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Repression of Cell-Cell Fusion by Components of the *C. elegans* Vacuolar ATPase Complex

Short Article

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Summary

Cell-cell fusion initiates fertilization, sculpts tissues during animal development, reprograms stem cells to new differentiated states, and may be a key step in cancer progression. While cell fusion is tightly regulated, the mechanisms that limit fusion to appropriate partners are unknown. Here, we report that the *fus-1* gene is essential to repress fusion of epidermal cells in *C. elegans*: in severe *fus-1* mutants, all epidermal cells, except the lateral seam cells, inappropriately fuse into a single large syncytium. This hyperfusion requires EFF-1, an integral membrane protein essential for fusion of epidermal cells into discrete syncytia. FUS-1 is localized to the apical plasma membrane in all epidermal cells potentiated to undergo fusion, whereas it is virtually undetectable in nonfusing seam cells. *fus-1* encodes the e subunit of the vacuolar H^+-ATPase (V-ATPase), and loss of other V-ATPase subunits also causes widespread hyperfusion. These findings raise the possibility of manipulating cell fusion by altering V-ATPase activity.

Introduction

Fusion of lipid bilayers between cells to create a single multinucleate cell not only marks the beginning of life for most multicellular organisms, but also features prominently in the subsequent formation of tissues and organs (Bischof et al., 2000; Dworak and Sink, 2002; Shemer and Podbilewicz, 2003; Wassarman et al., 2001; Witze and Rothman, 2002). During normal development, the fusion of myoblasts to form myotubes, trophoblasts to form the placental syncytium, and monocytes to form osteoclasts is pivotal to the genesis of muscle, placenta, and bone, respectively. Recent findings indicate that cell fusion may also underlie stem cell plasticity: bone marrow stem cells can be reprogrammed into multiple differentiated cell types, including hepatocytes, cardiocytes, and neural cells, by cell fusion (Wagers and Weissman, 2004). Mounting evidence suggests that inappropriate cell fusion contributes to cancer progression: various tumor cell types are fusogenic, and promiscuous fusion between tumor cells or between tumorigenic and normal cells can endow hybrids with new properties such as higher proliferation rates, metastasis, and resistance to apoptosis and drugs (Duelli and Lazebnik, 2003). Thus, the fusogenicity of tumor cells can increase tumor cell diversity, thereby enhancing their malignancy.

Fusion is a tightly regulated process normally restricted to only subsets of cells. While it is clear that the fusion machinery must be judiciously regulated to ensure that cells do not inappropriately fuse with their neighbors, the mechanisms that regulate this specificity are not understood. During *C. elegans* development, about one-third of somatic cells undergo a set of fusions to form an invariant pattern of syncytia (Podbilewicz and White, 1994; Shemer and Podbilewicz, 2003). This stereotyped cell fusion program is highly regulated, and even the particular side of a cell that fuses is highly reproducible. Extensive genetic screens have identified many alleles of a single gene, *eff-1*, that is essential for fusion: in *eff-1* mutants, all epidermal cells fail to fuse (Mohler et al., 2002; B. Podbilewicz and W. Mohler, personal communication). EFF-1 is a transmembrane protein with a sequence motif similar to those known to promote fusion of lipid bilayers and is therefore likely to be part of the machinery that mediates cell fusion. Although several transcription factors and signaling pathways are known to regulate cell fusion events and *eff-1* expression (Alper and Kenyon, 2002; Chen and Han, 2001; Koh and Rothman, 2001; Shemer and Podbilewicz, 2002), it is not known how the fusogenic action of EFF-1 is restricted to particular sets of cells to create many distinct and adjacent syncytia.

Results and Discussion

To investigate the molecular mechanisms that limit cell-cell fusion to the appropriate cells, we have identified genes required for normal cell-fusion patterns in *C. elegans*. Most cell fusion in *C. elegans* embryos occurs in the epidermis during body elongation. The epidermis is an epithelial sheet that is subdivided into pairs of dorsal, lateral, and ventral rows of cells (Priess and Hirsh, 1986). The anterior, dorsal, and ventral cells undergo limited, specific fusions to generate several distinct and stably maintained syncytia (Figure 1A). Neither P cells, the central cells in the ventral row, nor seam cells, the lateral cells, fuse during embryogenesis. The pattern of cell fusion can be monitored by following AJM-1, a protein of the apical junctions surrounding each epithelial cell (Alper and Kenyon, 2002; Koh and Rothman, 2001; Koppen et al., 2001; Mohler et al., 1998; Mohler et al., 2002; Podbilewicz and White, 1994). Fusion of two cells results in elimination of the AJM-1 boundary between them, while AJM-1 stably demarcates the boundary between adjacent syncytia.

