidi Shemer (Cassata et al., 2005).

Interestingly, cell fusion is also repressed in the ventral region of the epidermis by expression of other homeobox containing proteins *lin-39*/Dfd and *ceh-20*/Exd (Shemer and Podbilewicz, 2002) acting also through ELT-5 (Koh et al., 2002; see above). Thus, expression of distinct homeobox containing proteins in lateral and ventral hypodermal compartments may regulate cell fates through ELT-5-mediated repression of *eff-1* during embryonic and larval development (Figure 12D). While Wnt and Ras signaling pathways control the expression of *lin-39* in VPCs (Eisenmann and Kim, 2000; Eisenmann et al., 1998; Gleason et al., 2002; Maloof and Kenyon, 1998), it is not known what signaling pathways control *ceh-16* activity in the seam cells (Cassata et al., 2005). Very few effectors of Hox genes and *engrailed* have been identified in any organism. In *C. elegans*, a putative cell fusion protein (*eff-1*) is a strong candidate to be an effector of ELT-5/6, *lin-39-ceh-20*/Extradenticle and *ceh-16/engrailed* in distinct developmental regions and times (Cassata et al., 2005; Shemer and Podbilewicz, 2002).

5.3.3. Micro RNAs and regulation of the timing of postembryonic cell fusion

The timing of larval development is regulated by the expression of micro RNAs (Ambros, 2004; See *C. elegans* microRNAs). Expression of the *lin-4* micro RNA during the L1 is required for down-regulation of other heterochronic genes (e.g. *lin-14*) and execution of L2 developmental programs (Ambros and Horvitz, 1987; Lee et al., 1993). Likewise, expression of the *let-7* micro RNA during the L4 controls the levels of other heterochronic genes (e.g. *lin-29*) required for adult developmental programs (Reinhart et al., 2000). Mutations in heterochronic genes result in precocious or retarded phenotypes that affect the timing of cell fusion events throughout larval development. *lin-29* controls late fusion events in the L4 larvae, including the fusion between seam cells during the last molt (Ambros, 1989; Ambros and Horvitz, 1984; Slack and Ruvkun, 1997). *lin-29* regulates the timing of late gene expression during organogenesis of the lateral epidermis, vulva, uterus and male reproductive system (Bettinger et al., 1997; Bettinger et al., 1996; Euling et al., 1999; Newman et al., 2000; Rougvie and Ambros, 1995). *lin-29* activities are required for seam cells-vulval-uterine connections involving cell fusion events in the three interacting organs (Newman et al., 2000). Thus, *lin-29* controls cell cycle exit, secretion of adult cuticle and cell fusion. It is not known whether *eff-1* is an effector of *lin-29* and the heterochronic pathway.

5.4. Dorsal cell fusions

Dorsal hypodermal cells fuse during embryogenesis and most of the dorsal cells complete the fusion process halfway through morphogenesis. Very few genes controlling dorsal cell fusions have been identified. The Hox gene *ceh-13* controls the embryonic fusion of some anterior dorsal hypodermal cells (Brunschwig et al., 1999; Wittmann et al., 1997) and the Hox cofactor *nhr-25* (see above) may also affect the most anterior dorsal cell fusion in hyp7 (Chen et al., 2004).

idt-1 (*zu316*), an embryonic lethal mutation isolated in a screen for elongation defective embryos (M. Costa and J. Priess, personal communication), is characterized by a block in embryonic dorsal cell fusion. While the *idt-1* (Irregular Dorsal Fusion-1) phenotype is variable, all the dorsal cells can fail to fuse. Irregular cell fusion events occurring in *idt-1* mutants are retarded compared to wild-type. The anterior dorsal cells have a higher probability of

remaining unfused than posterior cells. However, all dorsal cells are able to remain unfused in the mutant, suggesting that *idf-1* is required as part of the fusion machinery or in its regulation and not only as a regional regulator of the process (T. Gattegno and BP, unpublished results; Gattegno, 1998; Gattegno, 2003; Gattegno et al., unpublished results).

