The story of cell fusion: big lessons from little worms

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Summary
The ability of two or more cells to unite to form a new syncytial cell has been utilized in metazoans throughout evolution to form many complex organs, such as muscles, bones and placentae. This requires migration, recognition and adhesion between cells together with fusion of their plasma membranes and rearrangement of their cytoplasmic contents. Until recently, understanding of the mechanisms of cell fusion was restricted to fusion between enveloped viruses and their target cells. The identification of new factors that take part in developmental cell fusion in *C. elegans* opens the way to understanding how cells fuse and what the functions of this process are. In this review, we describe current knowledge on the mechanisms and putative roles of developmental cell fusion in *C. elegans* and how cell fusion is regulated, together with other intercellular processes, to promote organogenesis. *BioEssays* 25: 672–682, 2003. © 2003 Wiley Periodicals, Inc.

Introduction

Exoplasmic cell fusion is a fundamental process that has arisen throughout evolution and which facilitates a wide variety of biological events. This process, which involves the merger of plasma membranes, can be either transient, as in the case of sperm–egg fusion, resulting in a diploid cell that continues to divide, or permanent resulting in the formation of syncytia — multinuclear cells. Such syncytia serve as essential components of several somatic tissues in metazoans, including the myotubes in muscle formation, osteoclasts in bone formation and resorption and syncytial trophoblasts in the formation of the mammalian placenta.[1–3]

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Abbreviations: AC, anchor cell; Antp, antennapedia; Dfd, deformed; EFF, epithelial fusion failure; EXD, extradenticle; GFP, green fluorescent protein; HA, haemagglutinin; HIV, human immunodeficiency virus; HLH, helix–loop–helix; Hox, homeotic; MTA, metastasis-associated factor; NuRD, nucleosome remodeling and deacetylation; PLA_2, phospholipase A_2; SynMuv, synthetic multivulva; TBE, tick borne encephalitis; VPC, vulval precursor cell.

Exoplasmic cell fusion also takes place during specific viral infections, as enveloped viruses (e.g., influenza, HIV) fuse their membrane with the host's plasma or endosomal membrane. Similar to exoplasmic cell–cell fusion, viral–cell fusion takes place between the external layers of the fusing membranes and, as such, differs in many aspects from endoplasmic fusion events that occur within a cell (e.g., vesicular membrane transport between organelles, Ref. 4).

Intensive research on fusogenic viruses has revealed major components of the exoplasmic fusion machinery. It has been shown that specific membrane glycoproteins, also known as fusogens, act to promote fusion between the virus and its target membrane. This, and additional evidence for the role of specific lipids in the fusion process, enabled researches to present several models for the mechanism of viral–cell fusion. However, it is still unclear if the same models and components apply for fusion between two or more cells during development. Moreover, while it is obvious that viral–cell fusion is beneficial for viruses and that there is a biological reason for sperm–egg fusion, it is not easy to understand the advantages of cell fusion as a mechanism for making somatic syncytia, rather than other mechanisms, such as mitosis without cytokinesis.

Most of the studies dealing with cell fusion in human cells and in model organisms, such as yeasts, worms, flies and mice were not aimed directly at finding fusion-machinery genes. Nevertheless, these studies did result in the identification of genes that may be involved in cell fusion in yeast mating and in the placenta.[5,6] They also led to the discovery of several genes that are important for the regulation of the fusion process. Especially constructive were studies of myotube formation in *Drosophila melanogaster* (for a review, see Ref. 2) and epithelial fusion in the nematode *Caenorhabditis elegans* (Table 1). In recent years, direct genetic screens, targeted to find the proteins that are involved directly in the fusion machinery (the "holy grail" of this field) have led to the isolation of such putative factors in *C. elegans*.

In this review, we will concentrate on developmental cell fusion and discuss what the new findings in *C. elegans* can teach us about the putative mechanisms of this process and the factors that regulate it. We will also question the possible

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

Gene	Protein structure and proposed function	Tissue and references
A. Promoting fusion		
lin-29	Zinc-finger TF-Heterochronic pathway	Hypodermal seam cells[73]
ceh-13	Hox (*labial*)	Embryonic epithelia[74]
tlp-1	Zinc-finger TF	Male-tip of the tail[75]
lin-11	LIM-homeobox TF	Vulval-uterine connection[76]
cog-2/*egl-13*	Sox-domain TF	Vulval-uterine connection[77]
ty10	*sec-18*/*nsf* homolog-intracellular vesicular fusion	Vulval-uterine connection*
lin-39 + *mab-5*	Hox (*Dfd*/*Scr* + *Antp*)	Male-ventral epidermis[47,78,79]
egl-27	Chromatin remodelling-inhibits *lin-39* and *mab-5*	Ventral epidermis[67]
ref-1	bHLH TF (*hairy*)-inhibits *lin-39* with MAB-5	Ventral epidermis[68]
SynMuv class A (e.g., *lin-15a*)	TFs, NURD complex components and chromatin remodelling-work with class B to antagonize Ras pathway signaling	Ventral epidermis[71,72,80]
SynMuv class B (e.g., *lin-15b*)	TFs, NURD complex components and chromatin remodelling-work with class A to antagonize Ras pathway signaling	Ventral epidermis[71,72,80]
let-60	Ras	Vulva-late organogenesis[36]
idf-1	nd	Embryonic epithelia[43]
eff-1	Novel trans-membrane-fusion machinery	Epithelia and mesoderm[39]
B. Inhibiting fusion		
lin-39	Hox (*Dfd*/*Scr*)-represses *eff-1* activity	Ventral epidermis, Vulva[47,59,60,78]
mab-5	Hox (*Antp*)	Male-Ventral epidermis[81]
ref-2	Zinc-finger TF-promotes mutual inhibition between *lin-39* and *mab-5*	Male-Ventral epidermis[82]
sem-4	Zinc-finger TF-positively regulates *lin-39*	Vulva[69]
bar-1	Wnt pathway (*beta-catenin*)-positively regulates *lin-39*	Vulva[63]
apr-1	Wnt pathway (APC related factor)-activates *bar-1*	Vulva[70]
let-60	Ras	Vulva[83]
ceh-20	Hox co-factor (*exd*/*pbx*)-represses *eff-1* activity	Vulva[48]
egl-18/*elt-6*	Gata factors	Vulva, Seam cells[66,84]
ceh-16	Homeobox TF (*engrailed*)	Seam cells**
lin-25	Novel protein-possibly part of a TF complex	Seam cells[85]

Class A of the SynMuv genes work redundantly with class B genes to promote fusion. Each one of these classes contains several genes. nd, not determined; TF, transcription factor.
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functions of fusion between somatic cells by examining how cell fusion affects other developmental events, such as animal body elongation and cell migration, and how it contributes to the formation of tissues and organs. Finally, we will discuss how cell fusion, along with other developmental processes, is tightly regulated to drive organogenesis of a model organ—the vulva of *C. elegans*.

