Programmed cell fusion in development and homeostasis

Yael Iosilevskii and Benjamin Podbilewicz*
Department of Biology, Technion—Israel Institute of Technology, Haifa, Israel
*Corresponding author: e-mail address: podbilew@technion.ac.il

Contents

1. Introduction 2
2. Cell fusion in embryogenesis 4
   2.1 Sperm-egg fusion in *C. elegans* 4
   2.2 AFF-1-mediated self-cell fusion and endocytic fission shape the excretory duct cell 5
   2.3 Auto-fusion at the pharynx-intestine valve 7
   2.4 Tail morphogenesis by fusion 7
   2.5 Hypodermis formation and sculpting 9
   2.6 The endocytic machinery as regulators of surface-exposed EFF-1 10
   2.7 Role of the cytoskeleton in cell-cell fusion 11
   2.8 Transcriptional regulation of embryonic cell-cell fusion 12
3. Cell fusion during larval development 12
   3.1 Hypodermal fusions during larval development 13
   3.2 Regulation of hypodermal fusions during larval development 13
   3.3 Cell-cell fusions sculpt the reproductive system 14
   3.4 Exoplasmic fusion in neuronal morphogenesis and homeostasis 15
4. Fusion in homeostasis 17
   4.1 Repair of wounded epithelia 19
   4.2 Neuronal sculpting and repair 19
5. Why does *C. elegans* need more than one fusogen? 20
   5.1 Do AFF-1 and EFF-1 interact in vivo? 20
   5.2 Is AFF-1 more precise than EFF-1? 21
6. Concluding remarks 22
Acknowledgments 22
References 22

Abstract

During multicellular organism development, complex structures are sculpted to form organs and tissues, which are maintained throughout adulthood. Many of these processes require cells to fuse with one another, or with themselves. These plasma

Current Topics in Developmental Biology
ISSN 0070-2153
https://doi.org/10.1016/bs.ctdb.2020.12.013

membrane fusions merge endoplasmic cellular content across external, exoplasmic, space. In the nematode *Caenorhabditis elegans*, such cell fusions serve as a unique sculpting force, involved in the embryonic morphogenesis of the skin-like multinuclear hypodermal cells, but also in refining delicate structures, such as valve openings and the tip of the tail. During post-embryonic development, plasma membrane fusions continue to shape complex neuron structures and organs such as the vulva, while during adulthood fusion participates in cell and tissue repair. These processes rely on two fusion proteins (fusogens): EFF-1 and AFF-1, which are part of a broader family of structurally related membrane fusion proteins, encompassing sexual reproduction, viral infection, and tissue remodeling. The established capabilities of these exoplasmic fusogens are further expanded by new findings involving EFF-1 and AFF-1 in endocytic vesicle fission and phagosome sealing. Tight regulation by cell-autonomous and non-cell autonomous mechanisms orchestrates these diverse cell fusions at the correct place and time—these processes and their significance are discussed in this review.

1. Introduction

Membrane fusion is essential for many functions in eukaryotes, from intracellular trafficking, to organ formation, to fertilization itself (Brukman, Uygur, Podbilewicz, & Chernomordik, 2019; Hernández & Podbilewicz, 2017; Petrany & Millay, 2019; Segev, Avinoam, & Podbilewicz, 2018). Such fusion requires special effort: a cell (or any membrane-covered compartment) is a stable structure, and has no "energetic incentive" to join its lipid membrane into a neighboring cell's membrane, nor to form an opening between them (Chernomordik & Kozlov, 2008; Markvoort & Marrink, 2011). A unique type of proteins, called fusogens, mediate this process by bringing the apposing membranes into close proximity, reducing the "effort" required to elicit membrane fusion (Chernomordik & Kozlov, 2008; Markvoort & Marrink, 2011). Cell-to-cell fusion also calls for dedication, and some degree of compatibility: Since a cell may or may not express a fusogen on its surface, cells differ in their relative "dedication" to fusion, and such fusogen expression may be regulated by numerous signaling pathways (del Campo et al., 2005; Shemer et al., 2004; Shemer & Podbilewicz, 2003). Moreover, some fusogens utilize a bilateral mechanism, and are required on both fusing membranes—this represents a measure of "compatibility" in the merger of adjacent cells. This unique property is absent in unilateral fusogens, such as those facilitating viral infection (Aguilar et al., 2013; Brukman et al., 2019; Podbilewicz, 2014; Segev et al., 2018; Shinn-Thomas & Mohler, 2011).

Developmental cell fusions are a driving force in the sculpting of organs and tissues, such as muscle and bone (Petrany & Millay, 2019; Shin et al., 2014; Verma, Leikina, Melikov, & Chernomordik, 2014; Yagi et al., 2005).

Such processes represent a unique angle into understanding membrane fusion on a more global level and are often tightly regulated in space and time. While the process of membrane fusion has been the focus of research in cell biology for over half a century (Palade, 1975; Rothman, 2014; White, 1992; Wickner & Schekman, 2008), we still do not fully understand the structural properties, temporal and spatial control, and diversity of roles that fusogens play in all aspects of life.

The nematode *Caenorhabditis elegans* (*C. elegans*) provides an excellent model for studying developmental fusion events: an invariant and fully characterized cellular lineage allows for the unique identification of every cell's fate during development (Sulston & Horvitz, 1977; Sulston, Schierenberg, White, & Thomson, 1983). Moreover, many of the cells are destined to fuse with others: the worm is covered by a skin-like tissue called hypodermis, which is formed by an intricate fusion of multiple epithelial cells to create a multinucleate layer (syncytium) (Podbilewicz & White, 1994; Sulston et al., 1983). In the young adult, approximately one-third of all somatic nuclei reside in multinucleate cells that originate by fusion (Podbilewicz & White, 1994). While such extensive developmental syncytiogenesis is to be found in other organisms, such as glass sponges (hexactinellids) (Leys, 2003), and during tissue formation of mammalian muscle (Petrany & Millay, 2019) and bone (Shin et al., 2014; Verma et al., 2014), *C. elegans* epithelia undergoes a reproducible and finite number of fusion events, which facilitates precise study.

A screen for epithelial fusion defects led to the discovery of the gene *eff-1* (*epithelial fusion failure-1*) (Mohler et al., 2002) and further studies indicated that EFF-1 is a bona fide fusogen (Podbilewicz et al., 2006; Shemer et al., 2004). A second *C. elegans* fusogen, AFF-1, was found to mediate anchor cell fusion (*aff-1*, *anchor cell fusion failure-1*) which forms part of the reproductive system (Sapir et al., 2007). Fusions mediated by EFF-1 or AFF-1 would not occur in their absence, making them entirely necessary for membrane merger (Mohler et al., 2002; Sapir et al., 2007). As bona fide fusogens, EFF-1 and AFF-1 are also sufficient in themselves to induce a fusion event which would otherwise not occur (Sapir et al., 2007; Shemer et al., 2004). The fusogenic activity of EFF-1 and AFF-1 requires their presence on both opposing membranes (Podbilewicz et al., 2006; Sapir et al., 2007), indicating their bilateral mechanism of activity. Subsequent characterization of both fusogens established that AFF-1 is similar enough to EFF-1 to enable their functional interaction in heterologous systems (Avinoam et al., 2011) and possibly in vivo (Meledin et al., 2020; Oren-Suissa, Gattegno, Kravtsov, & Podbilewicz, 2017), as will be discussed below.

The determination of EFF-1 atomic structure by X-ray crystallography (Pérez-Vargas et al., 2014) uncovered high structural similarity to viral class II fusogens, and more recently this homology was extended to include GCS1(HAP2) proteins which mediate sperm-egg fusion in plants and mating in protists (Fédry et al., 2017; Feng et al., 2018; Hirai et al., 2008; Liu et al., 2008; Mori, Kuroiwa, Higashiyama, & Kuroiwa, 2006; Pérez-Vargas et al., 2014; Valansi et al., 2017; von Besser, Frank, Johnson, & Preuss, 2006). These fusogens represent a protein family, evolutionarily conserved enough as to allow the nematode EFF-1 to functionally interact with plant GCS1(HAP2) (Valansi et al., 2017). Such a tight mechanistic resemblance, which is not directly mirrored in the proteins' constituent amino acids (Valansi et al., 2017), presents a challenge but also great promise in the quest to map ancestrally-distant orthologs and their phylogenetic relations (see Hernández & Podbilewicz, 2017; Vance & Lee, 2020). By piecing the morphological contexts involving *eff-1* and *aff-1* in *C. elegans* we hope to gain a better understanding of fusogens and their importance in nematodes and other organisms. This review covers the functions EFF-1 and AFF-1 play during development and in adult-stage homeostatic processes in *C. elegans*, and what is known about their regulation. Some topics were previously covered by Soulavie and Sundaram (2016), Giordano-Santini, Linton, and Hilliard (2016) and Alper and Podbilewicz (2008) and are updated here to present a more complete view of our current knowledge.

2. Cell fusion in embryogenesis

2.1 Sperm-egg fusion in *C. elegans*

Before discussing the roles cell fusion plays in the developing embryo, we must start at the beginning. All sexually reproducing animals begin life by the fusion of gametes (Brukman et al., 2019). This single ubiquitous event remains poorly understood in many organisms; the fusogens mediating sperm-egg fusion in vertebrates, fungi, and in *C. elegans*, remain unknown. Multiple genes in *C. elegans* were shown to result in sterility when mutated, on both sperm and oocyte (Marcello, Singaravelu, & Singson, 2013; Nishimura & L'Hernault, 2010); however, no protein to date was demonstrated to be directly sufficient to induce *C. elegans* gamete fusion. As mentioned, EFF-1 and AFF-1 are structurally similar to the established gamete fusogen GCS1(HAP2) (Hirai et al., 2008; Mori et al., 2006; Valansi et al., 2017); however, they do not assume this role in *C. elegans* (Sapir et al., 2007). Numerous promising candidates exist (to name but

a few): SPE-45, homologous (Nishimura, Tajima, Comstra, Gleason, & L'Hernault, 2015) to the vertebrate crucial gamete adhesion protein IZUMO1 (Inoue et al., 2013), and SPE-42 and its homolog SPE-49, which are similar (Wilson et al., 2018) to both the *Drosophila* sperm acrosomal protein Sneaky (*snky*) (Wilson, Fitch, Bafus, & Wakimoto, 2006), and to the vertebrate protein DC-STAMP which is important in osteoclast fusion (Yagi et al., 2005). While these proteins are all important in later stages of fertilization, their role as fusogens was never shown. The first and foremost fusion event, therefore, still remains a mystery, as we move onto embryonic development.

Around 800 minutes after fertilization, the *C. elegans* embryo forms, with 671 nuclei each assuming its correct location and fate (Sulston et al., 1983). These fates may involve programmed cell death, or, indeed, fusion (Sulston et al., 1983). Cell-to-cell fusions were shown to sculpt tubular elements in the excretory and the digestive system, as well as shape fine structures such as the tip of the tail. Conversely, fusions also mediate large-scale hypodermal syncytia formation, joining 23 epidermal cells in the embryo to form hyp7 syncytium (Fig. 1A and B). The following sections describe these processes and their regulation (Fig. 1); hypodermal morphogenesis continues through larval development (see Fig. 2), as will be discussed in the following parts.