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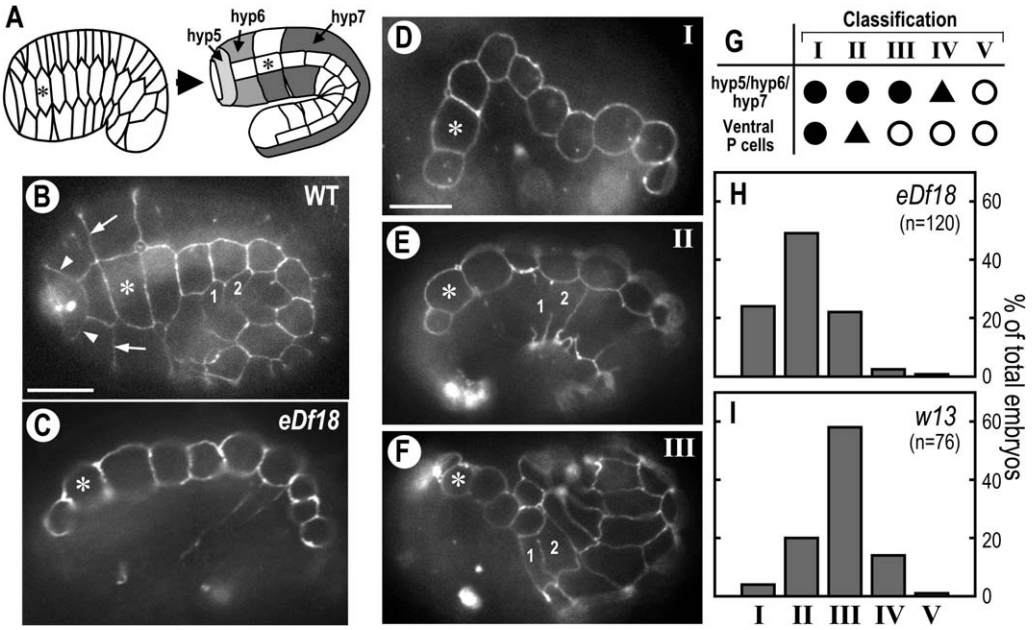

Figure 1. *fus-1* Mutants Undergo Hyperfusion of Embryonic Epidermal Cells

(A) The fusion of epidermal cells, occurring between the "comma" stage (~6.5 hr pf; left) and 2-fold stage (~7.5 hr pf; right) of normal embryonic development, is revealed by the pattern of apical junctions outlining each of these cells, as schematized. The positions of three (hyp5-hyp7) of the eleven epidermal syncytia are indicated.

(B–F) Immunostaining of embryos with AJM-1 reactive monoclonal antibody, MH27, to visualize cell boundaries in the epidermis. In all panels, the same seam cell is indicated by an asterisk and the first two ventral P cells are numbered when they have not undergone complete fusion.

(B) Wild-type embryo (~1.5-fold stage). Cell boundaries between hyp5 and hyp6 (arrowheads) and between hyp6 and hyp7 (arrows) are visible.

(C–G) *eDf18* arrested embryo showing the hyperfusion phenotype. The hyp5/hyp6 and hyp6/hyp7 boundaries and some of the ventral P cell boundaries are absent as a result of hyperfusion. Hyperfusion phenotypes of embryos were classified into five groups (G) on the basis of ectopic cell fusions (closed circle, severe hyperfusion; closed triangle, mild hyperfusion; open circle, no hyperfusion) in two major regions of the epidermis (hyp5/hyp6/hyp7 and ventral P cells) as described in the Experimental Procedures. (D) Class I (in which all except seam epidermal cells fuse to form a large syncytium), (E) Class II, and (F) Class III *fus-1* embryos are shown.

(H and I) Quantitative analysis of the hyperfusion phenotype of *eDf18* and *fus-1* embryos based on the classification described above.

Scale bars equal approximately 10 μm.

Previous screens in our lab and others have identified several chromosomal deficiencies in which epidermal cells apparently undergo hyperfusion (Labouesse, 1997; Terns et al., 1997). A particularly striking hyperfusion phenotype is observed with the deficiency *eDf18*, which deletes a segment of chromosome IV: in terminal *eDf18* embryos, most of the boundaries of the anterior, dorsal, and ventral epidermal cells disappear, revealing the hyperfusion phenotype (Figure 1C); however, the seam cell boundaries persist, such that in many *eDf18* embryos, AJM-1 delimits only a chain of ten cells on each side.