5.5. Repression of *eff-1*-mediated cell fusion by the vacuolar-ATPase

fus-1 gene protects dorsal and ventral cells from late embryonic cell fusions. In strong *fus-1* mutations (Terns et al., 1997), all epidermal cells fuse except the seam cells. *eff-1* is epistatic to *fus-1*. *fus-1* encodes a membrane e subunit of the vacuolar H⁺-ATPase localized to the apical membranes of epidermal fusogenic cells. Thus, *eff-1* may be mislocalized to cell membranes in the absence of V-ATPase activity and/or expression, resulting in ectopic hyperfusion (Kontani et al., 2005). The V-ATPase appears to be the first posttranscriptional repressor of *eff-1*-mediated cell fusion.

5.6. Non-epidermal *eff-1*-independent cell fusion and the uterine-vulval connection

The utse is a H-shaped cell that connects the uterus to the seams with a thin hymen separating the vulval and uterine lumens. This syncytium is formed in the late L4 stage by cell fusion of the AC and 8 out of 12 π daughters (Newman and Sternberg, 1996; Newman et al., 1996; Sharma-Kishore et al., 1999). The *cog-2/egl-13* SOX domain and the *lin-11* LIM domain transcription factors are expressed in π cells and their daughters (Cinar et al., 2003; Hanna-Rose and Han, 1999; Newman et al., 1999). In *lin-11* mutants, the AC fails to fuse with the utse and a functional uterine-vulval connection is not formed (Newman et al., 1999). In addition, *lin-11* is covalently bound to SUMO (*smo-1*) and this modification is required for AC-utse and intratoroidal vulval fusions (Broday et al., 2004). In *egl-13* mutants, the AC also fails to fuse to the utse (Cinar et al., 2003; Hanna-Rose and Han, 1999) because the π cell lineage is initiated but not completed correctly (Cinar et al., 2003). The disruption of π cell development is at an earlier stage in *egl-13* than in *lin-11* (Cinar et al., 2003). *lin-11* appears to be a target of LIN-12 signaling in the π cells (Gupta and Sternberg, 2002). Thus, both *lin-11* and *egl-13/cog-2* are required for the maintenance of π cell fate and the differentiation of its progeny and not directly for cell fusion of the AC to utse. In addition, this heterotypic cell fusion event has been shown to be *eff-1*-independent (Shemer et al., 2004), and a NEM-sensitive factor (NSF/SEC-18) appears to be required for the AC-utse cell fusion and the formation of the uterine-vulval connection (A. Newman, personal communication).

6. Open questions

C. elegans has become an excellent research system to study cell membrane fusion and to understand the functions, regulation and mechanisms of cell fusion. Mutations in *eff-1* result in a complete and specific block of cell fusion without affecting other cellular functions like cell migration and adhesion. While the mechanisms of cell fusion are poorly understood, it is already clear that *eff-1* has a central role in most cell fusions in *C. elegans*. A working model is that stochastic fluctuations in the levels of *eff-1* on the plasma membranes, coupled to transcriptional and post-transcriptional mechanisms that amplify these changes, may lead to fusion of some cells where the levels of activated *eff-1* reach a threshold that triggers membrane fusion (Figure 16). A large number of regulatory genes and many more still to be discovered control the formation of a giant multinucleate cell. Most of these genes appear to precisely regulate *eff-1* transcription throughout development. It is expected that numerous posttranslational regulators of cell fusion will also act on *eff-1* stability and localization. In particular, it appears that in the worm and probably in other organisms as well, there is a widespread repression of cell membrane fusion and not only specific activation of this process. This is probably due to the negative effects of hyperfusion on viability. As occurs in program cell death in worms, ectopic activation of cell fusion results in embryonic lethality, while cell fusion failure results in sub viable larvae and adults with many morphological and behavioral defects. Many fascinating questions are completely open and waiting for a critical mass of *C. elegans* biologists interested in answering them.