2.2 AFF-1-mediated self-cell fusion and endocytic fission shape the excretory duct cell

The excretory system in *C. elegans* assumes a kidney-like role. It consists of three interconnected hollow cells: the canal (or excretory) cell, which draws fluids and possibly waste products from the worm's body, connects with the duct cell, which bonds with the pore cell. The pore cell is attached to the outer cuticle on the ventral side and opens the system to the external environment (Buechner, 2002; Nelson, Albert, & Riddle, 1983) (Fig. 1B and C). Interestingly, each cell becomes hollow by a different mechanism: The pore cell wraps around itself in a C-shape to form a hollow center yet does not auto-fuse (Nelson et al., 1983), the canal cell merges multiple intracellular vacuoles forming an internal cavity which later opens to the cell exterior (Berry, Bülow, Hall, & Hobert, 2003; Buechner, 2002), and the duct cell wraps around itself and auto-fuses to form a toroid (Stone, Hall, & Sundaram, 2009) (Fig. 1C). Elegant work by Sundaram and colleagues has shown that duct cell auto-fusion depends on AFF-1 (Stone et al., 2009). AFF-1 is transduced by fate-determining pathways involving EGF/Ras/MAPK, and also participates in duct cell elongation independently of auto-fusion (Soulavie et al., 2018). Unusually for an exoplasmic fusogen, which acts

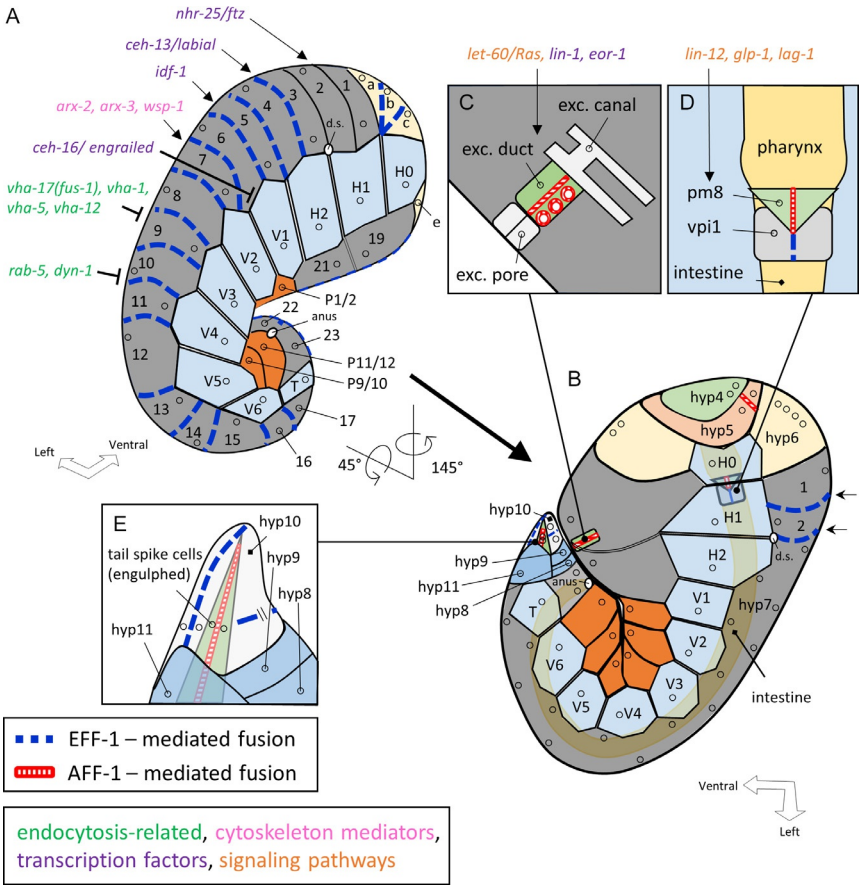

Fig. 1 Cell fusions in embryonic development. (A) Comma-stage embryo before dorsal fusions. Dashed blue: EFF-1-mediated fusions disassembling apical junctions. White encased and striped in red: AFF-1-mediated fusions disassembling apical junctions. Right dorso-lateral view (see Podbilewicz & White, 1994). Regulatory factors are colored according to the legend categories where applicable. d.s. deirid sensillum (see Brunschwig et al., 1999) (B) Left view of 1.5-fold embryo, after most dorsal fusions. Junctions 1/2 and 2/3 (black arrows; B) are largely maintained during the comma stage (Gattegno et al., 2007) and will fuse later (Podbilewicz & White, 1994). Light blue: seam cells (lateral). Dark orange: P cells (ventral). Black lines: apical junctions. Nuclei are represented as black empty circles. Insets C–E refer to specific fusions: (C) AFF-1 mediates auto-fusion of the excretory duct cell, as well as basolateral endocytic fission (white vesicles). *let-60/Ras* plays a key role in determining the fates of the duct cell: in gain-of-function (*gf*) *let-60* mutants, the future excretory pore cell instead assumes a duct fate, and the two ducts fuse together in an AFF-1-dependent fashion (Abdus-Saboor et al., 2011). *let-60/Ras* acts within the EGF/Ras/ERK pathway, promoting duct-cell fate through *lin-1* (an Ets transcription factor) and *eor-1* (zinc finger

in bridging two membranes across extracellular space, AFF-1 was also shown to facilitate duct cell basolateral-membrane endocytic fission of vesicles (Soulavie et al., 2018) (Fig. 1C).

2.3 Auto-fusion at the pharynx-intestine valve

Another example of auto-fusion forming hollow connections in the developing embryo is found in the interface between the pharynx (feeding organ) and the intestine. Connecting between the terminal bulb of the pharynx and the intestine is a valve, situated such that the posterior-most muscle cell of the pharynx, called pm8, and the anterior-most cell of the valve, called vpi1, are tightly bound (Albertson & Thomson, 1976; Rasmussen et al., 2008). pm8 and vpi1 each form a single mononucleated toroidal ring. This spatial configuration seems to stem from differential expression of the two different fusogens: auto-fusion of pm8 depends on AFF-1, while vpi1 auto-fusion depends on EFF-1, and the two adjacent cells do not merge (Rasmussen et al., 2008) (Fig. 1D).

Linking another crucial developmental signaling pathway (Greenwald, 2005) with fusogen regulation, Notch is highly expressed in pm8, but not in vpi1, and activates *aff-1* expression, possibly simultaneously repressing *eff-1* (Rasmussen et al., 2008). Mutants in *lag-1* (a downstream effector of the Notch pathway) unusually express *eff-1* in pm8, eliciting EFF-1-mediated aberrant fusion of pm8 with vpi1 (Rasmussen et al., 2008). Thus, the Notch pathway regulates EFF-1- and AFF-1-induced auto-fusions in the formation of the pharyngeal valve, preventing their cross-fusion (Rasmussen et al., 2008).

2.4 Tail morphogenesis by fusion

In the formation of the hermaphrodite *C. elegans* tail, two tail spike cells fuse and subsequently undergo *ced-3*-mediated programmed cell death (Chiorazzi et al., 2013; Sulston et al., 1983). This fusion requires *aff-1*, as *aff-1* mutants

transcription factor), conferring *aff-1* expression (Soulavie, Hall, & Sundaram, 2018). (exc.) excretory. Note the "H"-shaped canal cell is depicted for emphasis yet assumes this shape later (see Abdus-Saboor et al., 2011). (D) Auto-fusions of the pharynx-intestinal valve depend on AFF-1 in pm8 and EFF-1 in vpi1, with pm8 fusogen expression specified by Notch signaling. Two Notch proteins, LIN-12 and GLP-1, act through the DNA-binding CSL protein LAG-1. *lin-12;glp-1* or *lag-1* mutants show defects in valve morphogenesis (Rasmussen, English, Tenlen, & Priess, 2008). (E) Fusions which shape the tail. Note hyp8, hyp9 and hyp11 are mononucleate (Chiorazzi et al., 2013; Ghose et al., 2018; Nguyen, Hall, Yang, & Fitch, 1999; Sulston et al., 1983).

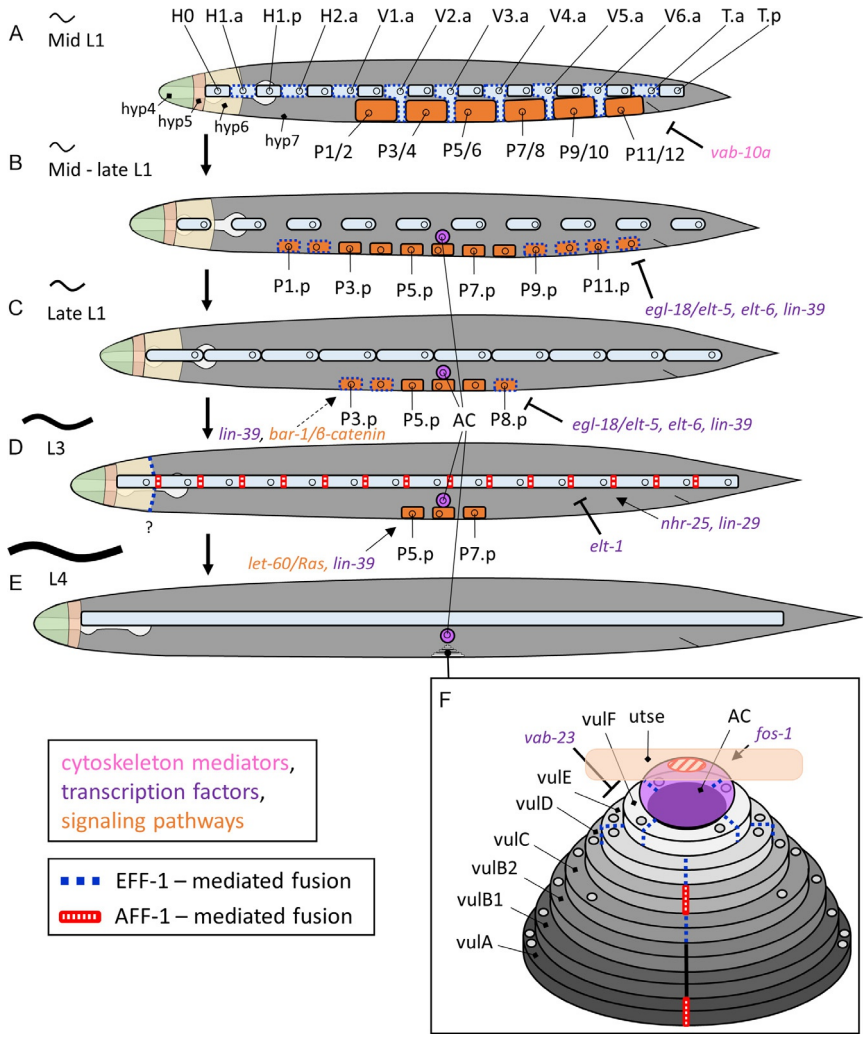

Fig. 2 Some cell fusions in larval development. Dashed blue—EFF-1 mediated-fusion. White encased and striped in red: AFF-1-mediated-fusion. Light blue: seam cells (lateral). Dark orange: P cells (ventral). Black lines: apical junctions. (A–E) Hyp7 depicted as gray background. (A) During mid L1 larval development, seam cells daughters displace P cells (see Podbilewicz & White, 1994). (B) Seam cell anterior daughters fuse into hyp7 via EFF-1, while two ventral rows of 6 P cells rotate forming a single row of 12 cells. These later divide anteriorly-posteriorly, and of the 12 posterior daughters (marked P[1 – 12].p), P[3–8].p form the vulval precursor cells (VPCs) (Schindler & Sherwood, 2013). Non-VPC posterior daughters fuse into hyp7. (C) Of the six VPCs, three typically end their fate early by EFF-1-mediated fusion. The homeotic gene *lin-39*/HoxD4 and its cofactor *ceh-20*/Exd homeobox-containing protein act cell-autonomously as negative regulators of EFF-1-mediated cell fusion, ensuring P[3–8].p do not fuse into hyp7

show morphologically abnormal and bifurcated tail tips (Chiorazzi et al., 2013). The distal ending of the fused tail spike is engulfed by the adjacent hyp10 syncytium (Ghose et al., 2018; Nguyen et al., 1999), which itself forms by EFF-1-mediated fusion (Ghose et al., 2018; Mohler et al., 2002). The phagosome formed by this engulfment must seal around itself, similarly to a cell auto-fusing into a toroid. Such phagosome sealing around apoptotic cell bodies was previously shown to involve specific phosphoinositides (Cheng et al., 2015), and more recently EFF-1 was demonstrated to be the mediator of hyp10 phagosome sealing around the engulfed fragment of the distal tail spike (Ghose et al., 2018) (Fig. 1E). Regulation of this novel function remains unclear. Future studies will have to confirm whether other cases of fusogen-mediated phagosome sealing exist, perhaps, for example, in the less-understood process of CEMVL (sex-specific neuron) dendrite clearance (Ghose et al., 2018).

