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The Type I Membrane Protein EFF-1 Is Essential for Developmental Cell Fusion

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Summary

Multinucleate cells are widespread in nature, yet the mechanism by which cells fuse their plasma membranes is poorly understood. To identify animal fusogens, we performed new screens for mutations that abolish cell fusion within tissues of *C. elegans* throughout development. We identified the gene *eff-1*, which is expressed as cells acquire fusion competence and encodes a novel integral membrane protein. EFF-1 sequence motifs suggest physicochemical actions that could cause adjacent bilayers to fuse. Mutations in the extracellular domain of EFF-1 completely block epithelial cell membrane fusion without affecting other prefusion events such as cell generation, patterning, differentiation, and adhesion. Thus, EFF-1 is a key component in the mechanism of cell fusion, a process essential to normal animal development.

Introduction

Cell fusion is a fundamental biological process involved in sexual reproduction and syncytium formation during organ development. A central problem of cell biology is how membrane bilayers fuse despite high repulsive energy between the hydrated polar heads of phospholipids. Models for virus-cell and intracellular vesicle-membrane fusion are based on activity of fusion proteins or fusogens that have been identified by a combination of genetic and biochemical methods (Hernandez et al., 1996; Jahn and Sudhof, 1999; Shemer and Podbilewicz, 2000). For intracellular membrane fusion reactions, the SNARE proteins, Rab-GTPases, and their cofactors promote prefusion membrane attachment, yet the mechanism of actual membrane merger remains controversial (Almers, 2001; Jahn and Sudhof, 1999; Peters et al., 2001; Weber et al., 1998). For extracellular membrane fusion reactions, there is strong evidence that viral fusogens reduce the activation energy for phospholipid-mediated fusion or organize the fusion site (Chernomordik et al., 1998).

Much less is known about the mechanisms that mediate plasma membrane fusion in fertilization and syncytium formation (Shemer and Podbilewicz, 2000). Fertilins and meltrins, members of the ADAM family of membrane glycoproteins, are no longer considered candidate fusogens (Cho et al., 2000; Hernandez et al., 1996). Syncytin, a proviral gene product expressed in primate placenta, has been shown to act as a fusogen in vitro, but its recent appearance in evolution cannot account for the ancient role of cell fusion in placentation (Mi et al., 2000). Several genes identified in yeast, flies, and mammals appear to mediate events that precede membrane fusion, including cell attachment, cell polarization, and cell wall reorganization (Shemer and Podbilewicz, 2000; Taylor, 2000; Wassarman, 1999). A membrane protein (Prm1p) may facilitate actual lipid bilayer fusion in yeast, although it is not absolutely required (Heiman and Walter, 2000).

To identify molecules acting in developmental cell fusion, we used *Caenorhabditis elegans*, a genetic animal system where cell fusion occurs in a variety of tissues and the process can be easily observed. One third of somatic cells fuse in a fully characterized and reproducible pattern during *C. elegans* development (Podbilewicz, 2000; Podbilewicz and White, 1994; Sulston and Horvitz, 1977; Sulston et al., 1983). Most fusions are between cells that constitute polarized epithelia (Figure 1). Several transcription factors are known to regulate specific cell fusion events in *C. elegans* (Alper and Kenyon, 2001; Ch'ng and Kenyon, 1999; Clark et al., 1993; Koh and Rothman, 2001). However, no proteins involved in the actual membrane fusion event have been identified. In this paper, we isolate two mutations that specifically block cell fusion in all epidermal and vulval epithelia in the worm, and we describe the phenotypic and molecular characterization of the novel *eff-1* gene.

Results

Generation of Cell Fusion Mutant Worms

To study the molecular mechanism of cell fusion and its developmental significance, we mutagenized transgenic *C. elegans* expressing AJM-1::GFP, a fluorescent protein that localizes to adherens junctions (zonulae adherens), revealing borders between epithelial cells (Köeppen et al., 2001; Mohler et al., 1998). We looked for mutants in which epithelial cells were properly differentiated and patterned but failed to fuse. Two such mutants, *eff-1(oj55)* and *eff-1(hy21)*, were isolated in independent screens and failed to complement (see Experimental Procedures). Both mutations result in viable worms that show severe body morphology defects associated with cell fusion failure. To assess the defects in *eff-1* mutants, we followed changes in their epithelial cell interactions throughout development (Podbilewicz, 2000; Podbilewicz and White, 1994).