From infective to developmental cell fusion: how do cells fuse?

In order for two opposed membranes to fuse, they must come into close contact and water must be excluded at the fusion site. Theoretical, biochemical and structural studies with isolated membranes suggested that these energetically costly events could be overcome by the formation of a stalk intermediate, a neck-like structure that connects only the outer leaflets (Fig. 1).[7–9]

Infective virus–cell fusion

Enveloped viruses enter specific host cells by fusing to their plasma membrane at neutral pH (e.g., HIV), or by fusing to the endosomal membrane at low pH following endocytosis (e.g., Influenza).[10] Over the past decades, in vitro studies with membranes, deciphering of the three-dimensional structures of viral fusion proteins, and isolation of viral fusion intermediates, all contributed to a better understanding of the fusion mechanism between enveloped viruses and their hosts.[11–14]

The fusion proteins are vital components of the process of virus–cell fusion. These viral membrane glycoproteins (fusogens) are essential for bilayer contact, stalk formation and creation of the fusion pore (for reviews, see Refs. 10,15–17). The known fusion proteins are divided into two classes that share several properties, including the presence of a short hydrophobic stretch (the fusion peptide), which is essential for fusogenic activity (Fig. 2A).[10] Class I fusogens are type-I membrane glycoproteins (e.g., haemagglutinin (HA) in influenza), sharing a coiled-coil helical domain that in the activated conformation extends the length of the spike glycoproteins, exposing the fusion peptide that promotes membrane fusion (Fig. 2A).[18] Class II fusion proteins (e.g., E glycoprotein in Tick Borne Encephalitis (TBE) flavivirus) share a β-barrel

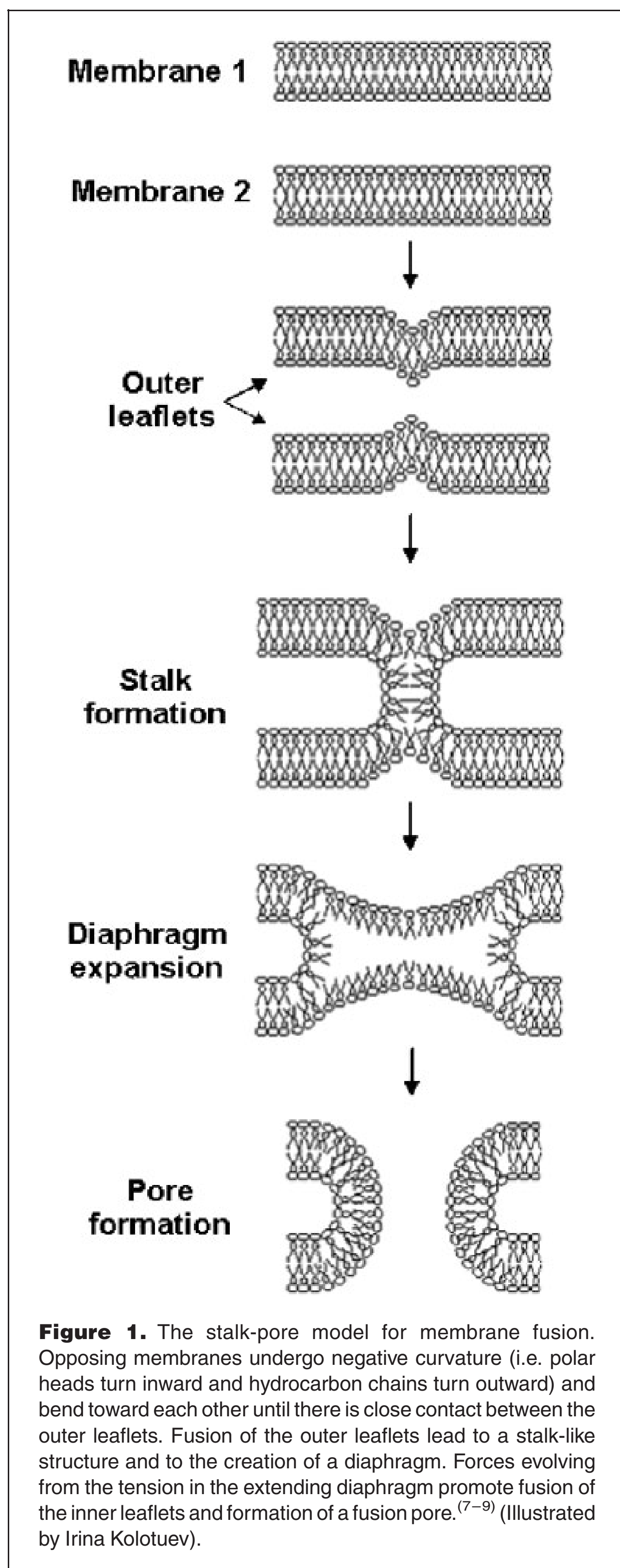

Figure 1. The stalk-pore model for membrane fusion. Opposing membranes undergo negative curvature (i.e. polar heads turn inward and hydrocarbon chains turn outward) and bend toward each other until there is close contact between the outer leaflets. Fusion of the outer leaflets lead to a stalk-like structure and to the creation of a diaphragm. Forces evolving from the tension in the extending diaphragm promote fusion of the inner leaflets and formation of a fusion pore.[7–9] (Illustrated by Irina Kolotuev).