2.5 Hypodermis formation and sculpting

The epidermal (hypodermal) tissue of *C. elegans* forms to create several distinct layers of multinucleated syncytia (reviewed in Altun & Hall, 2009a). During embryonic development, the largest syncytium that is formed is hyp7, mostly covering the mid-dorsal plane. Additional cells fuse into hyp7

(Alper & Kenyon, 2002; Shemer & Podbilewicz, 2002). Two GATA factors, *egl-18* (*elt-5*) and its paralog *elt-6* act downstream to *lin-39* in this process (Koh et al., 2002; Koh, Bernstein, & Sundaram, 2004). (D–E) Seam cells fuse together during L4 to young adult transition via AFF-1; the GATA transcription factor *elt-1* represses *eff-1* expression in seam cells to prevent their cross-fusion with hyp7 (Brabin, Appleford, & Woollard, 2011). *nhr-25*, expressed in the seam cells (Šilhánková, Jindra, & Asahina, 2005), facilitates their syncytiogenesis downstream to *lin-29* (Hada et al., 2010) possibly by promoting their adhesion (Šilhánková et al., 2005). hyp6 fuses with hyp7 (Yochem, Gu, & Han, 1998), possibly in an EFF-1-mediated fashion. Nuclei are represented as black empty circles, which disappear following cell fusion into a syncytium. Larva size is roughly sketched as a tilde. (F) Fusions of the vulva rings. Each ring in the stack (VulA to VulF) is created by two or four C-shaped cells which fuse together, except vulB1 and vulB2 which adhere but do not fuse; pairwise fusions of precursors of VulC and VulA occur earlier before the toroids are formed (Sharma-Kishore, White, Southgate, & Podbilewicz, 1999). The anchor cell (AC, purple) blocks the formed ring stack dorsally, and will fuse into the utse in an AFF-1-mediated fashion. *aff-1* expression in the anchor cell, but not in vulD or the seam cells, is mediated by *fos-1*, a Fos orthologue transcription factor (Sapir et al., 2007). The zinc finger transcription factor *vab-23* inhibits *eff-1* expression specifically in vulE and vulF (Pellegrino et al., 2011). Nuclei are represented as gray circles. Rings transition from dark to light gray. Only a small segment of the utse is shown. Diagonal top-down view (from the utse ventrally toward the exterior of the animal).

during larval development, totaling in 139-nuclei in the adult. Other hypodermal fusions create different, smaller syncytia (Podbilewicz & White, 1994; Yochem et al., 1998). Embryonic cell fusions into hyp7 and hyp6 are dependent upon EFF-1 (Mohler et al., 2002), whereas the binucleate hyp5 fusion requires AFF-1 (Sapir et al., 2007). The control over epithelial morphogenesis takes place at both a spatial and a temporal level (see reviews by Alper & Podbilewicz, 2008; Chisholm & Hardin, 2005; Podbilewicz, 2006). Regulatory factors divide roughly into four groups: signaling pathways (Fig. 1, orange), endocytic machinery (Fig. 1, green), cytoskeletal effectors (Fig. 1, pink), and transcription factors (Fig. 1, violet).

2.6 The endocytic machinery as regulators of surface-exposed EFF-1

For two cells to fuse together a fusogen must be present on the cell membrane in sufficient amounts, and possibly in a proper conformation (del Campo et al., 2005; Podbilewicz et al., 2006; Podbilewicz & Chernomordik, 2006; Sapir, Avinoam, Podbilewicz, & Chernomordik, 2008). The endocytic machinery participates in the removal of cell-surface elements from the membrane into the cytoplasm; this form of regulation has been well characterized in the context of signaling by cell-surface receptor recycling (e.g., Anggono & Huganir, 2012; Blitzer & Nusse, 2006; Pitulescu & Adams, 2014; Scholpp & Brand, 2004). However, it was not clear whether the endocytic removal of fusion machinery away from the cell membrane could regulate a cell's ability to fuse. The conserved GTPases RAB-5 and dynamin-1, crucial in endocytosis regulation (Grant & Hirsh, 1999) and endocytic vesicle fission (Bucci et al., 1992; Clark, Shurland, Meyerowitz, Bargmann, & Van Der Bliek, 1997), respectively, were indeed demonstrated to be important for the downregulation of fusion (Smurova & Podbilewicz, 2016, 2017). Mutants and knockdown of *rab-5* or *dyn-1* cause an epithelial hyper-fusion phenotype and EFF-1 accumulation in the hypodermal cells' surface (Smurova & Podbilewicz, 2016, 2017). EFF-1 is dynamically localized both in intracellular compartments and transiently on the cell surface (Smurova & Podbilewicz, 2016, 2017) and becomes enriched on the membrane site destined to fuse (del Campo et al., 2005; Yang, Zhang, et al., 2017). The endocytic machinery thus plays an important and novel regulatory role by removing surface-residing EFF-1 into intracellular vesicles, reducing a cell's fusion potential (Smurova & Podbilewicz, 2016, 2017). In contrast to this negative function on cell-to-cell fusion in *C. elegans*, dynamin has a positive role in the cell fusions which form osteoclasts and muscle fibers (Leikina et al., 2013; Shin et al., 2014;

Verma et al., 2014). The *Drosophila* dynamin (*shibire*) was also recently shown to stimulate myoblast fusion possibly by the bundling of actin filaments (Zhang et al., 2020).

The vacuolar H^+-ATPase (vATPase) complex acts as a proton pump which facilitates the acidification of intracellular compartments such as endosomes and lysosomes (Forgac, 1999). Impairment of various subunits of this complex in *C. elegans* elicits a hyper-fusion phenotype of the dorsal hypodermis; these include *vha-5* (Smurova & Podbilewicz, 2017), *vha-1* (B subunit), *vha-12* (c subunit) and *vha-17(fus-1)* (e subunit) (Kontani, Moskowitz, & Rothman, 2005). Severe *fus-1* mutants show EFF-1-mediated excessive fusions of all epidermal cells apart from the seam cells (Kontani et al., 2005). While the vATPase complex does not seem to mediate endocytosis itself, it does influence the endocytic degradative pathway in the transition between early and late endosomes (Hurtado-Lorenzo et al., 2006). This role and the similarity of phenotype to impairment in the endocytic machinery may suggest a model whereby the vATPase complex is involved in EFF-1 lysosomal degradation, upon endocytosis mediated by RAB-5 and DYN-1 (Smurova & Podbilewicz, 2017) (Fig. 1A, green). The vATPase complex is clearly an important negative regulator of EFF-1 during dorsal embryonic fusion, although the precise mechanism remains to be studied.

2.7 Role of the cytoskeleton in cell-cell fusion

The functions of the cytoskeleton in cell-to-cell fusion have been characterized in multiple systems, including *Drosophila* and mice myoblasts as well as in mating yeast (Dhanyasi et al., 2015; Gruenbaum-Cohen et al., 2012; Kim et al., 2007; Martin, 2016; Massarwa, Carmon, Shilo, & Schejter, 2007; Shinn-Thomas & Mohler, 2011). In *Drosophila*, actin protrusions from a fusion competent cell can bring a fusogen into close proximity with an adjacent founder cell, thereby initiating their merger (Kim et al., 2007; Önel, Dottermusch, Sickmann, Buttgereit, & Renkawitz-Pohl, 2011; Paululat, Holz, & Renkawitz-Pohl, 1999; Shilagardi et al., 2013). This process depends on the actin nucleation factor WASP as well as Ig-superfamily adhesion factors, SCAR, Arp2/3, and others (Önel et al., 2011; Önel & Renkawitz-Pohl, 2009; Richardson, Beckett, Nowak, & Baylies, 2007; Schäfer et al., 2007). Since the endogenous *Drosophila* fusogen remains unknown (Martin, 2016; Shilagardi et al., 2013), reconstructing the process in tissue culture requires the expression of *C. elegans*' EFF-1 (Shilagardi et al., 2013). In such

a system, fusion is enhanced by having an active actin cytoskeleton (Shilagardi et al., 2013). Mouse myoblast fusion also requires cytoskeletal elements (Gruenbaum-Cohen et al., 2012) and fusion itself involves two essential membrane proteins, Myomaker and Myomerger, neither of which is sufficient to induce fusion on its own (Leikina et al., 2018; Petrany & Millay, 2019).

In contrast to the work on myoblasts, in *C. elegans*, RNAi knockdown of the WASP (*wsp-1*) and Arp2/3 (*arx-2* and *arx-3*) actin nucleation factors delayed, but did not abrogate, dorsal fusions (Zhang, Yang, Zhu, & Ou, 2017) (Fig. 1A, pink). While the cytoplasmic region of the EFF-1 protein is important for its function (Shinn-Thomas, Del Campo, Wang, & Mohler, 2016), a connection with cytoskeletal elements does not appear to be crucial for its activity; knockdown and mutant analysis of other cytoskeletal elements such as WAVE/SCAR components *wve-1*, *arp-2, gex-2* and *gex-3*, revealed polarity defects but no effect on cell fusions (Patel et al., 2008; Xiong, Mohler, & Soto, 2011).

It remains unclear whether, similarly to mice (Petrany & Millay, 2019), more than one protein is required for *Drosophila* myoblast fusion.

2.8 Transcriptional regulation of embryonic cell-cell fusion

Several transcription factors were shown to directly influence epidermal fusions which occur during embryogenesis (Fig. 1A, violet). The homeobox domain gene *ceh-16/engrailed* negatively regulates *eff-1* in seam cells (Fig. 1, light blue), preventing their aberrant fusion into the adjacent hyp7 (Fig. 1, gray) (Cassata et al., 2005), while *idf-1* (*irregular dorsal fusion -1*) may positively regulate the correct EFF-1-mediated fusion events (Gattegno et al., 2007). There may be distinct regulation in some dorsal epithelial cells, but not in others: a Hox gene, *ceh-13/labial*, which also directs seam cell placements, specifically promotes the dorsal fusion of epithelial cells 3 and 4, although its effect on *eff-1* has not been examined (Brunschwig et al., 1999). Future studies may reveal novel regulatory mechanisms controlling the formation of smaller syncytia in the anterior, such as hyp6 and hyp5.

3. Cell fusion during larval development

The hypodermis continues to grow during the four larval stages of the worm until it has molted into a young adult, in parallel with the formation of complex organs such as the vulva (Fig. 2).