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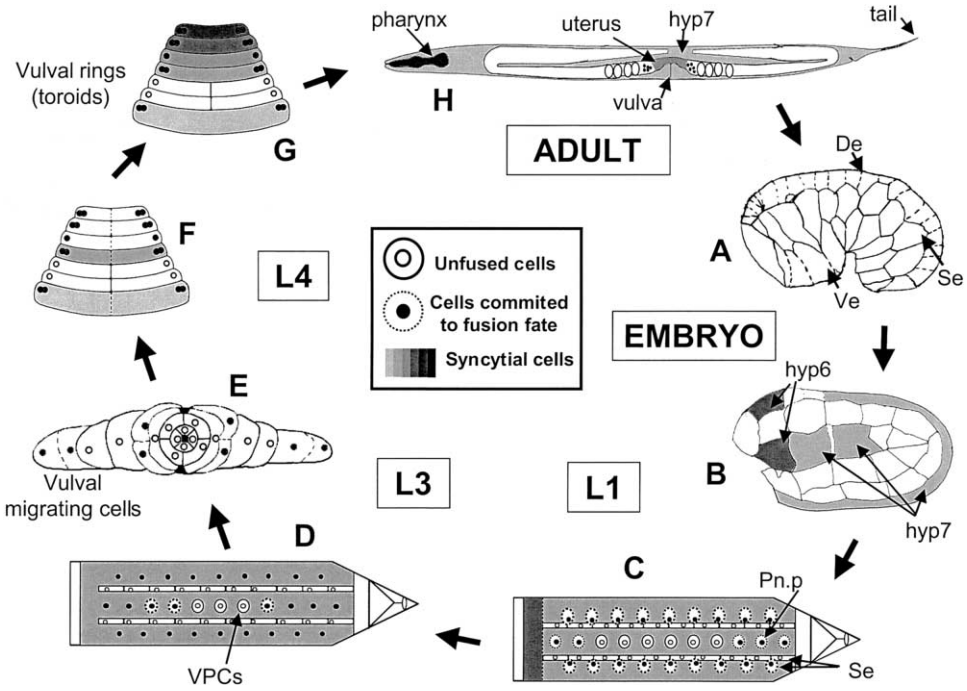

Figure 1. Cell Fusion in *C. elegans* Development

(A and B) As an embryo surrounded by dorsal epidermis (De), ventral epidermis (Ve), and bilateral seam cells (Se) elongates, dorsal and ventral epithelia fuse to yield syncytia hyp6 and hyp7.

(C and D) Ventral projections of L1 and L3 epidermal topology show Pn.p flanked by dividing seam cells (Se) and surrounded by hyp7 (gray). Five Pn.p cells fuse to hyp7 in L1 (C); the rest become vulva precursor cells (VPCs; [D] and [E]). By late L3, hyp6, three VPCs, and several daughters of ectoblastic seam cells also fuse to hyp7 (D).

(E–G) Fusions between some migrating cells and within toroids make five syncytial rings. In L4, vulval cells migrate to form a stack of rings (lateral view; [F]).

(H) Other organs containing syncytia are shown in the adult hermaphrodite (Shemer and Podbilewicz, 2000).

Epithelial Fusion Failure in *eff-1* Mutant Embryos

eff-1(oj55) and *eff-1(hy21)* mutant embryos fail in all epidermal cell fusions (Figure 2). In both wild-type and *eff-1* mutant embryos, the epidermal cells are born 210–230 min after first cleavage, and undergo identical migration and patterning events (Figures 1A, 2A, and 2B). However, all 43 cells that would normally form syncytia fail to fuse in *eff-1*, as the embryo elongates to form a worm ($n > 100$; Figures 1B, 2C, and 2D). In wild-type embryos, cells fuse 3 hr after they are generated (Podbilewicz and White, 1994), while in *eff-1* embryos, the block in epidermal fusion is maintained throughout embryogenesis, until L1s hatch 10 hr later.

eff-1 Mutant Epidermal Cells Fail in Plasma Membrane Permeabilization

Previous characterization of live epidermal cell fusion events showed that dissolution of the intercellular junction actually occurs minutes after the plasma membranes have disappeared between two fusing cells (Mohler et al., 1998). To exclude the possibility that *eff-1* mutations might block junction disassembly without blocking membrane fusion per se, we assessed the integrity of epidermal cell membranes by observing diffusion of soluble cytoplasmic GFP. We used a fragment of the *lbp-1* promoter (Plenefisch et al., 2000) that drives expression of GFP within only a subset of the precursors of hyp7. This approach revealed rapid mixing of cytoplasm between labeled and unlabeled cells in the earliest stages of cell fusion in wild-type embryos (Figures 2E–2G). In contrast, *eff-1* mutant embryos retained cytoplasmic GFP discretely within single epidermal cells, even through elongation and hatching (Figures 2H–2K).

Postembryonic Cell Fusion Defects in the Epidermis from the L1 to the Adult

In contrast to observations in embryos, we found that the two *eff-1* alleles differ in their effects on postembryonic fusions. In *eff-1(oj55)* at all temperatures and in *eff-1(hy21)* at 15°C or 20°C, most epidermal cells derived from lateral seam and the ventral P cells failed to fuse to hyp7 even well beyond 3 hr after they were born ($n > 300$). Yet many cells did eventually fuse during larval development. At 25°C, however, *eff-1(hy21)* larvae had a fully penetrant cell fusion defect ($n > 100$; Figure 3B). Similarly, in larvae with *eff-1* mutations placed in *trans* to a deficiency (*eff-1(oj55)/mnDf105* or *eff-1(hy21)/mnDf105*), all epidermal cells failed to fuse. Thus, residual function in *eff-1(oj55)* and *eff-1(hy21)* at 15°C appears to allow some successful fusion events during postembryonic development. Further loss of function in *eff-1(hy21)* at 25°C and in the *eff-1(−)* deficiency genotypes abrogates cell fusions entirely. We suspect that *eff-1(hy21)* approaches a functional null phenotype at 25°C,