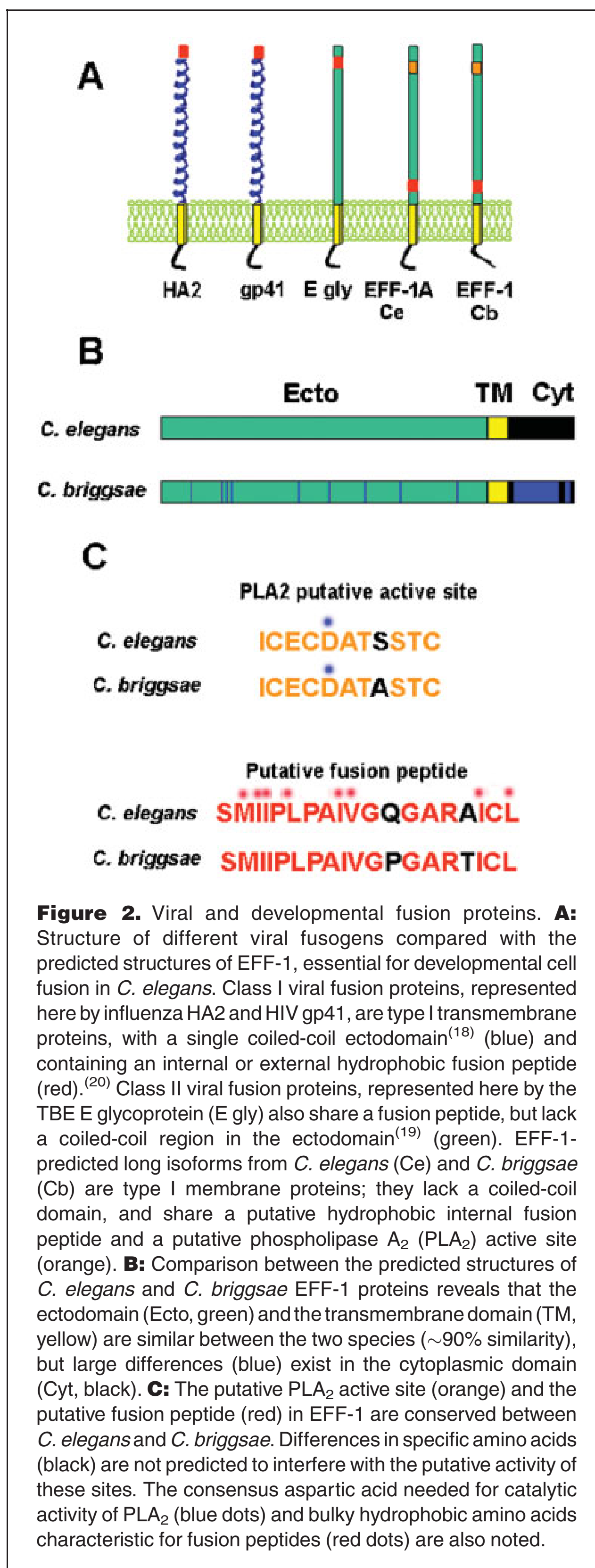

Figure 2. Viral and developmental fusion proteins. **A:** Structure of different viral fusogens compared with the predicted structures of EFF-1, essential for developmental cell fusion in *C. elegans*. Class I viral fusion proteins, represented here by influenza HA2 and HIV gp41, are type I transmembrane proteins, with a single coiled-coil ectodomain[18] (blue) and containing an internal or external hydrophobic fusion peptide (red).[20] Class II viral fusion proteins, represented here by the TBE E glycoprotein (E gly) also share a fusion peptide, but lack a coiled-coil region in the ectodomain[19] (green). EFF-1-predicted long isoforms from *C. elegans* (Ce) and *C. briggsae* (Cb) are type I membrane proteins; they lack a coiled-coil domain, and share a putative hydrophobic internal fusion peptide and a putative phospholipase A_2 (PLA_2) active site (orange). **B:** Comparison between the predicted structures of *C. elegans* and *C. briggsae* EFF-1 proteins reveals that the ectodomain (Ecto, green) and the transmembrane domain (TM, yellow) are similar between the two species (~90% similarity), but large differences (blue) exist in the cytoplasmic domain (Cyt, black). **C:** The putative PLA_2 active site (orange) and the putative fusion peptide (red) in EFF-1 are conserved between *C. elegans* and *C. briggsae*. Differences in specific amino acids (black) are not predicted to interfere with the putative activity of these sites. The consensus aspartic acid needed for catalytic activity of PLA_2 (blue dots) and bulky hydrophobic amino acids characteristic for fusion peptides (red dots) are also noted.

domain that probably undergoes structural changes during activation, allowing the exposure and functioning of the fusion peptide (Fig. 2A).[19] Two alternative models, based on research on HA-mediated fusion in influenza, describe how the fusion proteins promote the formation of a stalk-pore. Both hypotheses involve conformational changes in the fusion proteins triggered by specific conditions (e.g., acidic pH in HA-mediated fusion and ligand–receptor binding in HIV gp41). Whilst the first model predicts that the fusion proteins are present and active in the interacting zone,[20] the second model proposes that these proteins cluster outside this zone, driving bending forces and allowing close contact between the membranes.[17,21] Structural and functional studies with other proteins, such as the HIV gp41 protein,[22,23] suggest that the paradigm of HA-mediated fusion is not restricted to influenza, but rather is a more general mechanism common to class I fusion proteins (Fig. 2A). Class II fusion proteins are less understood and their action probably involves dimerization and formation of an icosahedral scaffold.[19,24]

Developmental cell–cell fusion

In contrast to the large amount of data that has been gathered from research on viral–cell fusion, very little is known about the mechanism and the key players that take part in developmental cell–cell fusion. This led researchers to look directly for fusion genes in model organisms suitable for fusion studies.[2,25,26]

In *C. elegans*, exoplasmic cell fusion is abundant during embryonic and post-embryonic development (Fig. 3). It is involved in the formation of epithelial syncytia in the hypodermis (epidermis), excretory glands, vulva (egg-laying and mating organ), uterus and pharynx as well as in the formation of the male tail that contains the male genitalia. Cell fusion in *C. elegans* is also responsible for the formation of syncytia that originate from the mesoderm, as in the case of the muscles in the pharynx.[26–37] Because of its suitability for genetic studies, *C. elegans* is thus an ideal platform for studying how cells fuse and the developmental functions of this process.[31] Studies in this system have shown that cell fusion