3.1 Hypodermal fusions during larval development

The epithelial seam cells that are born in the embryo form a stem-cell like repository, and divide during larval development (except H0) into one epithelial cell (anterior) and one seam cell (posterior) (Sulston & Horvitz, 1977). In the first larval stage (L1), anterior daughters of these divisions migrate ventrally and fuse into hyp7 in an EFF-1-mediated process (Mohler et al., 2002) (Fig. 2A, light blue), concurrent with the separation of the 12 ventral P cells from each other (Podbilewicz & White, 1994) (Fig. 2A, dark orange). Later, each P cell divides anteriorly-posteriorly, and the posterior daughters of P[3–8] (termed P[3–8].p) form the vulval precursor cells (VPCs) (Schindler & Sherwood, 2013; Sulston & Horvitz, 1977) discussed below (Fig. 2C) while the rest fuse into hyp7 (Fig. 2B). These fusions, however, are not essential for VPCs' ventral migration, as evident by the correct placement of the vulva in *eff-1* mutants (Mohler et al., 2002).

In the late fourth larval stage (L4), the remaining 15 seam cells on each side of the worm fuse among themselves to form a lateral syncytium (Podbilewicz & White, 1994; Sulston & Horvitz, 1977), in an AFF-1-mediated fashion (Sapir et al., 2007) (Fig. 2D and E). As in the embryo, epithelial fusions into hyp7 are *eff-1*-dependent (Mohler et al., 2002), although the fusion of hyp6 with hyp7 during late L3 (Yochem et al., 1998) (Fig. 2D), has not been studied with regard to *eff-1*.

Fusion also assists in creating sexually dimorphic features: in males during L4, EFF-1 is activated by the DM family genes *dmd-3* and *mab-3* (Mason, Rabinowitz, & Portman, 2008) to facilitate a male-specific fusion of hyp8–11, which later forms its specialized tail structure (Mason et al., 2008; Nguyen et al., 1999).

3.2 Regulation of hypodermal fusions during larval development

During larval epidermal modifications the seam cells are kept distinct from hyp7 by the GATA factor *elt-1* (Brabin et al., 2011) (Fig. 2D and E). The heterochronic genes *nhr-25* and *lin-29*, involved, among others, in L4 molting and vulva development, possibly regulate seam-to-seam cell fusions; transcriptional reporters indicate *lin-29* mediates *aff-1* expression (Friedlander-Shani & Podbilewicz, 2011; Hada et al., 2010; Harris & Horvitz, 2011) (Fig. 2D and E).

Fusion of seam cell descendants of the V lineages into hyp7 depends on the cytoskeletal scaffold protein spectraplakin VAB-10A, as mutation

in *vab-10a* reduced the accumulation of endogenous EFF-1 to the membrane of V.a cells and also delayed their fusion (Yang, Zhang, et al., 2017) (Fig. 2A, pink). This effect, of delayed, but not abrogated, fusion is in accordance with the role of other cytoskeletal elements during embryonic fusions (see above). Migration of V-cell descendants ventrally during early L1 was recently shown to involve the homeobox gene *pal-1/caudal*, regulating adherens junction segregation; this phenomenon, however, was not linked with fusion (Gilbert, Mullan, Poole, & Woollard, 2020).

3.3 Cell-cell fusions sculpt the reproductive system

The reproductive system in the hermaphrodite *C. elegans* consists of two gonad arms where germ cells gradually mature into oocytes, which are fertilized as they pass through a spermatheca and into the uterus (Hubbard & Greenstein, 2005). The embryos partly develop in the uterus before they are laid through the vulva and continue their development outside the mother (Hall, Herndon, & Altun, 2017; Wood, 1988). The vulva itself is a hollow stack of seven concentric rings which connect to the ventral exterior (Newman, White, & Sternberg, 1996) (see Fig. 2E and F). The first embryo laid would break a thin hymen-like syncytial membrane called the utse (the name derived from "uterine-seam cell"), which blocks the interface between the uterus and the vulva (Newman et al., 1996).

The vulva, one of the most studied organs in *C. elegans*, is formed by the interplay of several signaling pathways governing cellular shaping, division, migration, and fusion (Newman et al., 1996; Pénigault & Félix, 2011; Podbilewicz & White, 1994; Roiz, Escobar-Restrepo, Leu, & Hajnal, 2016; Schindler & Sherwood, 2013; Schmid & Hajnal, 2015; Sharma-Kishore et al., 1999; Sternberg, 2005; Weinstein & Mendoza, 2013; Weinstein & Podbilewicz, 2016). Cell-to-cell fusions help determine the correct fate of vulval precursor cells (VPCs) (Weinstein & Mendoza, 2013), as well as form the utse (Newman et al., 1996) and the vulva rings themselves (Sharma-Kishore et al., 1999). Regulation of vulva development has been extensively reviewed elsewhere (see Schindler & Sherwood, 2013; Weinstein & Mendoza, 2013; Weinstein & Podbilewicz, 2016); the process and some key regulatory players are presented in (Fig. 2C–F).

Of the six VPCs, only three (P[5–7].p) will form the vulva itself; the remaining three fuse into hyp7, thereby ending their developmental fate early (Sharma-Kishore et al., 1999). P3.p is uniquely stochastic in its fusion timing, and fuses into hyp7 either directly or following a subsequent division

(see Kroll, Tsiaxiras, & van Zon, 2020; Pénigault & Félix, 2011). A recent paper indicates that the stochastic nature of this fusion may result from variability in LIN-39, and interestingly, in BAR-1/β *catenin* peak levels: both factors inhibit EFF-1-mediated fusion, and temporal changes in their accumulation modulate the fusion decision (Kroll et al., 2020) (Fig. 2C).

In forming the vulva ring structures, EFF-1 and AFF-1 act in separate places. The fusion of the anchor cell to the utse, as well as the intra-toroidal cell fusions which seal the rings of vulA and vulD are mediated by AFF-1 (Sapir et al., 2007), while EFF-1 mediates the other vulval intra-toroidal fusions (Mohler et al., 2002) (Fig. 2F). A third player is missing: the creation of the utse syncytium by the fusion of eight π-cell daughters is mediated by a fusogen whose identity remains unknown (Choi, Richards, Cinar, & Newman, 2006; Sapir et al., 2007). A recent study also identified binucleate germ cells in the otherwise syncytial gonad, whose presence depends neither on *eff-1* nor *aff-1*. This particular case, however, may be the result of changes to membrane structure without cell-cell fusion (Raiders, Eastwood, Bacher, & Priess, 2018). This result also implies that the syncytial nature of the gonad does not seem to depend on EFF-1 or AFF-1 (Raiders et al., 2018).

The vulva remains a robust model for organogenesis in *C. elegans* and other nematodes (Kiontke et al., 2007; Kolotuev & Podbilewicz, 2004, 2008; Louvet-Vallée, Kolotuev, Podbilewicz, & Félix, 2003; Mereu et al., 2020; Yang, Roiz, Mereu, Daube, & Hajnal, 2017). Cell fusion failures in the reproductive system result in egg-laying defects due to morphogenetic deformities in the uterus, vulva and anchor cell, which are severe in *aff-1* mutant animals (Sapir et al., 2007). These roles of cell-cell fusion are important for understanding organogenesis as a global process, and the fine details of tube formation and cell-cell interactions. A similar dependence on fusion for organogenesis may be conserved in other nematode species, possibly mediated by fusogens related to EFF-1 and AFF-1 (Avinoam et al., 2011).

3.4 Exoplasmic fusion in neuronal morphogenesis and homeostasis

The *C. elegans* nervous system is composed of 302 neurons in the adult hermaphrodite (White, Southgate, Thomson, & Brenner, 1986). While multiple factors govern neuron migration, co-fasciculation and patterning (Ackley, 2014; Chisholm, Hutter, Jin, & Wadsworth, 2016; Inberg et al., 2019), fusogens also play surprising roles in the development and homeostasis of some neurons (Fig. 3) and glia.

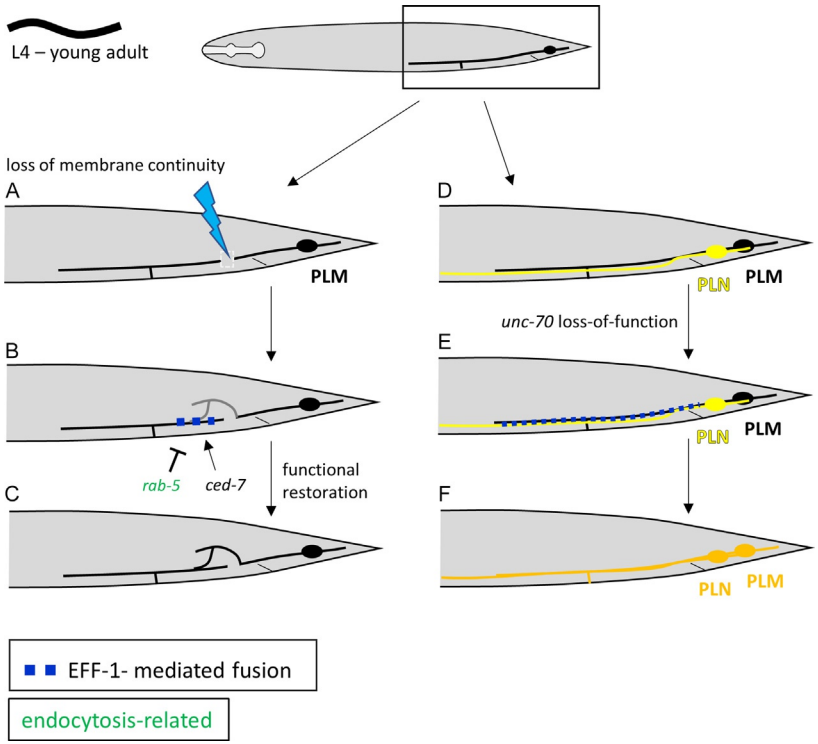

Fig. 3 Axonal repair by fusion in the PLM neuron. Dotted blue: EFF-1-mediated. (A–C) Upon axon severing by femtosecond laser pulses (Yanik et al., 2004, 2006), an outgrowth of ectopic branches is observed from the proximal site (Ghosh-Roy & Chisholm, 2010; Wu et al., 2007; Yanik et al., 2006). If a repair is accomplished by reconnection (Neumann, Nguyen, Hall, Ben-Yakar, & Hilliard, 2011) (as opposed to the distal fragment degenerating and the regrowth instead assuming its full functionality), EFF-1 facilitates the fusion of the regrown distal fragment with the proximal segment, leading to functional restoration (Abay et al., 2017; Basu et al., 2017). This process involves *ced-7*, inhibited by *let-7* miRNA (Basu et al., 2017), and is triggered by exposure of phosphatidylserine from the injured membrane (Abay et al., 2017) which is recognized by phosphatidylserine receptor *psr-1* (Basu et al., 2017). The reconnection is inhibited by *rab-5* (Linton et al., 2019). (D–F) Under an *unc-70* mutant background, which shows fragile axons (Hammarlund, Jorgensen, & Bastiani, 2007), neighboring PLM and PLN neurons may break and aberrantly fuse together, in an EFF-1-mediated fashion (Neumann et al., 2015). Left lateral view (showing PLML), in the area shown by top inset.

The inherent wiring of neuronal circuits requires specific connections between neurons (Chalfie et al., 1985; White et al., 1986). This implies neuronal fusion during development is often detrimental—when neurons are artificially linked by expression of vertebrate connexins to form novel gap

junctions, normal behavior is altered, and even reversed (Rabinowitch, Chatzigeorgiou, Zhao, Treinin, & Schafer, 2014). Such fusions were described for axons and for glia cells, but their extent and significance remain largely unknown. Upon entry to dauer state, a particularly resilient optional arrested state between L2 and L3 (Altun & Hall, 2009b; Riddle, 1988), the two amphid sheath (AMsh) glia cells fuse together (Albert & Riddle, 1983) in an *aff-1*-dependent fashion (Procko, Lu, & Shaham, 2011). Upon return to favorable conditions animals will exit dauer state (Altun & Hall, 2009b; Riddle, 1988), yet this fusion remains irreversible (Procko et al., 2011). Another unusual synthetic background for eliciting ectopic neuronal fusions during development is found in *unc-70*/β-spectrin mutants: it has been shown that *unc-70* mutants display fragile axons, which may break by muscle contractions alone (Hammarlund et al., 2007). The repair of some, but not all, of these severed axons is probably mediated by EFF-1. In around 15% of *unc-70* animals, fusion occurs between the tail neurons PLM and PLN, which is dependent upon *eff-1* (Neumann et al., 2015) (Fig. 3D–F). These rare cases stand in contrast to the role of fusogens in dendritic morphogenesis and neuronal repair, as described below.