We isolated a recessive zygotic lethal mutation in a gene we have called *fus-1* by screening for lethal mutations targeted to the region defined by *eDf18*. In *fus-1(w13)* mutant embryos, most of the epidermal cells that normally contribute to the 11 hyp cells (hyp1-11) undergo hyperfusion (Figures 1D–1F). In the most severely affected *fus-1* embryos, all epidermal cells except the seam cells fuse into a single, large syncytium (Figure 1D). The AJM-1 staining patterns of other epithelia, including those of the pharynx and intestine, appear to be unaffected in *fus-1* mutant (data not shown). Semiquantitative analysis of the hyperfusion phenotypes revealed that the expressivity of hyperfusion is somewhat stronger for *eDf18* than for *fus-1(w13)* (Figures 1H and 1I), suggesting that *fus-1(w13)* may be a partial loss-of-function mutant or that *eDf18* deletes additional gene(s) required for proper cell fusion.

To assess the dynamics of inappropriate cell fusion in *fus-1(w13)* embryos, we followed time-dependent changes in the AJM-1 staining pattern (Figures 2A–2H). *fus-1* homozygous embryos arrest in morphogenesis at the 2- to 2.5-fold stage (~8 hr postfertilization, pf). At this time, embryos initially show a normal AJM-1 staining pattern (Figures 2A and 2B). Hyperfusion becomes conspicuous at around the time that embryos normally hatch (~15 hr pf) (Figures 2C and 2D), and the proportion of embryos with the hyperfusion phenotype increases progressively until ~24 hr pf (Figures 2G and 2H). These observations suggest that *fus-1* is not essential for the initial pattern of cell fusions in the epidermis and that the hyperfusion phenotype arises from inappropriate activation of the cell fusion program per se in late-stage embryos, rather than as a consequence of abnormal patterning or specification of epidermal cells during early embryogenesis.

To examine further whether the hyperfusion observed

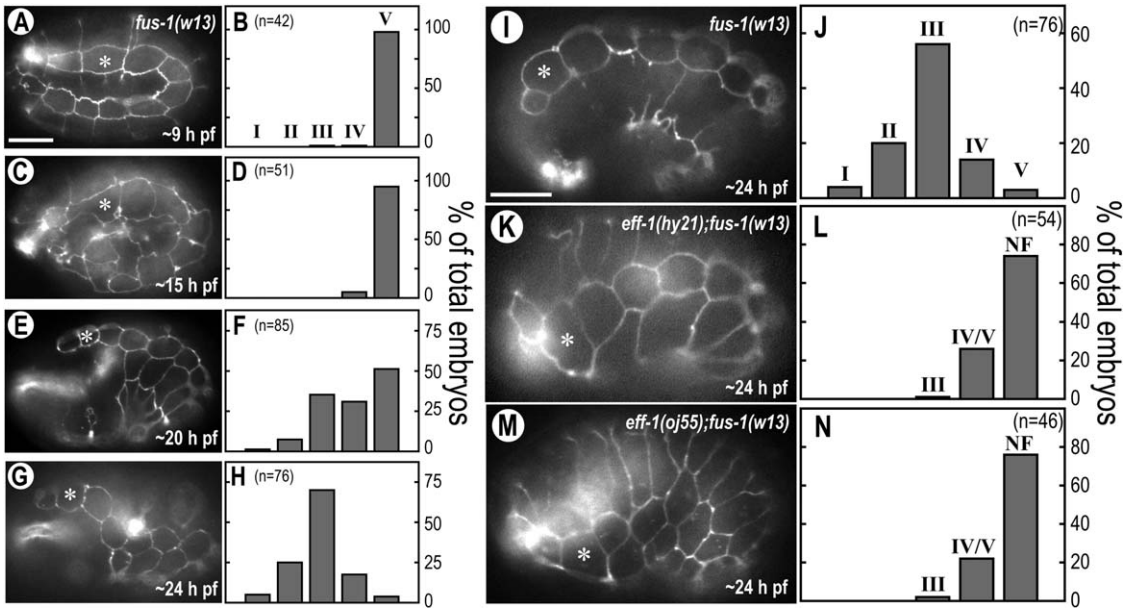

Figure 2. Time Course of *fus-1* Hyperfusion and Suppression by *eff-1* Mutations

Embryos were collected at various times (A, C, E, G, I, K, M) and immunostained with MH27 antibody. The same seam cell is indicated by an asterisk. Hyperfusion was quantified as described in the Experimental Procedures and the results are shown on the right of each micrograph (B, D, F, H, J, L, N). Time points were ~9 hr pf (A, B), ~15 hr pf (C, D), ~20 hr pf (E, F), and ~24 hr pf (G–N). The hyperfusion phenotype of *fus-1* is strongly suppressed by *eff-1* mutations: *fus-1(w13)* (I, J), *eff-1(hy21); fus-1(w13)* (K, L), and *eff-1(oj55); fus-1(w13)* (M, N). IV/V indicates that a low-level fusion was observed (it is not known if such fusion occurs in the normally fusing cells or ectopically, hence it is not possible to categorize these into class IV or V). NF, no fusion observed, as is typical for *eff-1* mutants. Scale bars equal approximately 10 μm.