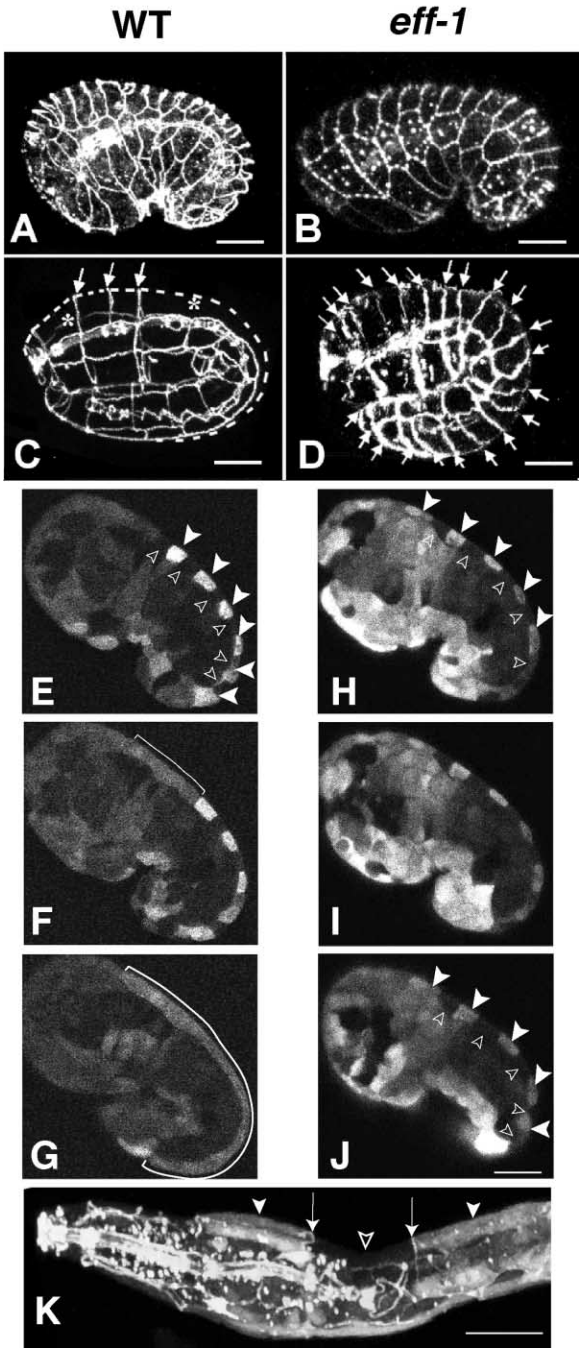

Figure 2. Defective Cell Fusion during Embryogenesis in *eff-1* Mutants

Anterior is left and dorsal is up.

(A–D) Adherens junctions in wild-type (A and C) and *eff-1(hy21)* (B and D) embryos labeled with MH27 (A and C) or AJM-1::GFP (B and D).

(A and B) Embryos at the onset of elongation.

(C and D) Two-fold elongated embryos. In wild-type (C), most cells have fused (asterisks), while three borders remain unfused (arrows). In *eff-1(hy21)* (D), all cells fail to fuse (arrows indicate dorsal junctions).

(E–J) Cytoplasmic diffusion between nonfluorescent cells (open arrowheads) and cells labeled with cytoplasmic *lbp-1p*::GFP (white arrowheads). Time lapse optical sections show the development of wild-type (E–G) and *eff-1(oj55)* (H–J) embryos from comma to 1.5-fold. Cells progressively fuse and mix their cytoplasms during wild-type elongation ([F] and [G]; syncytia are indicated by outline). Cytoplasms remain distinct during *eff-1(oj55)* elongation (I and J).

(K) *eff-1(oj55)* L1 expressing both AJM-1::GFP and *lbp-1p::gfp*. Adherens junctions (arrows) separate three unfused cells with distinct cytoplasms. The scale bar represents 10 μm.

and we have used this allele for all subsequent developmental studies.