Fusion was shown to play a crucial and positive role in the shaping of some dendrites. The PVD mechanosensory neuron pair develops postembryonically to span nearly the entire worm's body in an intricate yet highly ordered dendritic structure (Oren-Suissa, Hall, Treinin, Shemer, & Podbilewicz, 2010). Contrary to the wild-type, in *eff-1* mutants the young adult PVD dendritic tree is extremely hyperbranched and disordered (Oren-Suissa et al., 2010) (Fig. 4). This phenotype stems from a cell-autonomous role for *eff-1* in excessive branch pruning (Oren-Suissa et al., 2010) and/or from a non-cell-autonomous role in the localization of the neuronal guidance adhesion factor SAX-7/L1CAM in the epithelia, that is disrupted in *eff-1* mutants (Zhu, Liang, Wang, & Shen, 2017). In summary, despite our knowledge of neuron patterning mechanisms (see reviews by (Inberg et al., 2019; Soulavie & Sundaram, 2016), the regulation of neuron and glia structure by fusion and the importance of *eff-1* and *aff-1* in this process remain largely unknown.

4. Fusion in homeostasis

In standard laboratory growth conditions, *C. elegans* reaches adulthood within 4 days (Sulston & Horvitz, 1977), yet may live another 2 weeks longer (and twice as long under certain conditions) (Lin, Hsin, Libina,

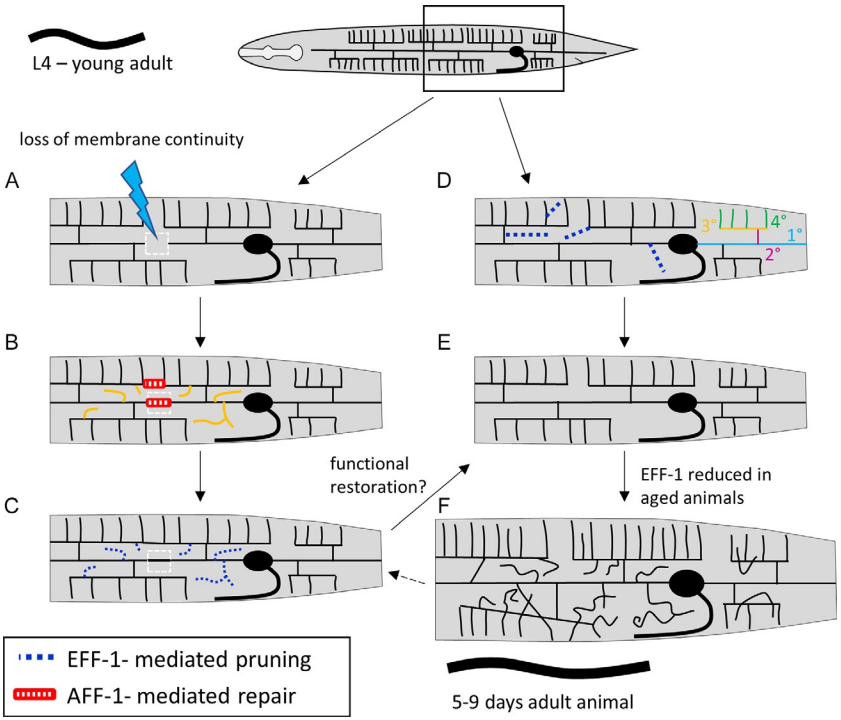

Fig. 4 Dendritic pruning and repair by fusion in the PVD neuron. Dotted blue: EFF-1-mediated. White encased and striped in red: AFF-1-mediated. (A and B) Upon dendritic severing by femtosecond laser pulses (Yanik et al., 2004, 2006), an outgrowth of ectopic branches is observed throughout the neuron; AFF-1 facilitates the repair by fusion, either by rejoining of the primary branch, and/or by bypassing the lesion through fusion of neighboring menorahs, in a non-cell-autonomous fashion (Oren-Suissa et al., 2017). (C) EFF-1 participates in the pruning of ectopic outgrowth, completing the regeneration (roughly similar to E) (Oren-Suissa et al., 2017). (D and E) The WT PVD neuron develops all four non-ectopic branching orders by the late L4 stage, as illustrated for one posterior menorah unit (see Oren-Suissa et al., 2010; Smith et al., 2010); ectopic branches are pruned by EFF-1. (F) During aging, a hyperbranching occurs, which may be partially rescued by cell-autonomous expression of EFF-1 (dashed arrow to C). Note the regenerative capability of an aged dendrite is too dependent on AFF-1, but, unlike young adults, may be rescued cell-autonomously (Kravtsov, Oren-Suissa, & Podbilewicz, 2017). Left lateral view (showing PVDL), in the area shown by top inset.

& Kenyon, 2001). Damaged cellular membranes must be repaired during this period; structures such as the PVD dendritic tree are maintained, to a certain extent, alongside neuron function, for several days (Kravtsov et al., 2017). Experimental approaches using needle wounding of the hypodermis (Chuang, Hsiao, Tong, Xu, & Chisholm, 2016; Xu & Chisholm, 2011) and

severing of axons and dendrites by femtosecond laser pulses (Abay et al., 2017; Basu et al., 2017; Oren-Suissa et al., 2017) have revealed that such homeostatic repair processes often involve cell fusions.

4.1 Repair of wounded epithelia

Studies have shown that during the repair of wounded hypodermis in the adult, an actin ring is assembled to facilitate membrane closure (Chuang et al., 2016; Xu & Chisholm, 2011). This sealing process has recently been shown to involve EFF-1, in a fashion dependent on a syntaxin (SYX-2), as well as on components of the ESCRT machinery which is involved in intracellular fusion and trafficking (Meng et al., 2020).

4.2 Neuronal sculpting and repair

The repair of axons following injury in *C. elegans* has been extensively reviewed and is briefly recounted here for convenience; readers are encouraged to turn to these reviews: (Chisholm et al., 2016; Giordano-Santini et al., 2016; Soulavie & Sundaram, 2016). Briefly, axonal auto-fusion is one of the mechanisms of repair in response to injury, providing functional restoration (Abay et al., 2017; Basu et al., 2017), and is cell-autonomously mediated by EFF-1 (Ghosh-Roy & Chisholm, 2010; Ghosh-Roy, Wu, Goncharov, Jin, & Chisholm, 2010; Neumann et al., 2015) (Fig. 3A–C). The final stage of neuronal reconnection by fusion has been recently shown to involve RAB-5 (Linton et al., 2019) (Fig. 3B), with decreased RAB-5 activity causing an accumulation of EFF-1 to neuron membranes, even forming extracellular vesicles (Linton et al., 2019). This regulatory effect is similar to that described for the endocytic machinery in embryonic epidermal cells (Smurova & Podbilewicz, 2016, 2017) (see above).

In contrast to the role of *eff-1* in axonal repair, in the PVD dendrite *eff-1* and *aff-1* seemingly have different roles; auto-fusion and repair rely on AFF-1, while EFF-1 prunes excessive outgrowth yet does not influence regenerative capability (Kravtsov et al., 2017; Oren-Suissa et al., 2017) (Fig. 4A–C). In aged animals, the PVD dendritic structure becomes hyperbranched (Fig. 4F), owing to a decrease in cellular EFF-1 levels (Kravtsov et al., 2017). *aff-1* contributes to young animals' regeneration non-cell-autonomously (Oren-Suissa et al., 2017). However, intriguingly, AFF-1 restores regenerative capabilities in aged animals from within the PVD (Kravtsov et al., 2017). The *daf-2*/IGF1R-*daf-16*/FOXO pathway, which promotes longevity (Lin et al., 2001), is important in the regenerative capabilities of the dendrite, yet does

not affect its aged dendritic structure (Kravtsov et al., 2017). This phenomenon, however, may be neuron-specific: studies of other neurons suggest modifying the *daf-2*/IGF1R pathway does mitigate morphological alterations associated with aging (Tank, Rodgers, & Kenyon, 2011; Toth et al., 2012). Moreover, while aging decreases the regenerative potential of the PVD dendrite (Kravtsov et al., 2017), it conversely improves the reconnection of the PLM axon (Abay et al., 2017). It is possible that aging pathways regulate *aff-1* and *eff-1* to different degrees in different contexts—perhaps similarly to regulation by heterochronic genes during larval development. In summary, fusogens play important functions in neuronal regeneration which are influenced by aging. These activities seem to differ between axons and dendrites and between neuron types. Understanding these processes may provide insights into neuronal functional and structural repair in other animals as well.

5. Why does *C. elegans* need more than one fusogen?

5.1 Do AFF-1 and EFF-1 interact in vivo?

EFF-1 and AFF-1 are type I single-pass transmembrane proteins (Mohler et al., 2002; Sapir et al., 2008), with EFF-1 crystallizing in trimers (Pérez-Vargas et al., 2014). While the similarity in amino acid properties between the two fusogens is below 50% (Sapir et al., 2007), most of the 23% identical residues seem located on the folded protein's surface, facilitating close structural similarity (Pérez-Vargas et al., 2014). The ability of EFF-1 and AFF-1 to interact in trans (i.e., "bridging the gap" from opposing membranes) has been demonstrated in heterologous in vitro systems, including insect (Sapir et al., 2007) and mammalian cell cultures (Avinoam et al., 2011) as well as pseudoviral infection of cell culture (Avinoam et al., 2011). While these and other results open new avenues for research in finding other structurally- and functionally-conserved fusogens (see Hernández & Podbilewicz, 2017), one wonders if such cross-interaction occurs in vivo.

Repair of the PVD dendrite in young animals involves AFF-1 acting from the seam cells but not from the PVD itself (Oren-Suissa et al., 2017). Observations of AFF-1-containing vesicles budding from the seam cells led to the hypothesis that such extracellular vesicles may interact in trans with PVD-expressed EFF-1 to facilitate repair, although this was not directly demonstrated (Oren-Suissa et al., 2017). Recent results using pseudotyped virus infection of *C. elegans* indicate such interaction is feasible in vivo (Meledin et al., 2020). Conversely, multiple lines of evidence mount to

suggest this heterotypic interaction does not occur during development; notably: the adjacent pm8 and vpi1 express AFF-1 and EFF-1, respectively, which seemingly prevents their merger (Rasmussen et al., 2008) (Fig. 1D); the tail spike cells fuse via AFF-1 while hyp10, which partially engulfs them, does so via EFF-1, yet no cross-fusion was noted (Ghose et al., 2018) (Fig. 1E); lastly, hyp7 syncytium, fused by EFF-1, does not fuse with adjacent AFF-1-expressing seam cells (Sapir et al., 2007) (Fig. 2E). Perhaps the two fusogens never meet on adjacent membranes in *C. elegans*, for instance by assuming apical vs basolateral localization. Experimentally altering cell fates or signaling pathways to induce same-fusogen expression in adjacent cells may bring about ectopic fusion, as occurs when *eff-1* is aberrantly expressed in pm8, which then fuses with vpi1 (Rasmussen et al., 2008) or when two *aff-1*-expressing duct cells (one synthetically-induced) come into contact and merge (Abdus-Saboor et al., 2011). These results point to the two fusogens serving mutually exclusive roles during development (also see discussion in Sapir et al., 2007), and illustrate the tight control involved in cell-to-cell fusion in developmental and homeostatic processes. Future studies may uncover unique settings which do require cross-interaction of different fusogens, perhaps showcasing certain phenotypes which persist in both *eff-1* and *aff-1* mutants.