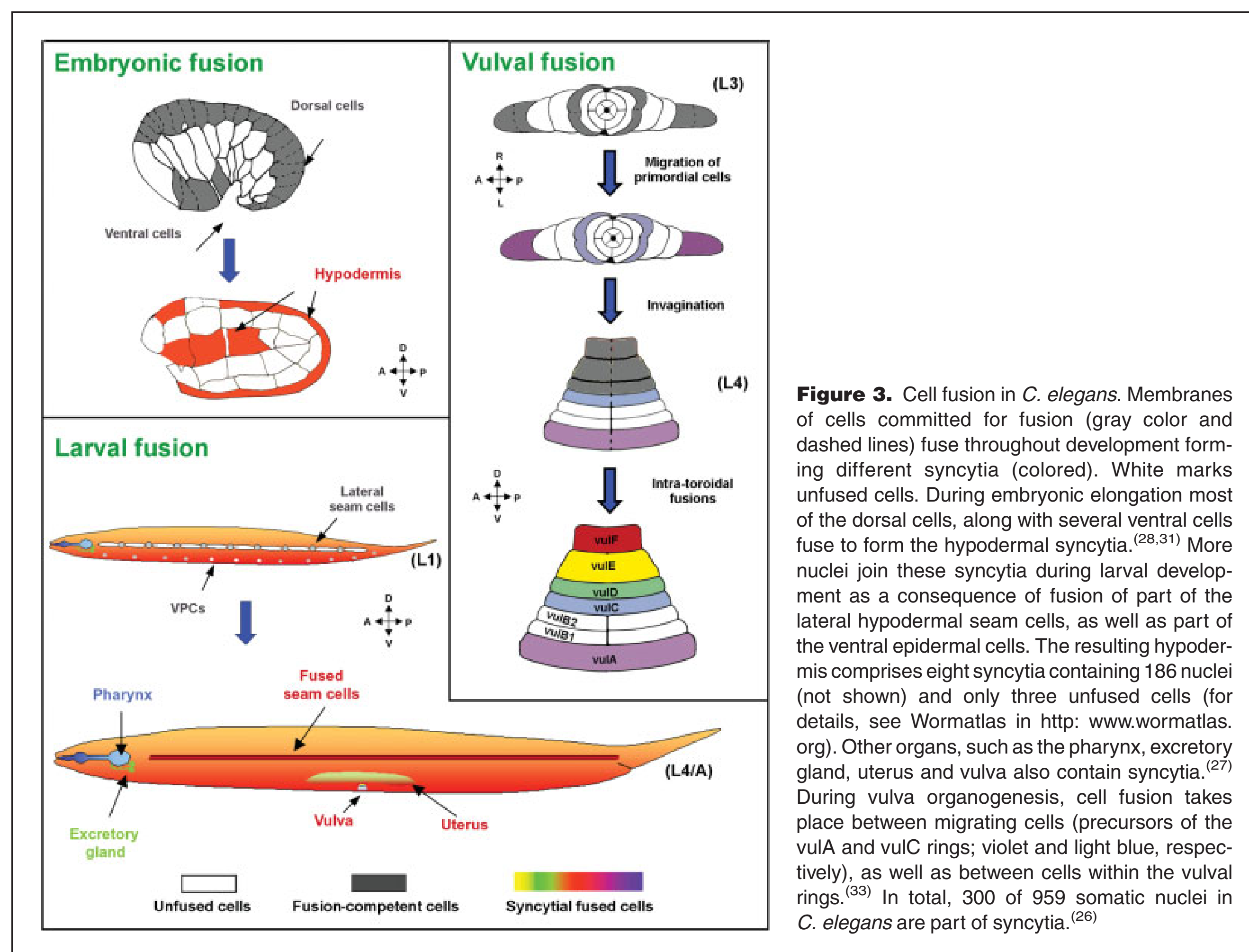

Figure 3. Cell fusion in *C. elegans*. Membranes of cells committed for fusion (gray color and dashed lines) fuse throughout development forming different syncytia (colored). White marks unfused cells. During embryonic elongation most of the dorsal cells, along with several ventral cells fuse to form the hypodermal syncytia.[28,31] 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 hypodermis comprises eight syncytia containing 186 nuclei (not shown) and only three unfused cells (for details, see Wormatlas in http: www.wormatlas.org). Other organs, such as the pharynx, excretory gland, uterus and vulva also contain syncytia.[27] 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.[33] In total, 300 of 959 somatic nuclei in *C. elegans* are part of syncytia.[26]

in the embryonic epithelia starts at the adherens junctions, probably with the formation of fusion pores, and that expansion of these pores involves vesiculation.[34,38] Several genes have been identified that regulate spatially and temporally developmental cell fusion in different tissues in the worm. The identity and putative modes of action of these regulators are presented in Table 1. The complex and tight regulation of cell fusion during the formation of the vulva in *C. elegans* will be discussed later in this review.

Two independent screens in *C. elegans*, the first for embryonic and the second for postembryonic fusion failures, resulted in the isolation of epithelial fusion failure-*1* (*eff-1*), a mutant that is completely and specifically defective in epithelial cell fusion.[39,40] Theoretically, it is possible that *eff-1* affects cell fusion in other manners (e.g., as a signaling molecule that drives other fusion genes). Several pieces of evidence suggest, however, that it does act as a structural gene in the fusion machinery. As such, analysis of its predicted structure can help us to address how the fusion machinery allows two cells to become one. First, *eff-1* mutants fail to promote any epithelial fusion event in vivo. Second, *eff-1* is expressed specifically in all epithelial cells committed to fusion. Third, *eff-1* does not appear to function in events prior to cell fusion (e.g., cell recognition, cell adhesion) as judged by the findings that the adherens junctions as well as the whole membranes remain intact in *eff-1* mutants. Fourth, in mutant *eff-1* embryos, a cytoplasmic green fluorescent protein (GFP) reporter fails to spread between the cells that fail to fuse.

eff-1 encodes at least four isoforms in *C. elegans*, two predicted type-I membrane proteins and two secreted proteins (WormBase, 2003 http://www.wormbase.org, release WS95). In addition, homologs of *eff-1* exist in the closely related nematode *C. briggsae* (contig cb25.fpc0011- NemaNet, http://www.nematode.net), in two plant-parasitic nematodes (*M. incognita* (EMBL number: BM881383) and *M. arenaria* (EMBL number: BI746953), Ref. 40) and in the sheep and cattle parasite *H. contortus* (EMBL number: CA869252). The EFF-1 predicted membrane proteins in *C. elegans* and *C. briggsae* share a single transmembrane domain and a large N-terminal ectodomain (Fig. 2A,B). This ectodomain contains 16 cysteines that may form disulfide bonds, potential N- and O-linked glycosylation sites and a 22 amino-acid internal putative fusion peptide (Fig. 2A,C).[10] Thus, EFF-1 may act like a viral fusogen. A predicted coiled-coil domain characteristic of viral class I fusogens was not found in either of the predicted EFF-1 isoforms (Fig. 2A). EFF-1 may, rather, utilize a mechanism similar to that of class II fusogens, involving oligomerization of the fusogen and exposure of the fusion peptide.[19]

EFF-1 isoforms in *C. elegans* and in *C. briggsae* contain in their ectodomain a predicted consensus phospholipase A_2 (PLA_2) aspartic acid active site (Fig. 2A,C). Although they lack a conserved histidine and/or serine in the predicted catalytic site,[41] the presence of the PLA_2 consensus raises two alternative functional possibilities: (1) that EFF-1 may associate with another protein to reconstitute a full PLA_2 active site, and (2) that this domain of EFF-1 could act in a membrane-binding step by interacting with phospholipids within the fusing membranes. These mechanisms are both plausible and consistent with other reports that show how the lipid contents of adjacent membranes can facilitate or inhibit membrane fusion.[9,14,17,42]

So far, four independent screens of ~20,000 haploid genomes for fusion defective mutants have yielded only alleles of *eff-1* in *C. elegans*.[26] The only other fusion-defective mutant isolated in these screens was irregular dorsal fusion-*1* (*idf-1*, also known as *duf-1*), but this mutant shows only partial embryonic fusion failure.[43] However, it is still unclear whether *eff-1* acts as a single fusogen, similar to some viral fusogens or whether developmental cell fusion requires not only a single fusion protein, but rather a dynamic complex of molecules that can assemble and disassemble at the correct timing, as in the case of the SNARE–NSF–SNAP complex, which is involved in intracellular fusion.[20,44,45] Do both cells need to express EFF-1? Preliminary results imply that, at the transcriptional level, at least in some epidermal embryonic cells, expression of *eff-1p::GFP* in only one of the fusing cells is sufficient for fusion (G.S. and B.P., unpublished results), suggesting that, as in the case of some viral fusion proteins, you do not need "two to tango" for fusion between two epithelial cells.