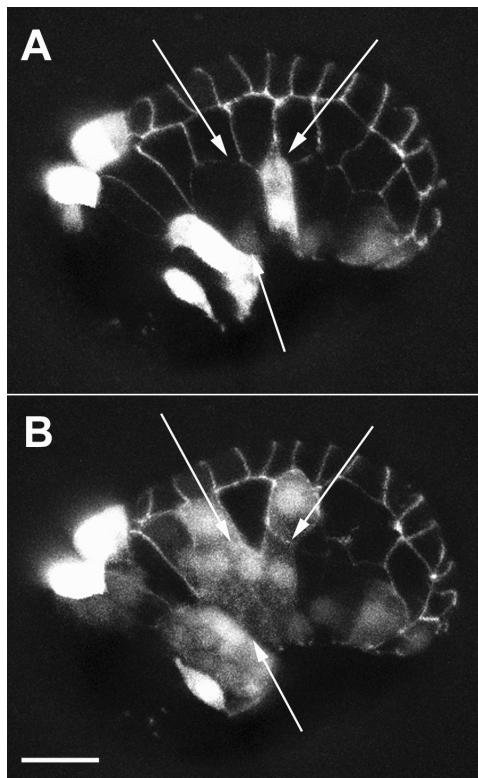


Figure 16. *eff-1*-mediated ectopic fusion leads to mixing of cytoplasmic contents. *eff-1(hy21); hsp::EFF-1* embryo expressing *ajm-1::gfp* and *eff-1p::gfp*. Following heat shock, ectopic fusion between normally nonfusing cells (A) (embryonic dorsal with lateral cells) allows mixing of cytoplasmic contents (B). *eff-1p::gfp* transcriptional construct served as a cytoplasmic marker. This marker is not expressed normally in the embryonic lateral seam cells of wild-type, *eff-1(-)*, and control animals. The cytoplasmic mixing is coupled to disappearance of apical junctions (arrows). Dorsal is up and anterior is left. The scale bar represents 10 μ m. Reproduced from Shemer et al. (2004), Copyright (2004), with permission from Elsevier.

6.1. Mechanistic questions

- Is there a trigger (e.g. binding, low pH, calcium) that initiates cell fusion?
- What are the intermediates and detailed kinetics of early and late fusion steps?
- What is the fate of the vesicles generated during cell fusion?
- How does *eff-1* affect intracellular membrane dynamics (see Figure 3 and Figure 18)?
- How does *eff-1* fuse cells?
- What domains of the *eff-1* protein have central roles in cell fusion?
- Which *eff-1* isoform(s) are essential and sufficient for cell fusion?
- Is *eff-1* a fusogen similar to viral class I or II fusogens or is it a class of its own?
- What is the structure of *eff-1*?
- Is there cooperativity between different *eff-1* proteins to fuse cells?
- Is *eff-1* the only effector of cell fusion in *C. elegans*?
- Are there *eff-1* receptors and co receptors?
- Does *eff-1* have functions independent of cell fusion?
- Does *eff-1* act as an anti-cell migration factor in the epidermis (see Figure 17 and Figure 18)?
- Does cell fusion have a role in larval elongation?
- Are the late defects that develop after fusion failure direct or indirect?
- Are there other genes required for cell attachment, pore formation, expansion of cytoplasmic bridge and rearrangement of cytoplasmic contents during cell fusion?

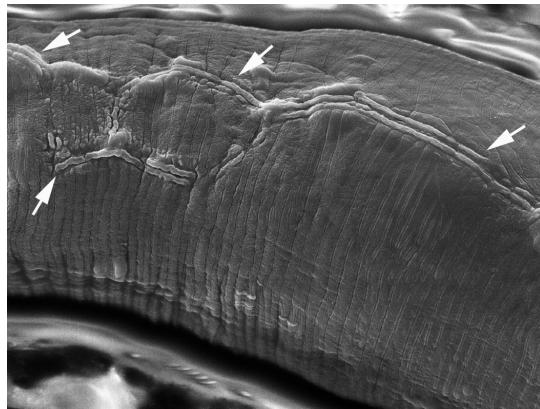


Figure 17. SEM of *eff-1* body cuticle shows defective alae. The alae are formed by the underlying seam cells, and show distinct differences at various developmental stages (singlet in L1, quartet in dauer, triplet in adult, no alae in L2, L3 or L4; D.H. Hall and R. Lints, wormatlas.org/hermaphrodite/cuticle/Cutframeset.html). In wild type the seam cells fuse into a single midline row, whereas in *eff-1* they fail to fuse and can form overlapping or parallel rows. Here in an adult *eff-1* animal, arrows indicate the malformed alae running lengthwise along the lateral body wall, generally as doublet or triplet ridges. In wild type the adult alae form one triplet ridge at the exact midline, whereas in the *eff-1* animals the alae wander and split repeatedly to form several parallel stripes. Reproduced from Shemer et al. (2004), Copyright (2004), with permission from Elsevier.