5.2 Is AFF-1 more precise than EFF-1?

AFF-1 tends to mediate small-scale fusions, which seem rather selected; for example, auto-fusions of a subset of the vulva rings and fusion of the anchor cell to utse (Sapir et al., 2007) (Fig. 2F). EFF-1, on the other hand, mediates vast epithelial syncytia formations such as the 139-nuclei hyp7 (Podbilewicz & White, 1994). During embryonic formation of hyp7, syncytiogenesis can begin with almost any cell pair, at equal probability, and proceeds somewhat stochastically (Gattegno et al., 2007). It may thus be tempting to define AFF-1 as "local" and EFF-1 as "global," yet EFF-1 acts in highly localized settings as well, inducing auto-fusion (e.g., in vpi; Fig. 1D (Rasmussen et al., 2008)) and phagosome sealing (in hyp10; Fig. 1E (Chiorazzi et al., 2013)). There seems to be no clear distinction between fusion of a cell into a syncytium and auto-fusion of a single cell, from a fusogen's point of view; the 138 fusion events which form hyp7 are all distinct cell-to-cell fusions. The difference in fusion behaviors may be merely the result of differential regulation and as such, EFF-1 is no less precise and no more promiscuous than AFF-1.

6. Concluding remarks

During embryonic and postembryonic development in *C. elegans*, multiple tissues form by cell-to-cell fusions. The majority of these events are mediated by two fusogens only (EFF-1 and AFF-1), which take on different roles in shaping sheets, tubes, and spikes, connecting severed fragments and pruning excess ones. Uniquely, we are now beginning to realize the same proteins may mediate events such as phagosome sealing and scission of endocytic vesicles—all in order to fulfill and maintain the invariant development which has made *C. elegans* one of the best-studied animals to date. While some fusion events in the worm remain uncharacterized, studies of fusogens have shed light on organogenesis, homeostasis, membrane repair and aging. Elucidating the regulatory pathways which govern these processes, and finding novel fusogens, will contribute to our understanding of neuronal repair, tissue morphogenesis, endocytosis, phagocytosis and fertilization in other organisms as well.

Acknowledgments

We would like to thank past and present members of the Podbilewicz lab for sharing their ideas, expertise and enthusiasm. Research in the BP lab is supported by the Israel Science Foundation (ISF grants 257/17, 2462/18, 2327/19, and 178/20). We are grateful to Dan Cassel, Amir Sapir and Ayelet Lamm for discussions and critical reading of the manuscript.

References

Abay, Z. C., Wong, M. Y.-Y., Teoh, J.-S., Vijayaraghavan, T., Hilliard, M. A., & Neumann, B. (2017). Phosphatidylserine save-me signals drive functional recovery of severed axons in *Caenorhabditis elegans*. *Proceedings of the National Academy of Sciences of the United States of America*, *114*, E10196–E10205.

Abdus-Saboor, I., Mancuso, V. P., Murray, J. I., Palozola, K., Norris, C., Hall, D. H., et al. (2011). Notch and Ras promote sequential steps of excretory tube development in *C. elegans*. *Development*, *138*, 3545–3555.

Ackley, B. D. (2014). Wnt-signaling and planar cell polarity genes regulate axon guidance along the anteroposterior axis in *C. elegans*. *Developmental Neurobiology*, *74*, 781–796.

Aguilar, P. S., Baylies, M. K., Fleissner, A., Helming, L., Inoue, N., Podbilewicz, B., et al. (2013). Genetic basis of cell-cell fusion mechanisms. *Trends in Genetics*, *29*, 427–437.

Albert, P. S., & Riddle, D. L. (1983). Developmental alterations in sensory neuroanatomy of the *Caenorhabditis elegans* dauer larva. *Journal of Comparative Neurology*, *219*, 461–481.

Albertson, D. G., & Thomson, J. N. (1976). The pharynx of *Caenorhabditis elegans*. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *275*, 299–325.

Alper, S., & Kenyon, C. (2002). The zinc finger protein REF-2 functions with the Hox genes to inhibit cell fusion in the ventral epidermis of *C. elegans*. *Development*, *129*, 3335–3348.

Alper, S., & Podbilewicz, B. (2008). Cell fusion in *Caenorhabditis elegans*. *Methods in Molecular Biology*, *475*, 53–74.

Altun, Z. F., & Hall, D. H. (2009a). In L. A. Herndon (Ed.), *Epithelial system, hypodermis* WormAtlas.

Altun, Z. F., & Hall, D. H. (2009b). In L. A. Herndon (Ed.), *Introduction* WormAtlas.

Anggono, V., & Huganir, R. L. (2012). Regulation of AMPA receptor trafficking and synaptic plasticity. *Current Opinion in Neurobiology*, *22*, 461–469.

Avinoam, O., Fridman, K., Valansi, C., Abutbul, I., Zeev-Ben-Mordehai, T., Maurer, U. E., et al. (2011). Conserved eukaryotic fusogens can fuse viral envelopes to cells. *Science*, *332*, 589–592.

Basu, A., Dey, S., Puri, D., Saha, N. D., Sabharwal, V., Thyagarajan, P., et al. (2017). let-7 miRNA controls CED-7 homotypic adhesion and EFF-1–mediated axonal self-fusion to restore touch sensation following injury. *Proceedings of the National Academy of Sciences of the United States of America*, *114*, E10206–E10215.

Berry, K. L., Bülow, H. E., Hall, D. H., & Hobert, O. (2003). A *C. elegans* CLIC-like protein required for intracellular tube formation and maintenance. *Science*, *302*, 2134–2137.

Blitzer, J. T., & Nusse, R. (2006). A critical role for endocytosis in Wnt signaling. *BMC Cell Biology*, *7*, 1–10.

Brabin, C., Appleford, P. J., & Woollard, A. (2011). The *Caenorhabditis elegans* gata factor ELT-1 works through the cell proliferation regulator BRO-1 and the fusogen EFF-1 to maintain the seam stem-like fate. *PLoS Genetics*, *7*, e1002200.

Brukman, N. G., Uygur, B., Podbilewicz, B., & Chernomordik, L. V. (2019). How cells fuse. *Journal of Cell Biology*, *218*, 1436–1451.

Brunschwig, K., Wittmann, C., Schnabel, R., Bürglin, T. R., Tobler, H., & Müller, F. (1999). Anterior organization of the *Caenorhabditis elegans* embryo by the labial-like Hox gene ceh-13. *Development*, *126*, 1537–1546.

Bucci, C., Parton, R. G., Mather, I. H., Stunnenberg, H., Simons, K., Hoflack, B., et al. (1992). The small GTPase rab5 functions as a regulatory factor in the early endocytic pathway. *Cell*, *70*, 715–728.

Buechner, M. (2002). Tubes and the single *C. elegans* excretory cell. *Trends in Cell Biology*, *12*, 479–484.

Cassata, G., Shemer, G., Morandi, P., Donhauser, R., Podbilewicz, B., & Baumeister, R. (2005). *ceh-16*/engrailed patterns the embryonic epidermis of *Caenorhabditis elegans*. *Development*, *132*, 739–749.

Chalfie, M., Sulston, J. E., White, J. G., Southgate, E., Thomson, J. N., & Brenner, S. (1985). The neural circuit for touch sensitivity in *Caenorhabditis elegans*. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *5*, 956–964.

Cheng, S., Wang, K., Zou, W., Miao, R., Huang, Y., Wang, H., et al. (2015). PtdIns(4,5)P2 and PtdIns3P coordinate to regulate phagosomal sealing for apoptotic cell clearance. *Journal of Cell Biology*, *210*, 485–502.

Chernomordik, L. V., & Kozlov, M. M. (2008). Mechanics of membrane fusion. *Nature Structural & Molecular Biology*, *15*, 675–683.

Chiorazzi, M., Rui, L., Yang, Y., Ceribelli, M., Tishbi, N., Maurer, C. W., et al. (2013). Related F-box proteins control cell death in *Caenorhabditis elegans* and human lymphoma. *Proceedings of the National Academy of Sciences of the United States of America*, *110*, 3943–3948.

Chisholm, A. D., & Hardin, J. (2005). Epidermal morphogenesis. In *The online review of C. elegans biology* (pp. 1–22). WormBook.

Chisholm, A. D., Hutter, H., Jin, Y., & Wadsworth, W. G. (2016). The genetics of axon guidance and axon regeneration in *Caenorhabditis elegans*. *Genetics*, *204*, 849–882.

Choi, J., Richards, K. L., Cinar, H. N., & Newman, A. P. (2006). N-ethylmaleimide sensitive factor is required for fusion of the *C. elegans* uterine anchor cell. *Developmental Biology*, *297*, 87–102.

Chuang, M., Hsiao, T. I., Tong, A., Xu, S., & Chisholm, A. D. (2016). DAPK interacts with Patronin and the microtubule cytoskeleton in epidermal development and wound repair. *eLife*, *5*, e15833.

Clark, S. G., Shurland, D. L., Meyerowitz, E. M., Bargmann, C. I., & Van Der Bliek, A. M. (1997). A dynamin GTPase mutation causes a rapid and reversible temperature-inducible locomotion defect in *C. elegans*. *Proceedings of the National Academy of Sciences of the United States of America*, *94*, 10438–10443.

del Campo, J. J., Opoku-Serebuoh, E., Isaacson, A. B., Scranton, V. L., Tucker, M., Han, M., et al. (2005). Fusogenic activity of EFF-1 is regulated via dynamic localization in fusing somatic cells of *C. elegans*. *Current Biology*, *15*, 413–423.

Dhanyasi, N., Segal, D., Shimoni, E., Shinder, V., Shilo, B. Z., VijayRaghavan, K., et al. (2015). Surface apposition and multiple cell contacts promote myoblast fusion in *Drosophila* flight muscles. *Journal of Cell Biology*, *211*, 191–203.

Fédry, J., Liu, Y., Péhau-Arnaudet, G., Pei, J., Li, W., Tortorici, M. A., et al. (2017). The ancient gamete fusogen HAP2 Is a eukaryotic class II fusion protein. *Cell*, *168*, 904–915.e10.

Feng, J., Dong, X., Pinello, J., Zhang, J., Lu, C., Iacob, R. E., et al. (2018). Fusion surface structure, function, and dynamics of gamete fusogen HAP2. *eLife*, *7*, e39772.

Forgac, M. (1999). Structure and properties of the vacuolar (H +)-ATPases. *Journal of Biological Chemistry*, *274*, 12951–12954.

Friedlander-Shani, L., & Podbilewicz, B. (2011). Heterochronic control of AFF-1-mediated cell-to-cell fusion in *C. elegans*. *Advances in Experimental Medicine and Biology*, *713*, 5–11.

Gattegno, T., Mittal, A., Valansi, C., Nguyen, K. C. Q., Hall, D. H., Chernomordik, L. V., et al. (2007). Genetic control of fusion pore expansion in the epidermis of *Caenorhabditis elegans*. *Molecular Biology of the Cell*, *18*, 1153–1166.

Ghose, P., Rashid, A., Insley, P., Trivedi, M., Shah, P., Singhal, A., et al. (2018). EFF-1 fusogen promotes phagosome sealing during cell process clearance in *Caenorhabditis elegans*. *Nature Cell Biology*, *20*, 393–399.