Developmental functions of cell fusion: what do cells fuse for?

A fundamental question in development is how a basic cellular process drives formation of tissues and organs. The classic approach to this question is to interfere with the basic cellular process and to analyze the effects of this block on developmental processes and on the whole animal. Until recently, cell fusion could not be studied in this way, since the only mutants isolated with abnormal fusion behavior were either not specific for fusion, or were involved only in a specific tissue.[5,6,46] Here we will discuss how cell fusion defects in *eff-1* mutants isolated in *C. elegans* lead to developmental alterations in the worm at the level of organ structure, behavior and basic intercellular processes such as cell migration, body elongation and cell fate determination (Figs. 3,4).

Cell fusion regulates intercellular events

Analysis of *eff-1* fusion defective mutants shows that, in *C. elegans*, developmental cell fusion is responsible for at least two major intercellular events: (1) to limit non-autonomously epithelial cell migration of cells surrounded by a syncytium and (2) to affect cell fate determination. The role of developmental cell fusion in regulating cell migration is carried out by elimination of potential migration paths. In wild-type worms,

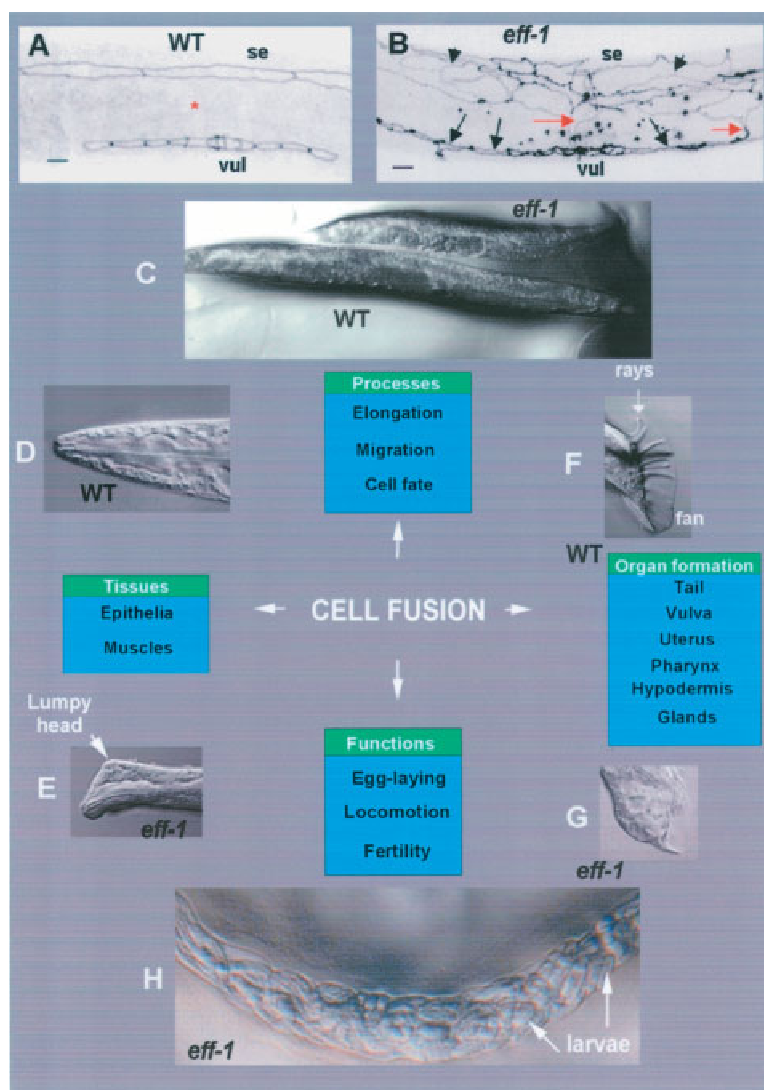

Figure 4. Cell fusion is essential for normal behavior and morphogenesis in *C. elegans*. Fluorescence negative images (A,B) and Nomarski (D-H) micrographs of wild-type and *eff-1* mutant *C. elegans*. In all pictures anterior is to the left and dorsal up. **A,B:** Staining of the apical domains of epithelial 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 to the surrounding hypodermis, the daughter cells of the seam cells migrate throughout the hypodermis (arrowheads) and form a misarranged net.[39] 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.[53] 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.[34] **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 escape from her body (a "bag of worms" phenotype).

specific vulval and lateral hypodermal (seam) cells fuse to the surrounding hypodermis. This hypodermal syncytium restricts the migration paths of the remaining non-fusing cells (Figs. 3,4A). In contrast, in fusion-defective worms, the hypodermal syncytium is not formed and, as a consequence, the unrestricted cells migrate throughout the hypodermis forming an ectopic aberrant net of apical extensions (Fig. 4B).

The second role of cell fusion, control of cell fates, is best demonstrated during vulva formation as each of six equivalent cells (VPCs) adopt either a vulval or a fusion fate.[27] When cell fusion is blocked in *eff-1* mutants, the abnormally unfused cell also responds to neighboring signals and adopts vulval fates. The result is the formation of an ectopic and non-functional vulva.[39] Thus, cell fusion could be regarded as a switch between different fates, in a manner similar to the process of programmed cell death in the nervous system of *C. elegans*.[47]

Previously, it was suggested that cell fusion could have a putative role as a proliferation blocker.[31] Although this is a logical hypothesis, since "fused cells do not proliferate", it seems that, in *C. elegans*, this is not the case. Independent evidence from analyses of vulval cells in *eff-1* mutants, *eff-1;lin-39/HoxD4/Dfd* double mutants (see below) and mutants expressing ectopic Ras function, show that, although different cells fail to fuse in these mutants, they also fail to proliferate.[36,39,48] Thus, cell fusion has not evolved as a mechanism to exit from the cell division cycle and unfused cells need additional cues to proliferate.

Cell fusion arrest is also not associated with cancer in *eff-1* mutants. However, this phenomenon could be limited to *C. elegans* and related organisms. The fact that cells escaping from programmed cell death lead to cancer in humans, but not in *C. elegans*,[49] may suggest a similar difference between worms and humans with regard to cell fusion. Thus, we predict that, if a syndrome causing specific failure of cell fusion in humans were found (e.g., in muscle, bone, placenta), it might be tumorogenic.