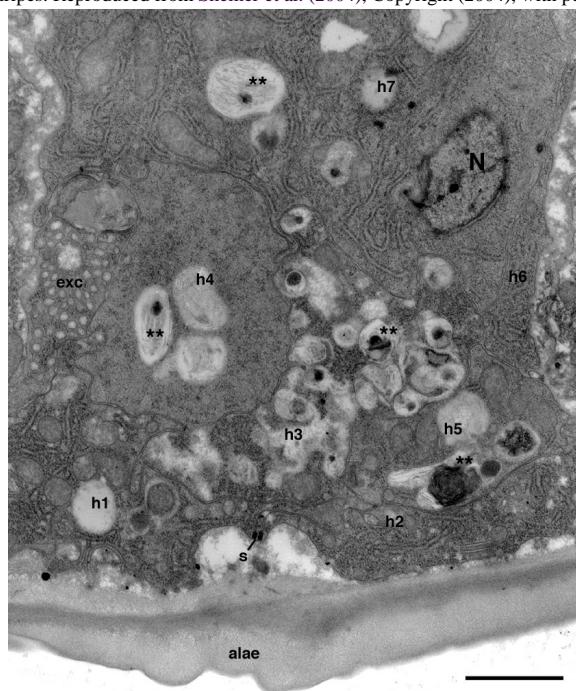


Figure 18. Autophagy and epithelial stratification in lateral hypodermal tissue. *eff-1* animal shows reduced alae formation, and fusion failure has caused hypodermal tissue to be divided by membranes into 7 separate compartments, numbered here as h1-h7, of which only h1 and h2 face the cuticle directly. Unlike normal syncytial epidermis, where each *hyp* compartment is exposed to the cuticle, this portion of lateral epidermis has become stratified and the interior hypodermal cells show signs of autophagy (* *)(Melendez et al., 2003), perhaps due to a buildup of cuticle-related products in cells h3-h7. N, hypodermal nucleus; exc, excretory canal; s, seam cell. Ventral is to the left, lateral side is down. Scale bar is 1 μ m. Reproduced from Shemer et al. (2004), Copyright (2004), with permission from Elsevier.

6.2. Regulatory questions

eff-1 is controlled by regulators of cell fusion (*ref-1* and *ref-2*), Hox genes and other cofactors in the ventral Pn.p cells and by the homeodomain gene *ceh-16/engrailed* and GATA factors ELT-5/EGL-18 in the lateral seam cells.

Are these regulators acting directly or indirectly on *eff-1* transcription?

Are there posttranscriptional mechanisms of regulation of *eff-1*?

How does a V-ATPase represses *eff-1*-mediated epidermal fusion?

How does the heterochronic pathway control cell fusion?
How are dorsal hypodermal cell fusions regulated in the embryo?

7. Implications of cell fusion research beyond worms

Cell fusion is a very frequent and naturally occurring process about which very little is known. We are beginning to understand why cells fuse during the formation of organs in *C. elegans* (Figure 5). Cell fusion research has potential applications in the fields of contraception, cancer, gene therapy and stem cell research. Cell fusions in the epidermis and vulva of *C. elegans* and during the formation of muscles, bones and placenta in humans, involve regulation of cell differentiation. Dividing cells that are potentially invasive (e.g. epidermal, muscle, placenta and stem cells) fuse to form part of multinucleated cells, stop dividing, and become differentiated. Thus, defects in cell fusion during development may result in cancerous growth in mammals (Duelli and Lazebnik, 2003). However, as with apoptosis, this is not the case in worms. Finally, laser-induced cell fusion in worms (Irle and Schierenberg, 2002; Schierenberg, 1984; Schierenberg, 1987) and other organisms (Avigan et al., 2004; Lundqvist et al., 2004) is an under-explored technique with great potential for understanding many basic and applied biological questions.

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