Ghosh-Roy, A., & Chisholm, A. D. (2010). *Caenorhabditis elegans*: A new model organism for studies of axon regeneration. *Developmental Dynamics*, *239*, 1460–1464.

Ghosh-Roy, A., Wu, Z., Goncharov, A., Jin, Y., & Chisholm, A. D. (2010). Calcium and cyclic AMP promote axonal regeneration in *Caenorhabditis elegans* and require DLK-1 kinase. *Journal of Neuroscience*, *30*, 3175–3183.

Gilbert, S. P. R., Mullan, T. W., Poole, R. J., & Woollard, A. (2020). Caudal-dependent cell positioning directs morphogenesis of the *C. elegans* ventral epidermis. *Developmental Biology*, *461*, 31–42.

Giordano-Santini, R., Linton, C., & Hilliard, M. A. (2016). Cell-cell fusion in the nervous system: Alternative mechanisms of development, injury, and repair. *Seminars in Cell and Developmental Biology*, *60*, 146–154.

Grant, B., & Hirsh, D. (1999). Receptor-mediated endocytosis in the *Caenorhabditis elegans* oocyte. *Molecular Biology of the Cell*, *10*, 4311–4326.

Greenwald, I. (2005). LIN-12/Notch signaling in *C. elegans*. In *The online review of C. elegans biology* (pp. 1–16). *WormBook*.

Gruenbaum-Cohen, Y., Harel, I., Umansky, K. B., Tzahor, E., Snapper, S. B., Shilo, B.-Z., et al. (2012). The actin regulator N-WASp is required for muscle-cell fusion in mice. *Proceedings of the National Academy of Sciences of the United States of America*, *109*, 11211–11216.

Hada, K., Asahina, M., Hasegawa, H., Kanaho, Y., Slack, F. J., & Niwa, R. (2010). The nuclear receptor gene nhr-25 plays multiple roles in the *Caenorhabditis elegans* heterochronic gene network to control the larva-to-adult transition. *Developmental Biology*, *344*, 1100–1109.

Hall, D. H., Herndon, L. A., & Altun, Z. F. (2017). *Introduction to C. elegans embryo anatomy*. WormAtlas.

Hammarlund, M., Jorgensen, E. M., & Bastiani, M. J. (2007). Axons break in animals lacking β-spectrin. *Journal of Cell Biology*, *176*, 269–275.

Harris, D. T., & Horvitz, H. R. (2011). MAB-10/NAB acts with LIN-29/EGR to regulate terminal differentiation and the transition from larva to adult in *C. elegans*. *Development*, *138*, 4051–4062.

Hernández, J. M., & Podbilewicz, B. (2017). The hallmarks of cell-cell fusion. *Development (Cambridge)*, *144*, 4481–4495.

Hirai, M., Arai, M., Mori, T., Miyagishima, S. Y., Kawai, S., Kita, K., et al. (2008). Male fertility of malaria parasites is determined by GCS1, a plant-type reproduction factor. *Current Biology*, *18*, 607–613.

Hubbard, E. J. A., & Greenstein, D. (2005). Introduction to the germ line. In *The online review of C. elegans biology* (pp. 1–4). WormBook.

Hurtado-Lorenzo, A., Skinner, M., El Annan, J., Futai, M., Sun-Wada, G. H., Bourgoin, S., et al. (2006). V-ATPase interacts with ARNO and Arf6 in early endosomes and regulates the protein degradative pathway. *Nature Cell Biology*, *8*, 124–136.

Inberg, S., Meledin, A., Kravtsov, V., Iosilevskii, Y., Oren-Suissa, M., & Podbilewicz, B. (2019). Lessons from worm dendritic patterning. *Annual Review of Neuroscience*, *42*, 365–383.

Inoue, N., Hamada, D., Kamikubo, H., Hirata, K., Kataoka, M., Yamamoto, M., et al. (2013). Molecular dissection of IZUMO1, a sperm protein essential for sperm-egg fusion. *Development*, *140*, 3221–3229.

Kim, S., Shilagardi, K., Zhang, S., Hong, S. N., Sens, K. L., Bo, J., et al. (2007). A critical function for the actin cytoskeleton in targeted exocytosis of prefusion vesicles during myoblast fusion. *Developmental Cell*, *12*, 571–586.

Kiontke, K., Barrière, A., Kolotuev, I., Podbilewicz, B., Sommer, R., Fitch, D. H. A., et al. (2007). Trends, stasis, and drift in the evolution of nematode vulva development. *Current Biology*, *17*, 1925–1937.

Koh, K., Bernstein, Y., & Sundaram, M. V. (2004). The nT1 translocation separates vulval regulatory elements from the *egl-18* and *elt-6* GATA factor genes. *Developmental Biology*, *267*, 252–263.

Koh, K., Peyrot, S. M., Wood, C. G., Wagmaister, J. A., Maduro, M. F., Eisenmann, D. M., et al. (2002). Cell fates and fusion in the *C. elegans* vulval primordium are regulated by the EGL-18 and ELt-6 GATA factors—Apparent direct targets of the LIN-39-Hox protein. *Development*, *129*, 5171–5180.

Kolotuev, I., & Podbilewicz, B. (2004). *Pristionchus pacificus* vulva formation: Polarized division, cell migration, cell fusion, and evolution of invagination. *Developmental Biology*, *266*, 322–333.

Kolotuev, I., & Podbilewicz, B. (2008). Changing of the cell division axes drives vulva evolution in nematodes. *Developmental Biology*, *313*, 142–154.

Kontani, K., Moskowitz, I. P. G., & Rothman, J. H. (2005). Repression of cell-cell fusion by components of the *C. elegans* vacuolar ATPase complex. *Developmental Cell*, *8*, 787–794.

Kravtsov, V., Oren-Suissa, M., & Podbilewicz, B. (2017). The fusogen AFF-1 can rejuvenate the regenerative potential of adult dendritic trees by self-fusion. *Development (Cambridge)*, *144*, 2364–2374.

Kroll, J. R., Tsiaxiras, J., & van Zon, J. S. (2020). Variability in β-catenin pulse dynamics in a stochastic cell fate decision in *C. elegans*. *Developmental Biology*, *461*, 110–123.

Leikina, E., Gamage, D. G., Prasad, V., Goykhberg, J., Crowe, M., Diao, J., et al. (2018). Myomaker and myomerger work independently to control distinct steps of membrane remodeling during myoblast fusion. *Developmental Cell*, *46*, 767–780.e7.

Leikina, E., Melikov, K., Sanyal, S., Verma, S. K., Eun, B., Gebert, C., et al. (2013). Extracellular annexins and dynamin are important for sequential steps in myoblast fusion. *Journal of Cell Biology*, *200*, 109–123.

Leys, S. P. (2003). The significance of syncytial tissues for the position of the hexactinellida in the metazoa. *Integrative and Comparative Biology*, *43*, 19–27.

Lin, K., Hsin, H., Libina, N., & Kenyon, C. (2001). Regulation of the *Caenorhabditis elegans* longevity protein DAF-16 by insulin/IGF-1 and germline signaling. *Nature Genetics*, *28*, 139–145.

Linton, C., Riyadh, M. A., Ho, X. Y., Neumann, B., Giordano-Santini, R., & Hilliard, M. A. (2019). Disruption of RAB-5 increases EFF-1 fusogen availability at the cell surface and promotes the regenerative axonal fusion capacity of the neuron. *Journal of Neuroscience*, *39*, 2823–2836.

Liu, Y., Tewari, R., Ning, J., Blagborough, A. M., Garbom, S., Pei, J., et al. (2008). The conserved plant sterility gene HAP2 functions after attachment of fusogenic membranes in *Chlamydomonas* and *Plasmodium* gametes. *Genes and Development*, *22*, 1051–1068.

Louvet-Vallée, S., Kolotuev, I., Podbilewicz, B., & Félix, M. A. (2003). Control of vulval competence and centering in the nematode *Oscheius* sp. 1 CEW1. *Genetics*, *163*, 133–146.

Marcello, M. R., Singaravelu, G., & Singson, A. (2013). Fertilization. In T. Schedl (Ed.), *Germ cell development in C. elegans* (pp. 321–350). New York, NY: Springer.

Markvoort, A. J., & Marrink, S. J. (2011). Lipid acrobatics in the membrane fusion arena. In *Current topics in membranes* Elsevier Inc.

Martin, S. G. (2016). Role and organization of the actin cytoskeleton during cell-cell fusion. *Seminars in Cell and Developmental Biology*, *60*, 121–126.

Mason, D. A., Rabinowitz, J. S., & Portman, D. S. (2008). dmd-3, a doublesex-related gene regulated by tra-1, governs sex-specific morphogenesis in *C. elegans*. *Development*, *135*, 2373–2382.

Massarwa, R., Carmon, S., Shilo, B. Z., & Schejter, E. D. (2007). WIP/WASp-based actin-polymerization machinery is essential for myoblast fusion in *Drosophila*. *Developmental Cell*, *12*, 557–569.

Meledin, A., Li, X., Matveev, E., Gildor, B., Katzir, O., & Podbilewicz, B. (2020). EFF-1 promotes muscle fusion, paralysis and retargets infection by AFF-1-coated viruses in *C. elegans*. *bioRxiv*. https://doi.org/10.1101/2020.05.17.099622.

Meng, X., Yang, Q., Yu, X., Zhou, J., Ren, X., Zhou, Y., et al. (2020). Actin polymerization and ESCRT trigger recruitment of the fusogens syntaxin-2 and EFF-1 to promote membrane repair in *C. elegans*. *Developmental Cell*, *54*, 624–638. https://doi.org/10.1016/j.devcel.2020.06.027.

Mereu, L., Morf, M. K., Spiri, S., Gutierrez, P., Escobar-Restrepo, J. M., Daube, M., et al. (2020). Polarized epidermal growth factor secretion ensures robust vulval cell fate specification in *Caenorhabditis elegans*. *Development*, *147*, dev175760.

Mohler, W. A., Shemer, G., Del Campo, J. J., Valansi, C., Opoku-Serebuoh, E., Scranton, V., et al. (2002). The type 1 membrane protein EFF-1 is essential for development cell fusion. *Developmental Cell*, *2*, 355–362.

Mori, T., Kuroiwa, H., Higashiyama, T., & Kuroiwa, T. (2006). GENERATIVE CELL SPECIFIC 1 is essential for angiosperm fertilization. *Nature Cell Biology*, *8*, 64–71.

Nelson, K. F., Albert, P. S., & Riddle, D. L. (1983). Fine structure of the *Caenorhabditis elegans* secretory-excretory system. *Journal of Ultrastructure Research*, *82*, 156–171.

Neumann, B., Coakley, S., Giordano-Santini, R., Linton, C., Lee, E. S., Nakagawa, A., et al. (2015). EFF-1-mediated regenerative axonal fusion requires components of the apoptotic pathway. *Nature*, *517*, 219–222.

Neumann, B., Nguyen, K. C. Q., Hall, D. H., Ben-Yakar, A., & Hilliard, M. A. (2011). Axonal regeneration proceeds through specific axonal fusion in transected *C. elegans* neurons. *Developmental Dynamics*, *240*, 1365–1372.

Newman, A. P., White, J. G., & Sternberg, P. W. (1996). Morphogenesis of the *C. elegans* hermaphrodite uterus. *Development*, *122*, 3617–3626.

Nguyen, C. Q., Hall, D. H., Yang, Y., & Fitch, D. H. A. (1999). Morphogenesis of the *Caenorhabditis elegans* male tail tip. *Developmental Biology*, *207*, 86–106.