To determine whether effects of *eff-1(hy21)* were specific to cell fusion, we followed the behavior of cells in larvae at different stages of 25°C development. We found that all epidermal cells were generated at the right stages of development, exhibited appropriate cell lineage patterns, and made normal cell contacts and short-range migrations (Figure 3). However, cells failed to fuse even in the adult, and ectopic adherens junctions connected all regions of the epidermis (Figures 3D, 3F, and 3H). We examined thin sections of L4 *eff-1(hy21)* animals by electron microscopy and found extra epithelial cells bounded by intact membranes and linked by adherens junctions (I. Kolotuev and B.P., unpublished data). Unfused seam cells also produced abnormal cuticle alae. In summary, the morphology and behavior of the unfused epithelial cells appear normal by light and electron microscopy, except that plasma membranes and cell junctions remain intact and unfused cells send processes that cross the boundaries between dorsal, ventral, and lateral epidermis.

eff-1-Mediated Cell Fusion Is Essential for Vulval Organogenesis, Tail Remodeling, and Normal Body Morphology

eff-1(hy21) was isolated in a screen for temperature-sensitive vulva defects. One hundred percent of adult *eff-1(hy21)* worms at 25°C are egg-laying defective ($n = 715$), 46% have a protruded vulva (142/306), 18% explode in the vulva region (137/751), and 2% have one or two additional pseudovulvae (14/784), suggesting that cumulative cell fusion failure results in significant defects in the egg-laying machinery. We followed the vulva precursor cell (VPC) lineage and all 25 fusion events involved in vulva development (Figures 1D–1G; Sharma-Kishore et al., 1999). In the mutant strain, VPCs were generated correctly in the L1 but remained surrounded by epidermal cells that had failed to fuse into hyp7 (compare Figures 3C and 3D). Nonetheless, during the mid-L3 stage, three of the VPCs were induced to undergo divisions resulting in the typical array of 22 cells. The other three VPCs divided only once, as in the wild-type, but failed to fuse to hyp7, forming part of an ectopic ventral midline composed of unfused cells (Figure 3F). In invagination and terminal vulva formation, migrations, cell contacts, and rearrangements of the 22 vulval cells occurred normally, but all fusions were blocked in *eff-1(hy21)* (Figure 3H).

One hundred percent of hermaphrodites displayed abnormalities in formation of the tail spike, which is known to involve a cell fusion event (Sulston et al., 1983). Several fusions have also been linked to retraction of the tail spike in males and formation of the mature copulatory bursa (Nguyen et al., 1999). We found that *eff-1*