In summary, cell fusion in worms limits cell migration and restricts cell fate determination. However, in *C. elegans*, cell fusion failure causes neither ectopic cell division nor cancer.

Cell fusion is essential for developmental and behavioral patterning

In wild-type *C. elegans*, embryos and larvae elongate to a final length of ~1 mm. Embryonic elongation is driven by contraction of circumferential actin microfilaments,[50] while larval elongation involves polyploidization, reorganization of the cytoskeleton, cuticle stretching and additional changes in the shape of the hypodermis.[51] Inspection of *eff-1* temperature-sensitive mutants revealed that a block of cell fusion in these animals causes severe embryonic and postembryonic elongation defects.[39] The elongation rate of *eff-1* embryos is decreased by 50% (B.P., unpublished results), resulting in the formation of dumpy and short worms (Fig. 4C). Elongation defects are also observed in *idf-1* embryonic fusion mutants,[43] suggesting that cell fusion normally promotes elongation in *C. elegans*.

How does cell fusion promote elongation? A plausible explanation is proposed in the fusomorphogenetic hypothesis. This model proposes that cell fusion between adjacent cells, taking place in the apical domains, is followed by vesiculation of the fusing membranes and targeting of these vesicles to other domains in the newly formed syncytia.[26] Such recycling and redistribution of membranes could be an alternative pathway to de novo membrane synthesis. This theory is supported by the fact that cell fusion blockage inhibits, but does not completely arrest, the process of elongation. Thus, one mechanism (synthesis) works less efficiently than two mechanisms (synthesis and fusion-dependent membrane recycling).

The fusomorphogenetic hypothesis can also explain how cell fusion contributes to the formation of the hypodermis, the tissue that envelopes the whole body of the worm. The hypodermis is formed during embryonic and postembryonic development by fusion of different epithelial cells including the dorsal and ventral epithelia, lateral seam cells and vulval cells.[31,52] Redistribution of membranes within the hypodermis can be used as a mechanism to promote the required enlargement of this tissue throughout development. Consistent with this, lack of cell fusion in specific epithelial cells in mutant animals results in abnormal hypodermal arrangement and protrusions of the hypodermis out of the body exactly in the unfused areas (Lumpy phenotype) (Fig. 4D,E).

The failure to promote normal arrangement of epithelia due to blockage of cell fusion also affects the locomotion behavior of the worms. In wild-type worms, the lateral seam cells secrete a specified well-patterned cuticle that is used for sliding above the ground. Since the seam cells fail to fuse in *eff-1* mutants and migrate throughout the hypodermis (Fig. 4B), the resulting secreted cuticle is disorganized and is probably responsible for the locomotion difficulties exhibited by these mutants.[40]

In *C. elegans*, embryos are produced either by self-fertilization or by mating between males and hermaphrodites. Except for the obvious role of cell fusion in fertilization, it is also required for the creation of the male mating organs and the hermaphrodite egg-laying system and genitalia. In hermaphrodites, cell fusion is essential for the formation of normal uterine as well as vulval structures, as discussed in detail in the next section (Fig. 4H). In males, cell fusion is needed for the formation of a patterned tail, which carries the sensilla (rays), organs that are essential for mating[26,34,53] (see Fig. 4F for details). Arrest of cell fusion causes mis-positioning of these sensilla (Fig. 4G) and, as a consequence, the males are completely infertile.

In summary, blocking cell fusion in *C. elegans* results in improper morphologies of organs such as vulva, uterus, hypodermis and tail spike (Fig. 4). These defects strongly suggest that cell fusion is essential in wild-type worms for correct organogenesis. Altered functions of organs and tissues due to fusion incompetence result in abnormal physiology, morphology and locomotion.

The vulva of *C. elegans*: a unified model for cell fusion in organ formation

Intensive research in past years used the vulva of *C. elegans* as a paradigm for studies on cellular behaviors and organogenesis.[33,48,54–58] This research has revealed that fundamental signaling pathways are involved in determining how primordial cells adopt cell fates, proliferate, differentiate and undergo morphogenesis. Based on the studies presented above, we propose a model that describes how all these pathways act together with cell fusion to promote the formation of rings/toroids that form a tube-shaped vulva in the nematode *C. elegans*. The model is presented in Figures 5 and 6.

Cell fusion is involved in four stages during vulva formation[33]: (1) a switch between vulval and epidermal fusion fates at the first larval stage (L1), (2) a similar fate determination during the L3 stage, (3) homotypic fusions between migrating primordial cells during the early L4 stage and (4) fusion within vulval rings during the late L4 stage (Fig. 3).

As cell fusion differentiates between cells that adopt epidermal (fusion) and vulval fates, it is important for this process to be firmly regulated. A key gene in this regulation is the Hox gene *lin-39*/*Dfd*/*HoxD4*. This gene is required already during the L1 stage in six out of eleven ventral epidermal cells (the six vulva precursor cells, VPCs) to prevent them from fusing with the surrounding hypodermal syncytial cell hyp7, a fate adopted by their sister cells, which lack *lin-39* activity[59,60] (Fig. 5A). This process appears to be conserved in evolution since in the nematode *Oscheius sp. 1 CEW, lin-39* also represses cell fusion in the VPCs.[61] However, in the nematode *Pristionchus pacificus*, *lin-39* represses programmed cell death in these cells instead of cell fusion.[62] Using genetic epistasis analysis and investigating the expression of *eff-1* RNA in different genetic backgrounds, it was shown that the fusion inhibitory activity of *lin-39* is gained by repressing *eff-1* expression in the non-fusing VPCs[48] (Fig. 5B–D). The unfused VPCs divide at mid-L3 followed by fusion of half of them to the large hypodermal syncytial cell (hyp7), omitting these cells from participating in vulva formation (Fig. 5A). Although basal activity of *lin-39* is present due to a Wnt signaling pathway,[63,64] this activity is not sufficient for *eff-1* repression, allowing *eff-1* fusogenic activity to promote fusion of these cells (Fig. 6A). In contrast, in other VPCs (daughters of P(5-7).p- 5-7 in Fig. 5A), the basal activity of the Hox gene *lin-39* is elevated due to a gonadal inductive signaling pathway

