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Cell Fusion: EFF Is Enough

Developmentally programmed cell–cell fusion in *Caenorhabditis elegans* requires the EFF-1 protein, which is sufficient to cause normally non-fusing cells to fuse. EFF-1 localizes to fusion-fated membranes, implicating it as a direct fusogen.

Kenji Kontani and Joel H. Rothman

If you are not a clone, you can be confident that the first instant of your life was initiated by a cell fusion: fertilization [1]. Cell fusion also serves to assemble and maintain many of our organs, including muscles, bones and placentas [2–5]. Recent attention has been drawn to cell fusion as a process that can promote differentiation of stem cells: bone-marrow stem cells can differentiate into several distinct cell types, including hepatocytes, cardiocytes and neurons, as a result of cell–cell fusion [6]. Cell fusion has also been implicated in the progression of cancer, causing tumor cells to become more malignant or resistant to therapeutic agents [7].

Our understanding of the mechanisms by which two apposed lipid bilayers fuse has come from extensive analysis of intracellular vesicle fusion and virus–host cell fusion [8,9]. Conformational changes of fusogenic SNARE proteins in intracellular vesicle fusion, and hemagglutinin in virus–host fusion, bring two apposing lipid bilayers into close proximity, causing them to meld into a single bilayer. In contrast, the mechanisms underlying the cell–cell fusions that occur during development are poorly understood. Although many genes required for normal cell fusion in animals have been reported [1,3,4,10], none has been demonstrated to encode a *bona fide* fusogen capable of joining the plasma membranes of two cells.

Recent findings by Shemer *et al*. [11] and del Campo *et al*. [12] on the EFF-1 protein, which is essential for developmental cell fusion in the nematode *Caenorhabditis elegans* [13], have shown that EFF-1 is sufficient to activate fusion of cells that normally never fuse, implicating it as a possible fusogenic molecule. Moreover, EFF-1 dynamically localizes to fusion-fated membranes prior to, and during, cell fusion [12], suggesting that EFF-1 may act directly at the membrane to promote conjoining of lipid bilayers during developmental cell fusion.

Although cell fusion functions in the formation of organs, the primary role of developmental cell fusion remains generally unknown. Fusion may provide a mechanism for cells to acquire a new identity and functional properties. For example, whereas transdifferentiation of unfused bone-marrow stem cells into different cell types might require specialized stimuli, such as cytokines and cell–cell interactions that direct their differentiation, bone-marrow stem cells that undergo fusion could exploit existing cellular components present in a fusion partner to reprogram their identity.

But fusion-competent cells walk a tightrope, as inappropriate fusion can have disastrous consequences. Fusion creates polyploid cells, and thereby instability and chromosome loss, which can result in tumorigenicity. In fact, many tumor cell types are fusogenic, and promiscuous fusion between tumor cells, or between tumorigenic and normal cells, endows the hybrids with new properties, which can include higher proliferation rates, metastasis and resistance to apoptosis and drugs [7]. Thus, the fusogenicity of tumor cells can increase tumor cell diversity, thereby enhancing their malignancy.

As with any process that dramatically affects cellular function, developmental cell fusion must be spatiotemporally regulated. Detailed anatomical and developmental analyses of *C. elegans* have revealed that about one-third of this nematode's somatic cells fuse with other cells to generate a number of epidermal and muscle syncytia, providing an excellent model system for analyzing the *in vivo* mechanisms of cell fusion [4,14,15]. This pattern of cell fusions is highly invariant, and even the particular side of a cell that undergoes fusion is highly reproducible.

Extensive genetic screens in *C. elegans* have identified many alleles of a single gene, *eff-1*, that is essential for epithelial cell fusions [5,12,13]. In *eff-1* mutants, all epidermal cells fail to fuse. The *eff-1* gene encodes a

transmembrane protein that contains a sequence motif similar to one occurring in proteins known to promote fusion of lipid bilayers. Although developmental cell fusion is regulated at least in part at the level of *eff-1* transcription [13,16], it is not known whether EFF-1 functions as a fusogen that directly promotes the mixing of lipid bilayers, nor how EFF-1 activity is restricted to particular sets of cells, such that many adjacent and distinct syncytia are created.