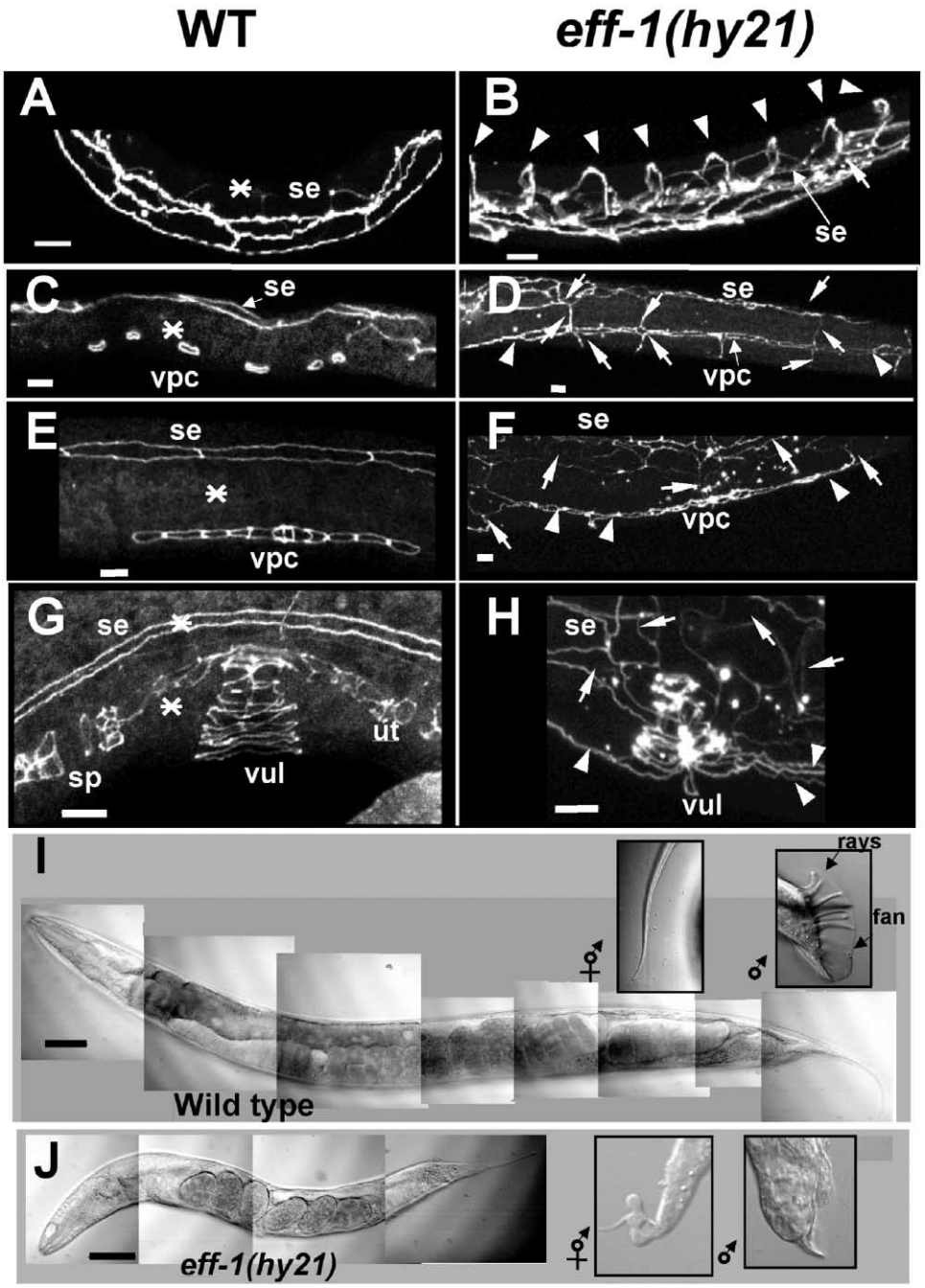

Figure 3. Lack of Postembryonic Cell Fusion and Defective Morphogenesis in *eff-1* Mutants

Confocal reconstructions (A–H) and Nomarski pictures (I and J) of wild-type (A, C, E, G, and I) and *eff-1(hy21)* (B, D, F, H, and J) worms. Asterisks mark syncytial cells. Arrowheads (dorsal or ventral cells) and arrows (seam cells) mark junctions that fail to fuse.

(A and B) L1 stage. In the wild-type, dorsal epithelial cells and daughters of the seam cells fuse into the syncytial cell hyp7 (A). In the mutant, these cells fail to fuse (B).

(C and D) Mid-L2 stage. In (C), Pn.p and migrating seam cells fuse, leaving six cells that will form the vulva (vpc). Pn.p and seam cell daughters fail to fuse in *eff-1(hy21)* (D).

(E and F) Mid-L3 stage. Unfused Pn.p cells in the mutant remain attached to VPCs (F), and seam cell daughters also fail to fuse.

(G and H) Late L4.

(G) Syncytia in the wild-type seam, hyp7, and vulva.

(H) In *eff-(hy21)*, extra unfused VPCs and Pn.p cells remain attached to seven unfused vulval rings, and postmitotic seam cells also remain unfused.

(I and J) Morphological defects resulting from lack of cell fusion. Compared with wild-type (I), the mutant adult (J) is dumpy. Inserts show tails of adult worms, with deformed hermaphrodite tail tip and absence of male tail rays and fan in the mutant (J). Lateral views (A–C and F–J); ventrolateral views (D and E). Anterior is left. Se, seam cells; vpc, vulva precursor cells; vul, toroidal cells comprising the vulva; sp, spermatheca; ut, uterus. The scale bar represents 5 μm in (A)–(H) and 50 μm in (I) and (J).

males are unable to mate efficiently, and that male tail morphogenesis was defective in all L4s and adults analyzed ($n > 50$). Patterning of the rays was abnormal and the tip had a spike reminiscent of the hermaphrodite leptoderan pointed tail (Figures 3I and 3J).

General anatomy and behavior are also compromised. *eff-1(hy21)* mutants at 25°C are Deformed, Dumpy ($n > 3000$), Uncoordinated ($n > 1000$), and small with Scrawny body morphology (49/957; Figure 3J). These defects are much less severe in *eff-1(hy21)* at 15°C and in *eff-1(oj55)* at all temperatures, showing a correlation between the severity of cell fusion failure and abnormal body morphology, behavior, and growth. To determine the temperature-sensitive period (TSP) for the *eff-1(hy21)* allele, we used shiftup and shiftdown experiments between 15°C and 25°C (see Supplemental Figure S1 at http://www.developmentalcell.com/cgi/content/full/2/3/355/DC1). The TSP for dorsal cell fusion starts at the comma stage embryo, 1 hr before the first embryonic cell fusion event in morphogenesis, and ends in the mid-L1. The TSP for adult body length starts during late embryogenesis, 4 hr after the TSP for dorsal cell fusion, suggesting that elongation defects in *eff-1(hy21)* are a consequence of the cell fusion block.

The *eff-1* Gene Encodes Novel Membrane Glycoproteins

We cloned the *eff-1* gene by standard methods and found that it corresponds to the predicted gene C26D10.5 (GenBank accession numbers NM_063356 [genomic] and Z54327 [cDNA]. See Supplemental Data on cloning and Supplemental Figures S2A–S2D). C26D10.5 is predicted to contain eight exons, encoding a 658 amino acid type I integral membrane glycoprotein (EFF-1A), with eight predicted pairs of disulfide-linked cysteines, six potential sites for N-linked glycosylation, and two potential sites for O-linked glycosylation. We also found evidence in cDNAs of alternative splicing. In addition to a pattern encoding EFF-1A, a second splicing pattern shortens the seventh and largest exon, encoding a 596 amino acid protein (EFF-1B). EFF-1A and EFF-1B share common extracellular and transmembrane domains, but differ completely in their C-terminal cytoplasmic tails (Supplemental Figures S2E and S2F). Sequencing the C26D10.5 coding regions from homozygous *eff-1* mutant worms revealed mutations in the extracellular domain, from Pro183 to Leu in *eff-1(hy21)* and from Ser441 to Leu in *eff-1(oj55)*.