in *fus-1* mutants results from inappropriate activation of the normal cell fusion program, we examined whether it requires *eff-1*, the only gene known to be essential for all epidermal cell fusions in *C. elegans*. Indeed, we found that hyperfusion in *fus-1(w13)* and *eDf18* homozygous embryos is strongly suppressed by two different mutations in *eff-1* (Figures 2I–2N and data not shown). This epistasis of *eff-1* to *fus-1* indicates that hyperfusion in *fus-1* mutants does not simply reflect nonspecific fusion of lipid bilayers, but instead occurs by an EFF-1-directed process. In *eff-1* mutants, all epidermal cells fail to fuse during embryogenesis; however, a minor fraction of epidermal cells fuse in *eff-1;fus-1* double mutants (Figures 2L and 2N). While it is conceivable that some *eff-1*-independent cell fusion may occur in the *fus-1* mutant, the *eff-1* mutations used here are not null (Mohler et al., 2002; B. Podbilewicz, personal communication), and it may be that a much smaller activity of *eff-1* is sufficient to induce cell fusion in *fus-1(-)* mutants than in wild-type. Although *eff-1* mutations suppress hyperfusion in *fus-1* mutants, they do not suppress its embryonic lethality (data not shown), implying that hyperfusion is not the exclusive cause of the embryonic lethality.

We identified the *fus-1* gene molecularly by standard positional cloning and rescue experiments (Figure 3A). A genomic fragment encompassing the predicted gene *F49C12.13* efficiently rescues both the hyperfusion and embryonic lethality of the *fus-1(w13)* mutant. The *w13* allele carries a G to A transition at the consensus splice-acceptor site of the third exon in *F49C12.13*. Analysis of a cDNA of this gene prepared from *fus-1* homozygotes revealed that the *w13* mutation results in a misspliced transcript; the use of a new acceptor site results in a premature stop codon in the predicted translation product (Figure 3B). RNAi of *F49C12.13* results in hyperfusion of the epidermal cells that is suppressed by the *eff-1* mutation (Figure 3E and data not shown).

The predicted FUS-1 protein shows significant similarity to the vacuolar H^+-ATPase (V-ATPase) e subunit from other species. The V-ATPases are multisubunit proton pumps composed of two functional sectors, V1 and V0 (Nishi and Forgac, 2002; Stevens and Forgac, 1997): a peripheral membrane V1 domain is responsible for ATP hydrolysis, which drives proton transport through an integral membrane V0 domain (Figure 3D). The V-ATPase e subunit is a highly hydrophobic protein with two putative transmembrane domains. While the function of the V-ATPase e subunit remains unknown, recent studies have suggested that Vma9p, the e subunit of the yeast *S. cerevisiae*, is a functionally essential part of the V-ATPase and is involved in assembly of the V-ATPase complex (Davis-Kaplan et al., 2004; Sambade and Kane, 2004). Sequence alignments of the human, *Drosophila melanogaster*, and *C. elegans* proteins reveal high similarity in their carboxy-terminal (C-terminal) regions, immediately following the second transmembrane domain (Figure 3C). We confirmed the importance of the C-terminal region in rescue experiments using wild-type and C-terminal-deleted constructs of FUS-1: while the wild-type *fus-1* efficiently