At which step(s) in cell fusion is EFF-1 activity required? Shemer *et al.* [11] performed ultra-structural studies of *eff-1* mutants and found that the unfused epithelial cells are separated by intact plasma membrane borders. The separation between these membranes is indistinguishable from that between adjacent non-fusing cells in wild-type animals, implying that EFF-1 is required for pre-initiation and/or initiation stages of epithelial cell fusion. In addition to epithelial cells, pharyngeal muscle cells undergo cell fusion in wild-type animals. Although most plasma membranes in muscle cells remain intact in *eff-1* mutants, cell fusion is initiated and small pores are formed in some plasma membranes of this cell type. These small pores, however, arrest without further expansion, which would otherwise complete cell fusion.

EFF-1 thus appears to be required for both initiation of cell fusion and for expansion of fusion pores in pharyngeal muscle cells. These observations also suggest that fusion and EFF-1 activity are differentially regulated in two different cell types. Consistent with different mechanisms acting in distinct cell types, EFF-1 is not at all required for at least two heterotypic cell fusions: fusion of the gonadal anchor cell and uterine cells, and joining of sperm and oocytes at fertilization. Thus, it is apparent that, while *eff-1* is required for homotypic cell fusions in epidermis and muscles, other molecule(s) function in heterotypic cell fusions.

Both Shemer *et al.* [11] and del Campo *et al.* [12] demonstrated that EFF-1 is not only essential, but is also sufficient to promote cell fusion: ectopic expression of EFF-1 causes cells in both embryos and larvae to fuse inappropriately. During normal development, cell–cell fusion is observed only in differentiated tissues. While ectopic EFF-1 can direct inappropriate fusion of fully differentiated cells, remarkably, it is also capable of triggering fusion of undifferentiated early blastomeres well before any fusions normally occur during embryogenesis; thus, EFF-1 expression is sufficient to promote both ectopic and heterochronic cell–cell fusion.

The fusion events triggered by ectopic EFF-1 appear similar to normal fusions: the kinetics of membrane permeability, estimated by the diffusion rate of cytoplasmic GFP in fusing cells, reveal that ectopic EFF-1 induces small pores with permeability similar to that observed during normal membrane fusion. These observations suggest that cell fusion may be directly induced by the fusogenic action of EFF-1, rather than as a consequence of EFF-1-mediated activation of unknown fusogenic component(s). Interestingly, overexpression of EFF-1 in *eff-1* mutants revealed that restoration of fusion between cells normally fated to fuse during development precedes the ectopic fusion between cells that normally do not fuse; these observations suggest that the fusogenic action of EFF-1 may be enhanced by other component(s) that predispose epidermal cells to fusion.

How does a fusion-fated cell find its appropriate partner among several choices of surrounding cells and achieve specific cell fusion? The specificity of cell fusion is regulated, at least in part, by transcriptional control of *eff-1* expression [13,16]. But *eff-1* also appears to be post-transcriptionally regulated, as the gene is expressed in some cells that never fuse during normal development. Inappropriate cell fusion appears to be prevented in part by inter-tissue barriers, in particular basal laminae.

Our recent findings [17] also indicate that components of the vacuolar ATPase prevent widespread cell fusion, perhaps by functioning at the plasma membrane to restrict EFF-1 action. Moreover, del Campo *et al.* [11] suggest that EFF-1 localization plays a key role in the specificity of cell fusion. The restriction of fusion to particular sets of cells apparently involves redistribution of EFF-1 to the boundary of cells fated to fuse: analysis of an EFF-1::GFP reporter protein revealed that it concentrates to cell–cell contacts between fusion-fated epidermal cells. Given that cell fusion

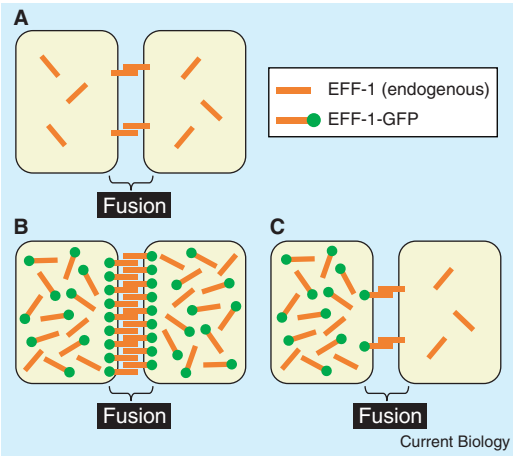

Figure 1. Model for EFF-1-mediated cell fusion in *C. elegans*.