Neither EFF-1 isoform shows appreciable similarity to any protein of known function. The EFF-1 proteins have detectable similarity to two other predicted *C. elegans* proteins, C26D10.4 (89% for both EFF-1A and EFF-1B) and C44B7.3 (26% for EFF-1A and 28% for EFF-1B). However, no evidence exists for expression of C26D10.4 (a possible pseudogene duplication of C26D10.5), and C44B7.3 has not been tested functionally. No other sequenced organisms appear to have genes with sequence homology to *eff-1*. Interestingly, however, both EFF-1 protein isoforms are predicted to contain a region similar to known phospholipase A_2 (PLA_2) aspartic acid active sites. In addition, a short hydrophobic stretch within the extracellular domain is reminiscent of known "fusion peptides" within fusogenic viral glycoproteins (see Supplemental Figure S2F for sequence information).

eff-1 Expression Is Induced in Epidermal Cells Preparing to Fuse

We used a 7.5 kb fragment of *eff-1* promoter to drive GFP in transgenic worms. Expression of *eff-1p::gfp* was silent through the first third of embryogenesis, first appearing about 230 min after first cleavage in a subset of epidermal precursor cells (Figure 4A). Over the next 3 hr, these and additional fluorescent cells were observed to migrate over the ventral and dorsal surfaces of the embryo, and the majority of GFP-positive cells fused to form the hyp6 and hyp7 syncytia. As elongation progressed, GFP was also expressed in a pair of cells that fused to form the binucleate "tail spike" (Figures 4B and 4C; see also Supplemental Movie S1). After hatching, *eff-1p::gfp* expression persisted in large epidermal syncytia through adulthood. Mononucleated epidermal cells—including the seam cells and the VPCs—remained nonfluorescent until shortly before undergoing larval fusion events (Figures 4D and 4F). More specifically, GFP was seen in (1) nonstem daughters of the seam cells shortly before they fused into hyp7 (Figure 4D); (2) vulval cells invaginating to form toroids during morphogenesis (Figure 4G); and (3) the rays and fan of the adult male tail (Figure 4I). Expression was also seen in nonepidermal organs known to contain syncytia, including the pharynx and uterus (Figures 4E and 4H). Interestingly, a few cells that express *eff-1p::gfp* have never been observed to fuse, such as some ventral epidermal precursors in the embryo and several neurons (Figures 4B and 4E).

Discussion

Epidermal and vulval cell fusions fail in *eff-1* mutants. The mutant cells appear normal in all aspects of differentiation and development other than cell fusion. Thus, we conclude that the *eff-1* gene is required specifically for the process of membrane fusion. Three lines of evidence demonstrate that *eff-1* mutant cells are blocked at or before the earliest stage of membrane fusion: cytoplasm remains contained within individual epidermal cells without spreading through a growing syncytium (Figures 2H–2K), adherens junctions remain between cells (Figures 2 and 3), and membranes between unfused cells appear intact by transmission electron microscopy (data not shown). Thus, EFF-1 function must be necessary prior to permeabilization of plasma membranes, diffusion of cytoplasm between cells, and ultimate rearrangement of membrane, cortical, and cytoplasmic structure (Figure 4K). EFF-1 has a strict requirement and specificity for the process of developmental cell fusion.

The phenotypes of *eff-1* mutants shed new light on the mechanism by which cells fuse. Cells must actively acquire fusion competence by expressing a nonredundant set of proteins, of which EFF-1 is an essential component. Furthermore, the cell fusion mechanism is separable from the rest of the biological function of the cell within the organism. The more specific question of how