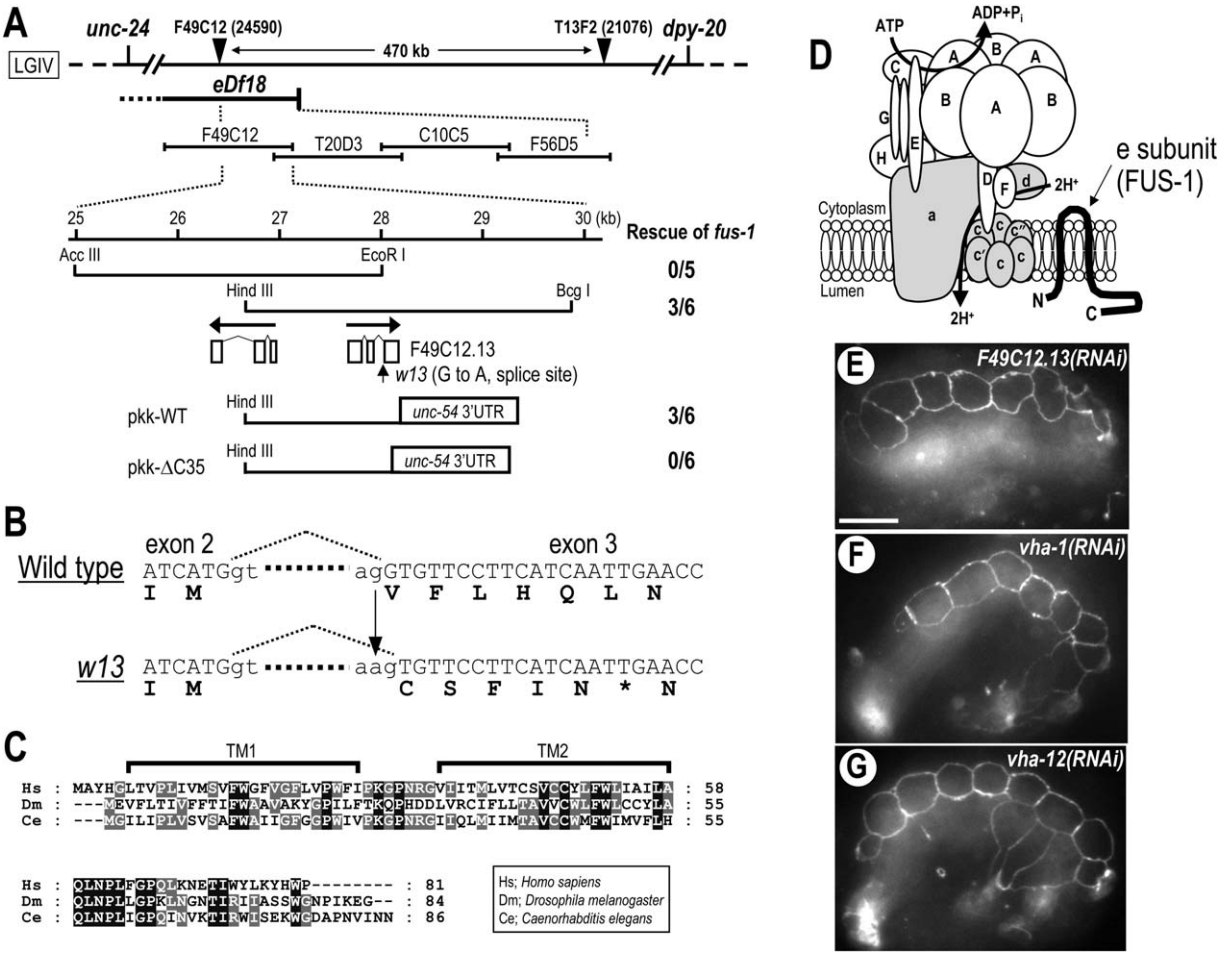

Figure 3. Molecular Characterization of *fus-1* and Induction of Hyperfusion by RNAi of Other V-ATPase Subunits

(A) Positional cloning of *fus-1*. The *w13* mutation maps between two SNPs (arrowheads) on chromosome IV and is not complemented by *eDf18*. Among four overlapping cosmid clones covering the mapped genomic region, only F49C12 is able to rescue the *fus-1* phenotype. An F49C12-derived genomic fragment encompassing the predicted gene *F49C12.13* rescues the *fus-1* phenotype. The position of the *w13* mutation in *F49C12.13* is indicated with a vertical arrow. Plasmid constructs containing wild-type *F49C12.13* (pkk-WT) and a deletion mutant of the gene (pkk-ΔC35) were used in rescue experiments. The number of rescued transgenic lines out of the total number of lines scored is indicated at the right of each construct tested.

(B) Sequence analysis of cDNAs prepared from *fus-1* homozygous embryos showed that the *w13* mutation results in a misspliced transcript containing a premature stop codon, resulting from the use of a new splice acceptor site.

(C) ClustalW sequence alignment of the human V-ATPase e subunit (GenBank accession number Y15286), *Drosophila melanogaster* VhaM9.7-1 (accession number NM_139611) and *C. elegans* FUS-1. Two putative transmembrane domains (TM1 and TM2) predicted by the SOSUI program (http://sosui.proteome.bio.tuat.ac.jp/sosuiframe0.html) are indicated with a line.

(D) Schematic of V-ATPase subunits (modified from Nishi and Forgac, 2002). The peripheral (V1) and integral (V0) membrane sectors are represented by white and gray, respectively.

(E) MH27 staining of a terminal *F49C12.13 (RNAi)* embryo, in which the hyperfusion phenotype of *fus-1(w13)* embryos is phenocopied with a penetrance of ~20%. The strongly affected embryos arrest at early stages with a variable undifferentiated morphology, suggesting that maternal contribution of FUS-1 is required for early embryogenesis. Scale bar equals approximately 10 μm.

(F and G) MH27 staining of *vha-1(RNAi)* (F) and *vha-12(RNAi)* (G) embryos also reveals a hyperfusion phenotype with ~20% penetrance in both cases, similar to the results with *fus-1(RNAi)*.

rescues the *fus-1* phenotype, the deletion mutant (pkk-ΔC35, carboxyl 35 amino acid deletion) does not (Figure 3A). Moreover, the *w13* allele apparently results in a C-terminally deleted protein (Figure 3B), further underscoring the importance of this domain.