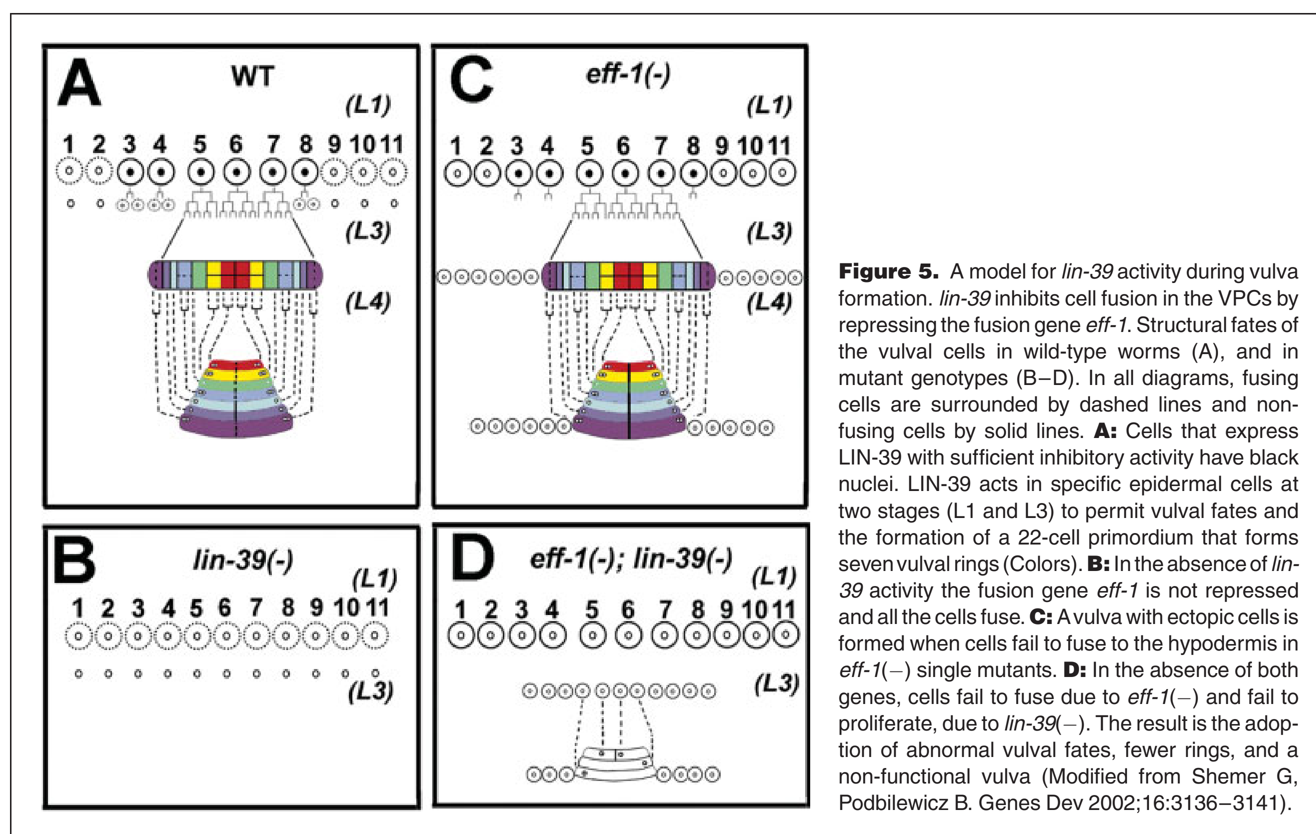

Figure 5. A model for *lin-39* activity during vulva formation. *lin-39* inhibits cell fusion in the VPCs by repressing the fusion gene *eff-1*. Structural fates of the vulval cells in wild-type worms (A), and in mutant genotypes (B–D). In all diagrams, fusing cells are surrounded by dashed lines and non-fusing cells by solid lines. **A:** Cells that express LIN-39 with sufficient inhibitory activity have black nuclei. LIN-39 acts in specific epidermal cells at two stages (L1 and L3) to permit vulval fates and the formation of a 22-cell primordium that forms seven vulval rings (Colors). **B:** In the absence of *lin-39* activity the fusion gene *eff-1* is not repressed and all the cells fuse. **C:** A vulva with ectopic cells is formed when cells fail to fuse to the hypodermis in *eff-1*(−) single mutants. **D:** In the absence of both genes, cells fail to fuse due to *eff-1*(−) and fail to proliferate, due to *lin-39*(−). The result is the adoption of abnormal vulval fates, fewer rings, and a non-functional vulva (Modified from Shemer G, Podbilewicz B. Genes Dev 2002;16:3136–3141).