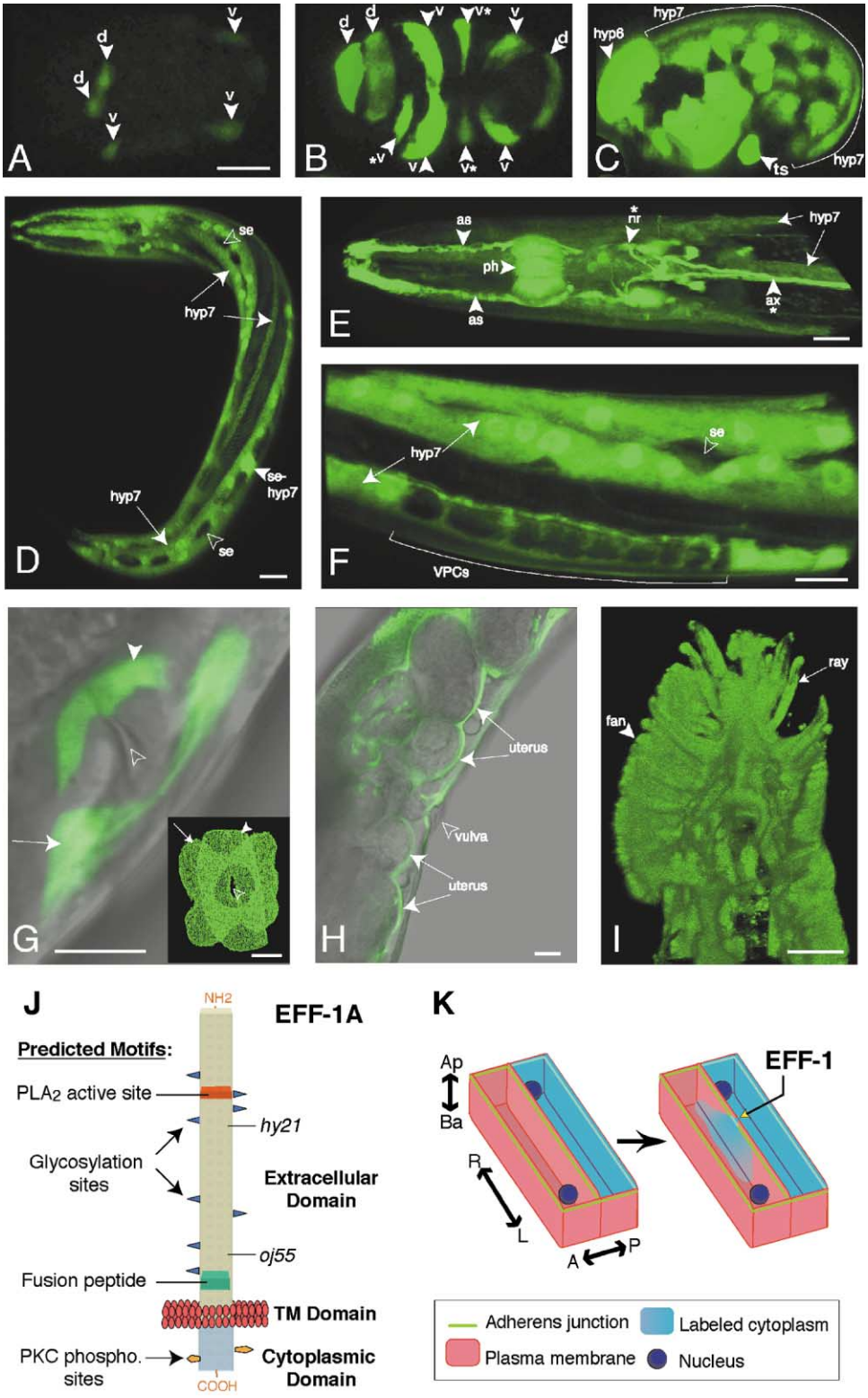

Figure 4. *eff-1* Expression in Fusing Cells and Models of EFF-1 Structure and Function in Cell Fusion

Expression of *eff-1p::gfp* in wild-type embryos and worms.

(A–C) Time course of expression in a single embryo.

(A) An approximately 500-cell embryo (250 min) showing first expression of GFP in epidermal precursors.

(B) Ventral view of enclosed embryo (340 min) with GFP in ventral (v) and dorsal (d) epidermal cells.

(C) Lateral view at 1.5-fold stage (430 min), with GFP throughout hyp6 and hyp7 syncytia and in tail spike cells (ts).

EFF-1 promotes membrane fusion cannot yet be precisely answered. However, analysis of the *eff-1* gene structure indicates two extracellular motifs that might play physicochemical roles in the function of the EFF-1 membrane glycoprotein (Figure 4J). First, a predicted PLA_2 aspartic acid active site could drive catalytic changes in the chemistry, stability, or local curvature of membrane leaflets. A PLA_2 enzyme has been implicated in mammalian fertilization (Wassarman, 1999), and it is conceivable that PLA_2 interfacial catalysis or binding to lipid receptors may drive EFF-1-mediated membrane fusion. Second, a hydrophobic-sided α helix within EFF-1 might act similarly to essential "fusion peptides" in a number of viral fusogens (Hernandez et al., 1996), binding to target membranes and driving the merger of adjacent bilayers. Both of these possibilities will have to be tested directly in future experiments.

Why Do Cells Fuse?

The functions of somatic cell fusion have until now been a subject of speculation because of the lack of mutants that specifically block developmental cell fusion (Podbilewicz, 1996; Podbilewicz and White, 1994). The isolation and characterization of *eff-1* mutants allow us to test previously proposed functions of cell fusion in morphogenesis (Podbilewicz, 2000; Shemer and Podbilewicz, 2000). It does not appear that cell fusion is normally used by *C. elegans* as a way to interrupt proliferative divisions; none of the cells deviate from the normal lineage pattern or numbers of cells generated when cell fusion is blocked. Unfused cells also continue to form structurally sound epidermis and cuticle in these mutants. However, morphological abnormalities that accumulate during development point to crucial functions for cell fusion in determining both overall body shape and fine detail of specialized structures in *C. elegans*. First, cell fusion is required for normal changes in body length as the worm increases in size. This could reflect a decreased plasticity in epidermis that has failed to form the large hyp7 syncytium. Ectopic extensions of filopodia by unfused mutant cells (see, for example, Figure 3H) indicate that fusion may also normally restrict potential avenues for cell migration, thereby promoting body elongation along the correct axis. Second, fusion can extinguish the developmental potential of a cell and delimit the range of inductive interactions. In 2% of mutant hermaphrodites there is a multivulva phenotype, suggesting that spurious interaction of unfused hyp7 cells with the vulva primordium induces the epidermal cells to change to a vulval fate. Thus, cell fusion normally acts to isolate an interactive group of cells (e.g., the VPCs) and to prevent inappropriate inductions. By further studying the *eff-1* phenotype, we expect to elucidate the role of cell fusion in tail morphogenesis, pharyngeal function, and elaboration of the reproductive tract.