Although the inappropriate fusion in *fus-1* mutants requires EFF-1 activity, this hyperfusion could be a secondary consequence of the embryonic lethality; for example, inappropriate cell fusion might result from degeneration of dying embryos. However, of the large number of embryonic lethal mutants studied, very few show hyperfusion (e.g., Labouesse, 1997; Terns et al., 1997; J.H.R., unpublished observation), and most embryonic lethal mutants known to undergo hyperfusion are defective in components of the V-ATPase (see below). Thus, it is clear that embryonic lethality and degradation per se does not generally lead to cell fusion. In an effort to separate the hyperfusion phenotype of

the *fus-1* mutation from embryonic lethality or degradation, we assayed for inappropriate cell fusion in living larvae that escaped the embryonic lethality of the *fus-1(w13)* mutation, but which contained subnormal levels of FUS-1. We found that a substantial fraction of *fus-1* homozygotes rescued for embryonic lethality with a *fus-1* genomic fragment that is expressed at low levels undergo inappropriate epidermal cell fusion (Supplemental Figure S1): while postembryonic cell fusion at the boundary between hyp6 and hyp7 is normally suppressed until the mid-L2 stage in wild-type animals (Yochem et al., 1998), these cells inappropriately fuse during the early L1 stage (no later than 5 hr after hatching) in the rescued *fus-1* homozygotes. Thus, *fus-1* is not only required to inhibit fusion of cells in the embryo that normally never fuse, as described above, but it is also necessary to repress fusion of cells that normally do undergo fusion later in development. This premature fusion was seen in larvae that by Nomarski microscopy appeared healthy and showed no signs of necrosis or deterioration (e.g., Supplemental Figure S1). Moreover, while this weakly expressed *fus-1* transgene is insufficient to rescue the mutants to adulthood, the larvae remained alive for at least 3 days after the time at which this precocious fusion was observed. Although we cannot rule out the possibility that inappropriate cell fusion in *fus-1*-arrested embryos is a consequence of the embryonic lethality, these observations suggest that *fus-1* is required to repress cell fusion during development.

To address whether FUS-1 is sufficient to prevent cell fusion, we expressed it from a heat shock driven construct and found that its overexpression alone does not suppress epidermal cell fusion (not shown). However, FUS-1 overexpression did not increase the levels of at least one other V-ATPase subunit (not shown); thus, assessing whether elevated expression of the V-ATPase is sufficient to prevent cell fusion might require overexpressing the entire V-ATPase, which consists of at least 13 subunits.

The localization of FUS-1 supports a role for its action at the plasma membrane in cells that are potentiated to fuse. Anti-FUS-1 antibody first detects FUS-1 in a punctate pattern within the gut cells of embryos at approximately the comma stage (~6 hr pf); it is undetectable in epidermal cells at this early stage (Figures 4B and 4E). FUS-1 is also detected in the excretory cell (Figure 4K) and the apical membrane of gut cells (not shown) starting later in embryogenesis. In the epidermis, the protein is first conspicuously detected in 2-fold stage embryos shortly after most epidermal cell fusions occur (data not shown). Initially, FUS-1 expression is seen at approximately equal levels throughout the dorsal, ventral, and anterior epidermal cells (not shown); it is specifically excluded from the lateral seam cells. As the embryo develops, FUS-1 levels increase in these epidermal cells, where it concentrates almost exclusively on the apical membrane (Figures 4H and 4K) and some colocalizes with apical junctions (Figures 4L–4N). FUS-1 remains virtually undetectable in seam cells (Figure 4H), which neither fuse in wild-type embryos and *fus-1* mutants nor express EFF-1. Thus, all cells that inappropriately fuse in *fus-1* mutants express *eff-1*, consistent with the epistasis results. Together with the finding that *eff-1*-mediated hyperfusion occurs in anterior, dorsal, and ventral epidermal cells in *fus-1(-)* embryos, it is therefore likely that FUS-1 functions as a suppressor of EFF-1-dependent cell fusion in these cells. The localization of FUS-1 at the plasma membrane of epidermal cells raises the possibility that it might do so by directly acting on EFF-1, also an apparent cell surface protein (W. Mohler, personal communication).