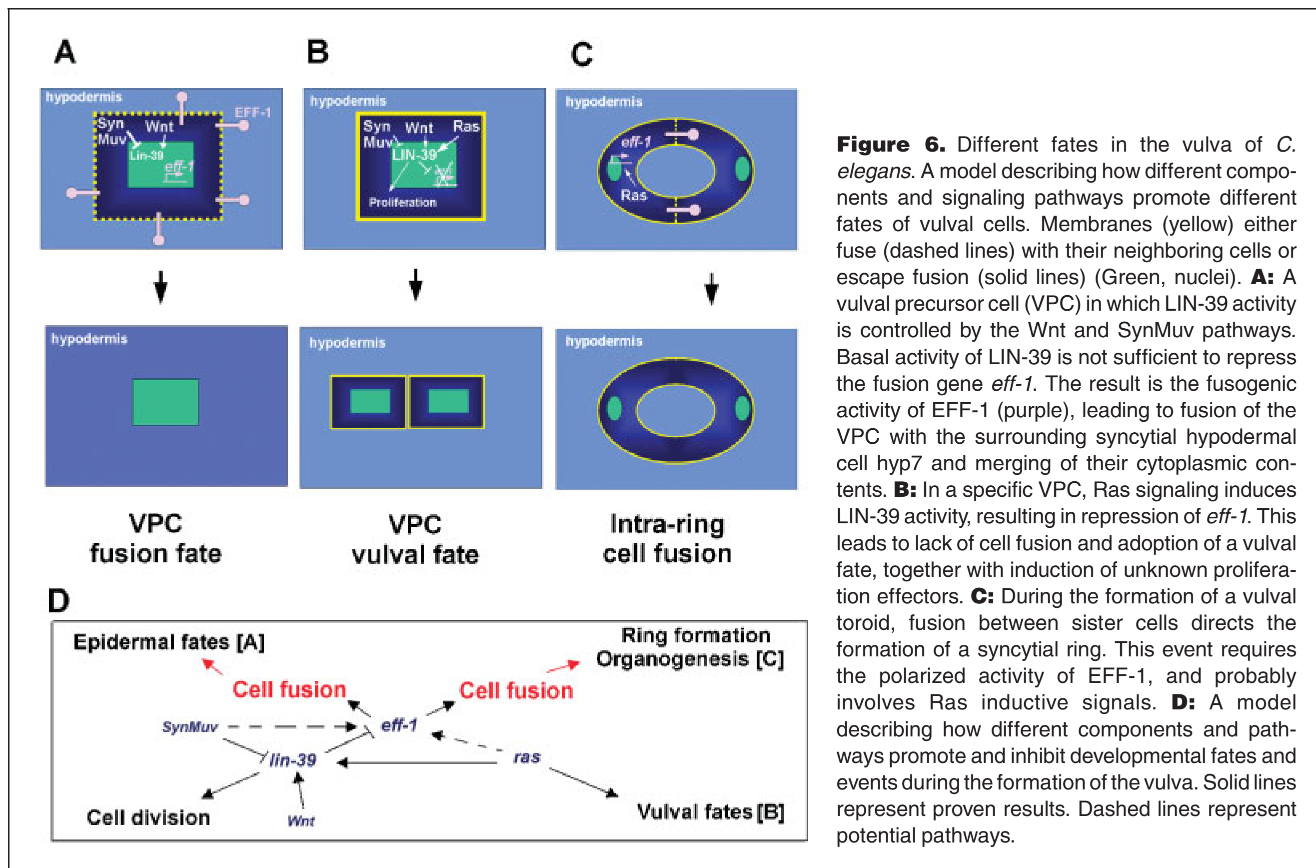

Figure 6. Different fates in the vulva of *C. elegans*. A model describing how different components and signaling pathways promote different fates of vulval cells. Membranes (yellow) either fuse (dashed lines) with their neighboring cells or escape fusion (solid lines) (Green, nuclei). **A:** A vulval precursor cell (VPC) in which LIN-39 activity is controlled by the Wnt and SynMuv pathways. Basal activity of LIN-39 is not sufficient to repress the fusion gene *eff-1*. The result is the fusogenic activity of EFF-1 (purple), leading to fusion of the VPC with the surrounding syncytial hypodermal cell hyp7 and merging of their cytoplasmic contents. **B:** In a specific VPC, Ras signaling induces LIN-39 activity, resulting in repression of *eff-1*. This leads to lack of cell fusion and adoption of a vulval fate, together with induction of unknown proliferation effectors. **C:** During the formation of a vulval toroid, fusion between sister cells directs the formation of a syncytial ring. This event requires the polarized activity of EFF-1, and probably involves Ras inductive signals. **D:** A model describing how different components and pathways promote and inhibit developmental fates and events during the formation of the vulva. Solid lines represent proven results. Dashed lines represent potential pathways.

that involves Ras and MAPK.[60] This elevated activity is sufficient to repress *eff-1* expression. In the absence of *eff-1* activity, the cells do not fuse and continue to divide in different patterns, yielding a 22-cell primordium.[48] This proliferation stage is induced by the activity of *lin-39*[48] (Fig. 6B).

The repression of *eff-1* action in the VPCs also results from the activity of the *pbx*/*extradenticle (exd) ortholog ceh-20,* a homeobox-containing transcription cofactor that has been shown to act as a co-activator with *lin-39* in the mesoderm of *C. elegans*.[65] *lin-39*, together with *ceh-20*, probably regulates the activity of the fusion regulators *egl-18* and *elt-6*, GATA factors that work redundantly to inhibit cell fusion in the vulval cells.[66] In addition, the *engrailed ortholog ceh-16*, another homeobox-containing gene, may repress fusion in the seam cells (G. Cassata and R. Baumeister, personal comm.) Given all this data (Table 1), it is tantalizing to hypothesize that *eff-1* activity is inhibited or induced by Hox genes, depending on the presence and identity of the cofactors that act in specialized transcriptional complexes in different cells and at different times of development.

At the onset of the L4 stage, the vulval primordial cells migrate toward the center and invaginate forming a stack of seven multicellular rings[33] (Fig. 3). Five of these rings then undergo intratoroidal fusions, which are promoted by *eff-1* activity and which are *lin-39*-independent. Evidence for ectopic fusion of vulval cells in *let-60*/*ras* gain-of-function mutants during these stages suggests that the intratoroidal fusions are facilitated by the activity of the Ras signaling pathway[36] (Fig. 6C). Although the activity of *lin-39* is not essential at this stage, it still may be important for establishing the correct sculpture of the seven-ringed vulva.[37] Finally, a gonadal cell (the anchor cell, AC) penetrates the apex of the vulva and fuses with the uterus, which is located dorsally.[33] This event is probably promoted by the activity of *sec-18*/*nsf*, a homolog of the universal NEM-sensitive factor (nsf) involved in endoplasmic membrane fusion (A. Newman, personal comm.). Following plastic changes (eversion) of this stack of rings, a tube-shape vulva that connects the uterus and the outside is formed.[33]

What are the factors that determine the spatial and temporal expression and activity of *lin-39*? It was found that, at the first larval stage, *lin-39* expression is suppressed in the posterior body region by the chromatin regulatory factor *egl-27*[67] as well as by the redundant activity of *ref-1*, an *hairy* homolog with two helix–loop–helix (HLH) domains and the Hox gene *mab-5*[68] (Table 1). At later stages, *lin-39* expression is elevated due to activity of the transcription factor *sem-4*,[69] and members of the Wnt pathway.[63,70] Additional

factors that regulate vulval cell fusion, probably via control of *lin-39* activity, belong to the synthetic multivulva (SynMuv) genes—a family of genes (e.g., *lin-53* RbAp and *lin-40* MTA, members of the NuRD complex and *lin-15a,b*) that act together to promote cell fusion of epidermal non-vulval cells.[71,72]

In summary, the formation of the vulva sculpture in *C. elegans* requires the combined activities of several intercellular events, such as cell adhesion, cell migration, cell fusion and ring formation. Serving as a key process throughout the steps of vulva formation, cell fusion is regulated in a stringent way by factors, such as Ras, Hox and Wnt, which are involved in various conserved pathways (Fig. 6D).

Concluding remarks

Cell fusion is a widespread process in metazoans. Although our understanding of how cell–cell fusion takes place is limited compared to our understanding of virus–cell fusion, newly identified key players that participate in cell–cell fusion help us understand the mechanism of this process. Most of the critical questions are yet to be answered. It is still not known whether *eff-1* is unique to nematodes or whether we can extrapolate from *C. elegans* to higher organisms because the only *eff-1* homologs identified so far are in other nematode species. A challenging issue that remains to be resolved is how *eff-1* acts. Identification of putative active domains suggests that *eff-1* may work in a manner similar to class I or, more likely, to class II viral fusogens. Alternatively, membrane fusion could be the consequence of lipid binding and/or modification by a PLA_2 active site. Future structure–function analyses will help in deciphering this issue. It is still not known whether there are other components of the fusion machinery complex. Identification of proteins that interact with EFF-1 could help in revealing the mechanism of such a complex. Finally, as more and more regulators of cell fusion are being found, it is very import to map the interactions between these factors and to better understand how cell fusion is regulated in a spatial and temporal way to form syncytia and organs. There is no doubt that, in the near future, genetic and biochemical research in a variety of model organisms will shed much more light on these fundamental questions.

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