EFF-1 is the first protein known to be required specifically for the fusion of somatic cell membranes in *C. elegans*. However, EFF-1 is unlikely to act alone. Many fusogens in other systems require binding ligands on their targets. Furthermore, each *C. elegans* epidermal cell displays remarkable specificity in fusing with one correct cell out of more than ten adjoining neighbors. Thus, even if hypothetical fusogenic motifs in EFF-1 (PLA_2 or fusion peptide) prove to be intrinsically active, it is certain that other cellular pathways governing cell polarity and intercellular communication must guide EFF-1 in targeting the appropriate cell border for destruction. Identification of factors interacting with EFF-1 in the process of cell fusion will be a powerful approach to solving both the biophysical and cell biological mechanisms that control this irreversible and developmentally crucial process.

Experimental Procedures

General Methods

All experiments were done at 20°C unless otherwise stated. *C. elegans* strain N2 served as the wild-type strain (Brenner, 1974). Other strains used are listed in Supplemental Data. When *eff-1; ajm-1::gfp* genotypes were used, the *ajm-1::gfp* strain was used as a control to eliminate possible effects of reporter transgenes on morphogenesis.

Mutagenesis and Screening and Complementation Tests

L4 hermaphrodites with parental genotype *unc119(e2498)* III; *ajm-1::gfp* IV were treated with EMS, and F1 progeny were plated singly. After 3 days, F2 progeny were viewed using a 60× oil immersion objective. Approximately 700 colonies (~1400 haploid genomes) were screened for embryos or larvae retaining extra AJM-1::GFP-labeled junctions normally lost during cell fusion. One colony containing ~25% affected larvae was isolated, and the mutant worms were found to be viable. The mutation was backcrossed more than five times away from *unc119(e2498)*, and was named *eff-1(oj55)*.

In a conditional screen for abnormal vulval morphologies, L4 *ajm-1::gfp* hermaphrodites were treated with EMS and 1472 F1 daughters were isolated to separate plates at 15°C. After 3 days, F2 progeny were screened for Pvl (protruded vulva) or Muv (multivulva) phenotypes. 111 Pvl and ten Muv F2 worms were analyzed for retention of adherens junctions. One Pvl mutant expressed such fusion defects. The mutation was outcrossed 12 times and was named *eff-1(hy21)*.

(D) L2 showing GFP in lateral and dorsal hyp7, but no expression in the seam cells (se). A seam daughter cell (se-hyp7) expresses GFP intensely before fusing to hyp7.

(E) Head of adult hermaphrodite, with GFP in pharyngeal metacorpus (ph), dorsal hyp7, amphid sheath cells (as), and neuronal axons (ax) projecting from the nerve ring (nr).

(F) Midsection of mid-L3 with GFP in lateral (top) and ventral (bottom) hyp7. Dividing unfused VPCs remain unlabeled.

(G) Vulval morphogenesis in mid-L4. Subset of vulval cells express GFP (green overlaid on DIC). Three-dimensional reconstruction of two tetranucleated toroids (inset, rotated 90°) shows lumen (open arrowhead) forming through centers of toroids (arrow and filled arrowhead).

(H) Midsection of gravid hermaphrodite showing GFP in a uterus containing embryos.

(I) Copulatory bursa of adult male tail with GFP in fan and rays.

Asterisks show cells not known to participate in cell fusion. The scale bars represent 10 μm.

(J) Model of EFF-1 primary structure, indicating known point mutations, putative functional domains, and sites of possible modification.

(K) Model of EFF-1 action in an early stage of a cell fusion event (Mohler et al., 1998). Two cells lie in close contact. EFF-1 is required for formation of an initial opening through the double bilayer at the adherens junction, and rapid diffusion of cytoplasm between cells. Anterior-posterior (A-P), left-right (L-R), and apical-basal (Ap-Ba) axes are shown.

To check complementation between *oj55* and *hy21* alleles, *eff-1(oj55)/+; ajm-1::gfp /+* males were crossed with *eff-1(hy21)* hermaphrodites. 41% of Roller progeny [*eff-1(oj55)/ eff-1(hy21); ajm-1::gfp*] showed tail and cell fusion defects, confirming that the alleles do not complement.

Strains, Immunofluorescence, Microscopy, TSP Experiments, and Cloning of *eff-1*

See Supplemental Experimental Procedures at http://www.developmentalcell.com/cgi/content/full/2/3/355/DC1.

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Accession Numbers

The GenBank accession numbers for open reading frames encoding EFF-1A and EFF-1B are AF480492 and AF480493, respectively.