The identification of FUS-1 as the e subunit of the V-ATPase motivated us to investigate whether the loss of other V-ATPase subunits (Oka and Futai, 2000; Oka et al., 2001) affects cell fusion. We performed RNAi experiments against *vha-1* and *vha-12*, which encode the *C. elegans* homologs of the c and B subunits of the V-ATPase, respectively. As shown in Figures 3F and 3G, *vha-1(RNAi)* and *vha-12(RNAi)* embryos show a hyperfusion phenotype that is indistinguishable from that of the *fus-1* mutant on the basis of the AJM-1 staining pattern. In both cases, the hyperfusion phenotype was suppressed by an *eff-1* mutation (not shown). These results suggest that the V-ATPase complex functions as a negative regulator of *eff-1*-mediated cell fusion in the *C. elegans* embryo. Screens of chromosomal deficiencies have revealed several loci that result in inappropriate fusion of epidermal cells (Labouesse, 1997; Terns et al., 1997). Genetic mapping data shows that most of these deficiencies delete genes encoding V-ATPase subunits (not shown), suggesting that the genes responsible for the hyperfusion phenotype of these deficiencies are likely to encode V-ATPase subunits.

How might components of the V-ATPase, or perhaps the entire complex, repress EFF-1-mediated cell fusion? EFF-1 is the only protein known to be essential for all epidermal cell fusions, and its forced expression is sufficient to promote fusion of cells that normally do not fuse (Shemer et al., 2004). Negative regulation of *eff-1* transcription is known to be important for *C. elegans* development: for example, *eff-1* is transcriptionally repressed to prevent inappropriate fusion of seam cells (Mohler et al., 2002) and the precursor cells that divide to give rise to the vulva postembryonically (Shemer and Podbilewicz, 2002). To examine possible transcriptional effects of FUS-1 on *eff-1*, we analyzed the expression of an *eff-1* transcriptional reporter construct and found that both the patterns and levels of *eff-1* are unaffected by a *fus-1* mutation (not shown). *eff-1* is expressed not only in fusion-competent epidermal cells but also in some ventral epidermal cells that normally never fuse during embryogenesis (Mohler et al., 2002). Thus, repression of fusion in cells expressing the fusogenic EFF-1 protein must involve inhibition of its activity. We have shown that FUS-1 concentrates at the apical membrane of these EFF-1-expressing epidermal cells and that V-ATPase subunits are essential to prevent inappropriate fusion of the epidermal cells; thus, the V-ATPase performs a critical function in restricting cell fusion by EFF-1 in the epidermis.

Given that cell fusion is known to initiate at a site along the apical edge of the fusing border and progresses basally (Mohler et al., 1998) and that the e subunit of V-ATPase has also been shown to be localized in the apical membrane of epidermal cells (Choi et al., 2003), it is reasonable to suppose that the V-ATPase components may act in the plasma membrane to re-

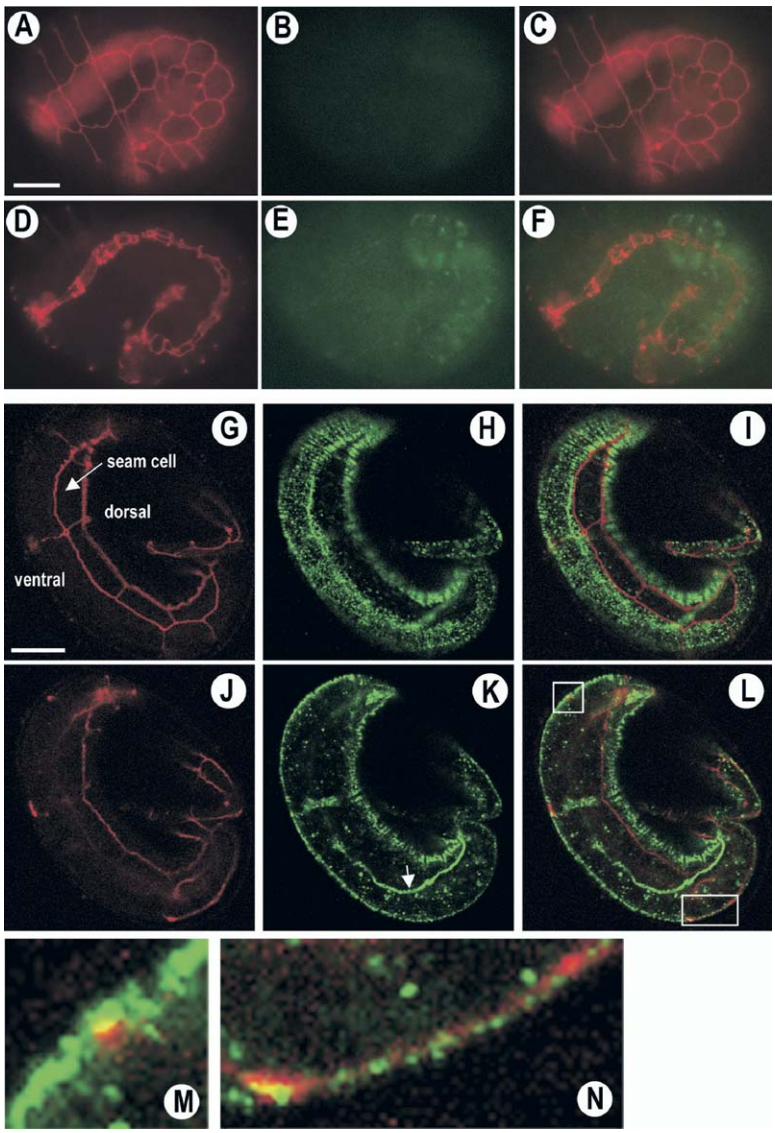

Figure 4. FUS-1 Localizes to the Apical Membrane of Epidermal Cells in Late-Stage Embryos