(A) Cell fusion in wild-type animals. Homotypic interactions between EFF-1 on juxtaposed plasma membranes of fusion-fated cells bring the two membranes into close proximity, facilitating the mixing of lipid bilayers and catalyzing cell fusion. (B,C) Accumulation of GFP fluorescence in adjacent membranes of fusion-fated cells expressing EFF-1::GFP. Strong GFP signal can be seen at the boundary of apposed membranes of fusing cells when both express high levels of EFF-1::GFP. (B) Only a very weak GFP signal is seen at the boundary between a cell expressing a high level of EFF-1::GFP and its fusion partner that expresses only the low endogenous levels of EFF-1. (C) These findings suggest that the accumulation of EFF-1 at the boundary of fusion-fated cells depends on stoichiometric *trans* interactions between EFF-1 molecules in each of the membranes destined to fuse. Model adapted from del Campo *et al*. [12].

initiates at a site along the apical edge of the fusing border, and that EFF-1::GFP also accumulates along the apical edge of the cell boundary, prior to fusion, it is likely that accumulation of EFF-1 at this boundary is a critical step in the initiation of fusion.

Analysis of the dynamics of EFF-1::GFP localization revealed two modes by which it accumulates at cell boundaries. In some cases, for example in epidermal cells that migrate toward each other and meet at the ventral midline, the protein is first expressed and present intracellularly; it then moves to the membrane and accumulates at the boundary after the cells have contacted each other. In other examples, such as with two adjacent dorsal epidermal cells, EFF-1::GFP appears at pre-existing cell–cell contacts, concomitant with the onset of its expression. The amount of EFF-1::GFP accumulating at cell boundaries exceeds the sum of the amount present in each membrane of the two adjacent cells before the boundary accumulation is observed, suggesting that the protein is actively transported specifically to cell boundaries, and/or that its endocytosis is inhibited in that region. Interestingly, accumulation of EFF-1::GFP at the cell boundaries is not observed in cells containing high levels of the protein ('bright' cells) at the boundary with cells that do not express the reporter protein ('dark' cells), even when the bright–dark pair undergoes fusion.

del Campo *et al.* [12] suggest that accumulation of EFF-1 at the boundary between cells destined to fuse is achieved by stoichiometric *trans*-interaction between EFF-1 molecules on juxtaposed membranes (Figure 1). Such *trans*-interactions of EFF-1 molecules may facilitate membrane fusion by bringing the membranes into close proximity, in a manner analogous to SNARE-mediated fusion of vesicles and target membranes.

The extracellular domain of EFF-1 contains two sequence motifs that might be involved in lipid bilayer fusion: a phospholipase A_2 (PLA_2) active site consensus, and a short hydrophobic stretch similar to a viral fusion peptide. Analysis of variant EFF-1 forms carrying point mutations in the putative PLA_2 active site domain that are analogous to mutations known to abolish the enzymatic activity of PLA_2 indicated that this region is not essential for EFF-1 function. In contrast, deletion of the hydrophobic fusion peptide-like region eliminates the fusogenic activity of EFF-1 [12]. These latter mutants fail to localize to the boundary of normally fusing cells, suggesting that the potential fusogenic activity of this fusion peptide domain may be intimately coupled to movement of the protein to the boundary between fusion-fated membranes. Further structure–function analyses of EFF-1, combined with *in vitro* reconstitution of membrane fusion in a liposome fusion reaction, should help to reveal the molecular mechanism underlying fusion of juxtaposed plasma membranes and make it possible to assess whether EFF-1 is a genuine fusogen that is capable of acting alone to induce membrane fusion.

Much of our mechanistic understanding of intracellular membrane fusion in the eukaryotic secretory pathway derived from genetic studies in yeast, combined with *in vitro* reconstitution assays [9,18]. Similarly, genetic analyses in animals have begun to uncover the key components of the molecular machinery for cell–cell fusion [4,19]. It will be of great interest to understand how EFF-1, a key fusogenic regulatory molecule, is restricted in its action to particular cell partners, and to what degree the molecular details of extracellular membrane fusion are related to those underlying intracellular fusion of lipid bilayers. Moreover, access to the molecules that mediate cell–cell fusion may prove instrumental in developing methods for clinically intervening in processes as disparate as fertility, tumorigenesis and stem cell differentiation. The ability to direct fusion of cells at will by expressing fusogenic molecules such as EFF-1 represents a substantial first step toward manipulating this critical cellular process.

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