(A–L) Images of wild-type embryos stained with MH27 (A, D, G, J), anti-FUS-1 (B, E, H, K), and the merge of both (C, F, I, L). External (A–C) and internal (D–F) focal planes of ~1.5-fold embryo show no detectable FUS-1 in the epidermis (B) and punctate staining in gut cells (E). Surface (G–I) confocal images of a ~3-fold embryo shows FUS-1 localized to the apical surface of dorsal and ventral epidermal cells, with virtually no staining detected in seam cells (H). FUS-1 is also observed in the excretory cell (arrow in K), as seen in an internal (J–L) focal plane of the same embryo.

(M and N) Enlarged images of the region shown in the white rectangles in (L). Magnified images of the area around the apical junctions between hyp6 and hyp7 (M) and between the P1 and P2 cells (N) show that FUS-1 is localized to apical membranes of the epidermal cells and partially colocalizes with AJM-1. Scale bar equals approximately 10 μm.

press EFF-1-mediated membrane fusion. Extracellular acidification by plasma membrane V-ATPases is important for a number of cell-surface processes (Nishi and Forgac, 2002; Stevens and Forgac, 1997) (e.g., bone resorption); the fusion-promoting activity of EFF-1 may be suppressed by local extracellular acidification catalyzed by the plasma membrane-localized V-ATPase. Alternatively, subunits of the ATPase might directly repress fusion by an acidification-independent mechanism. Such a possibility is bolstered by findings that implicate the V-ATPase directly in a membrane fusion event: a trans-complex between V_0 domains of adjacent intracellular yeast vacuole membranes promotes their fusion (Bayer et al., 2003; Peters et al., 2001). However, in this intracellular fusion process, the V-ATPase activates membrane fusion in contrast to its repressive role in cell-cell fusion reported here. A possible resolution for this apparent paradox may be that the membrane topology of the V-ATPase is reversed relative to the membrane faces that initiate fusion in these two distinct membrane fusion processes. Alternatively, the V-ATPase may be required to restrict EFF-1 localization to regions of cell fusion. V-ATPases play an important role in various membrane transport, protein sorting, and degradation processes (Nishi and Forgac, 2002; Stevens and Forgac, 1997). A recent report shows that inhibition of the V-ATPase prevents the degradation of a membrane type 1 matrix metalloproteinase and increases its activity at the cell surface (Maquoi et al., 2003). It is thus possible that EFF-1 is mislocalized to inappropriate cell membranes in the absence of V-ATPase function, leading to ectopic cell fusion.

Finally, our findings suggest that it may be possible to intervene in cell fusion in vivo by modulating V-ATPase activity, perhaps by exploiting a variety of pharmacological agents known to affect V-ATPase function. For example, bone marrow stem cells can adopt new fates by fusing with other cells; manipulating V-ATPase might enhance the fusion of stem cells, thereby promoting their differentiation. Alternatively, the

cell fusion-dependent steps in cancer progression might be inhibited by elevating the levels or activity of V-ATPase. If V-ATPase acts generally to repress cell fusion, modulating the V-ATPase could provide a mechanism for enhancing or preventing fertility.

Experimental Procedures

Classification of Embryonic Hyperfusion Phenotypes

Embryos were immunostained with monoclonal antibody MH27 to visualize cell boundaries (see Supplemental Experimental Procedures), and cell fusion patterns were classified based on the extent of ectopic cell fusion in a defined region of the epidermis: severe hyperfusion, most (>80%) epidermal cells ectopically fuse; mild hyperfusion, some epidermal cells (at least two adjacent cells) ectopically fuse; no hyperfusion, no epidermal cells ectopically fuse (i.e., wild-type cell fusion pattern). Hyperfusion phenotypes were then classified into five groups on the basis of the cell-fusion patterns in the two major regions of the epidermis (hyp5/hyp6/hyp7 and ventral P cells), as defined in Figure 1G.

Strains and Genetics, Molecular Identification of *fus-1*, RNAi Experiments, and Antibody Preparation and Immunostaining

See Supplemental Experimental Procedures.

Supplemental Data

Supplemental Data include one figure and Supplemental Experimental Procedures and can be found with this article online at http://www.developmentalcell.com/cgi/content/full/8/5/787/DC1/.

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