Nishimura, H., & L'Hernault, S. W. (2010). Spermatogenesis-defective (*spe*) mutants of the nematode *Caenorhabditis elegans* provide clues to solve the puzzle of male germline functions during reproduction. *Developmental Dynamics*, *239*, 1502–1514.

Nishimura, H., Tajima, T., Comstra, H. S., Gleason, E. J., & L'Hernault, S. W. (2015). The immunoglobulin-like gene spe-45 acts during fertilization in *Caenorhabditis elegans* like the mouse Izumo1 gene. *Current Biology*, *25*, 3225–3231.

Önel, S.-F., Dottermusch, C., Sickmann, A., Buttgereit, D., & Renkawitz-Pohl, R. (2011). Role of the actin cytoskeleton within FuRMAS during *Drosophila* myoblast fusion and first functionally conserved factors in vertebrates. In L.-I. Larsson (Ed.), *Cell fusions* (pp. 139–170). Netherlands, Dordrecht: Springer.

Önel, S. F., & Renkawitz-Pohl, R. (2009). FuRMAS: Triggering myoblast fusion in *Drosophila*. *Developmental Dynamics*, *238*, 1513–1525.

Oren-Suissa, M., Gattegno, T., Kravtsov, V., & Podbilewicz, B. (2017). Extrinsic repair of injured dendrites as a paradigm for regeneration by fusion in *Caenorhabditis elegans*. *Genetics*, *206*, 215–230.

Oren-Suissa, M., Hall, D. H., Treinin, M., Shemer, G., & Podbilewicz, B. (2010). The fusogen EFF-1 controls sculpting of mechanosensory dendrites. *Science*, *328*, 1285–1288.

Palade, G. (1975). Intracellular aspects of the process of protein synthesis. *Science (New York, N.Y.)*, *189*, 347–358.

Patel, F. B., Bernadskaya, Y. Y., Chen, E., Jobanputra, A., Pooladi, Z., Freeman, K. L., et al. (2008). The WAVE/SCAR complex promotes polarized cell movements and actin enrichment in epithelia during *C. elegans* embryogenesis. *Developmental Biology*, *324*, 297–309.

Paululat, A., Holz, A., & Renkawitz-Pohl, R. (1999). Essential genes for myoblast fusion in *Drosophila* embryogenesis. *Mechanisms of Development*, *83*, 17–26.

Pellegrino, M. W., Farooqui, S., Fröhli, E., Rehrauer, H., Kaeser-Pebernard, S., Müller, F., et al. (2011). LIN-39 and the EGFR/RAS/MAPK pathway regulate *C. elegans* vulval morphogenesis via the VAB-23 zinc finger protein. *Development*, *138*, 4649–4660.

Pénigault, J. B., & Félix, M. A. (2011). High sensitivity of *C. elegans* vulval precursor cells to the dose of posterior Wnts. *Developmental Biology*, *357*, 428–438.

Pérez-Vargas, J., Krey, T., Valansi, C., Avinoam, O., Haouz, A., Jamin, M., et al. (2014). Structural basis of eukaryotic cell-cell fusion. *Cell*, *157*, 407–419.

Petrany, M. J., & Millay, D. P. (2019). Cell fusion: Merging membranes and making muscle. *Trends in Cell Biology*, *29*, 964–973.

Pitulescu, M. E., & Adams, R. H. (2014). Regulation of signaling interactions and receptor endocytosis in growing blood vessels. *Cell Adhesion & Migration*, *8*, 366–377.

Podbilewicz, B. (2006). Cell fusion. In *The online review of C. elegans biology* (pp. 1–32). WormBook.

Podbilewicz, B. (2014). Virus and cell fusion mechanisms. *Annual Review of Cell and Developmental Biology*, *30*, 111–139.

Podbilewicz, B., & Chernomordik, L. V. (2006). Cell fusion in development and disease. In L. K. Tamm (Ed.), *Protein-lipid interactions: From membrane domains to cellular networks* (pp. 219–244). WILEY-VCH Verlag GmbH & Co.

Podbilewicz, B., Leikina, E., Sapir, A., Valansi, C., Suissa, M., Shemer, G., et al. (2006). The *C. elegans* developmental fusogen EFF-1 mediates homotypic fusion in heterologous cells and in vivo. *Developmental Cell*, *11*, 471–481.

Podbilewicz, B., & White, J. G. (1994). Cell fusions in the developing epithelia of *C. elegans*. *Developmental Biology*, *161*, 408–424.

Procko, C., Lu, Y., & Shaham, S. (2011). Glia delimit shape changes of sensory neuron receptive endings in *C. elegans*. *Development*, *138*, 1371–1381.

Rabinowitch, I., Chatzigeorgiou, M., Zhao, B., Treinin, M., & Schafer, W. R. (2014). Rewiring neural circuits by the insertion of ectopic electrical synapses in transgenic *C. elegans*. *Nature Communications*, *5*, 1–6.

Raiders, S. A., Eastwood, M. D., Bacher, M., & Priess, J. R. (2018). Binucleate germ cells in *Caenorhabditis elegans* are removed by physiological apoptosis. *PLoS Genetics*, *14*, e1007417.

Rasmussen, J. P., English, K., Tenlen, J. R., & Priess, J. R. (2008). Notch signaling and morphogenesis of single-cell tubes in the *C. elegans* digestive tract. *Developmental Cell*, *14*, 559–569.

Richardson, B. E., Beckett, K., Nowak, S. J., & Baylies, M. K. (2007). SCAR/WAVE and Arp2/3 are crucial for cytoskeletal remodeling at the site of myoblast fusion. *Development*, *134*, 4357–4367.

Riddle, D. L. (1988). The dauer larva. In W. B. Wood (Ed.), *The nematode C. elegans* (pp. 393–412). New York: Cold Spring Harbor Laboratory Press.

Roiz, D., Escobar-Restrepo, J. M., Leu, P., & Hajnal, A. (2016). The *C. elegans* hox gene lin-39 controls cell cycle progression during vulval development. *Developmental Biology*, *418*, 124–134.

Rothman, J. E. (2014). The principle of membrane fusion in the cell (Nobel Lecture). *Angewandte Chemie (International Ed. in English)*, *53*, 12676–12694.

Sapir, A., Avinoam, O., Podbilewicz, B., & Chernomordik, L. V. (2008). Viral and developmental cell fusion mechanisms: Conservation and divergence. *Developmental Cell*, *14*, 11–21.

Sapir, A., Choi, J., Leikina, E., Avinoam, O., Valansi, C., Chernomordik, L. V., et al. (2007). AFF-1, a FOS-1-regulated fusogen, mediates fusion of the anchor cell in *C. elegans*. *Developmental Cell*, *12*, 683–698.

Schäfer, G., Weber, S., Holz, A., Bogdan, S., Schumacher, S., Müller, A., et al. (2007). The Wiskott-Aldrich syndrome protein (WASP) is essential for myoblast fusion in *Drosophila*. *Developmental Biology*, *304*, 664–674.

Schindler, A. J., & Sherwood, D. R. (2013). Morphogenesis of the *Caenorhabditis elegans* vulva. *Wiley Interdisciplinary Reviews: Developmental Biology*, *2*, 75–95.

Schmid, T., & Hajnal, A. (2015). Signal transduction during *C. elegans* vulval development: A NeverEnding story. *Current Opinion in Genetics and Development*, *32*, 1–9.

Scholpp, S., & Brand, M. (2004). Endocytosis controls spreading and effective signaling range of Fgf8 protein. *Current Biology*, *14*, 1834–1841.

Segev, N., Avinoam, O., & Podbilewicz, B. (2018). Fusogens. *Current Biology*, *28*, R378–R380.

Sharma-Kishore, R., White, J. G., Southgate, E., & Podbilewicz, B. (1999). Formation of the vulva in *Caenorhabditis elegans*: A paradigm for organogenesis. *Development*, *126*, 691–699.

Shemer, G., & Podbilewicz, B. (2002). LIN-39/Hox triggers cell division and represses EFF-1/fusogen-dependent vulval cell fusion. *Genes and Development*, *16*, 3136–3141.

Shemer, G., & Podbilewicz, B. (2003). The story of cell fusion: Big lessons from little worms. *BioEssays*, *25*, 672–682.

Shemer, G., Suissa, M., Kolotuev, I., Nguyen, K. C. Q., Hall, D. H., & Podbilewicz, B. (2004). EFF-1 is sufficient to initiate and execute tissue-specific cell fusion in *C. elegans*. *Current Biology*, *14*, 1587–1591.

Shilagardi, K., Li, S., Luo, F., Marikar, F., Duan, R., Jin, P., et al. (2013). Actin-propelled invasive membrane protrusions promote fusogenic protein engagement during cell-cell fusion. *Science*, *340*, 359–363.

Shin, N. Y., Choi, H., Neff, L., Wu, Y., Saito, H., Ferguson, S. M., et al. (2014). Dynamin and endocytosis are required for the fusion of osteoclasts and myoblasts. *Journal of Cell Biology*, *207*, 73–89.

Shinn-Thomas, J. H., Del Campo, J. J., Wang, J., & Mohler, W. A. (2016). The EFF-1A cytoplasmic domain influences hypodermal cell fusions in *C. elegans* but is not dependent on 14–3-3 proteins. *PLoS One*, *11*, 1–23.

Shinn-Thomas, J. H., & Mohler, W. A. (2011). New insights into the mechanisms and roles of cell-cell fusion. In *International review of cell and molecular biology* (1st ed.). Elsevier Inc.

Šilhánková, M., Jindra, M., & Asahina, M. (2005). Nuclear receptor NHR-25 is required for cell-shape dynamics during epidermal differentiation in *Caenorhabditis elegans*. *Journal of Cell Science*, *118*, 223–232.

Smith, C. J., Watson, J. D., Spencer, W. C., O'Brien, T., Cha, B., Albeg, A., et al. (2010). Time-lapse imaging and cell-specific expression profiling reveal dynamic branching and molecular determinants of a multi-dendritic nociceptor in *C. elegans*. *Developmental Biology*, *345*, 18–33.

Smurova, K., & Podbilewicz, B. (2016). RAB-5- and DYNAMIN-1-mediated endocytosis of EFF-fusogen controls cell-cell fusion. *Cell Reports*, *14*, 1517–1527.

Smurova, K., & Podbilewicz, B. (2017). Endocytosis regulates membrane localization and function of the fusogen EFF-1. *Small GTPases*, *8*, 177–180.

Soulavie, F., Hall, D. H., & Sundaram, M. V. (2018). The AFF-1 exoplasmic fusogen is required for endocytic scission and seamless tube elongation. *Nature Communications*, *9*, 1–11, 1741. https://doi.org/10.1038/s41467-018-04091-1.

Soulavie, F., & Sundaram, M. V. (2016). Auto-fusion and the shaping of neurons and tubes. *Seminars in Cell and Developmental Biology*, *60*, 136–145.

Sternberg, P. W. (2005). Vulval development. In *The online review of C. elegans biology* (pp. 1–28). WormBook.

Stone, C. E., Hall, D. H., & Sundaram, M. V. (2009). Lipocalin signaling controls unicellular tube development in the *Caenorhabditis elegans* excretory system. *Developmental Biology*, *329*, 201–211.

Sulston, J. E., & Horvitz, H. R. (1977). Post-embryonic cell lineages of the nematode, *Caenorhabditis elegans*. *Developmental Biology*, *56*, 110–156.

Sulston, J. E., Schierenberg, E., White, J. G., & Thomson, J. N. (1983). The embryonic cell lineage of the nematode *Caenorhabditis elegans*. *Developmental Biology*, *100*, 64–119.

Tank, E. M. H., Rodgers, K. E., & Kenyon, C. (2011). Spontaneous age-related neurite branching in *Caenorhabditis elegans*. *Journal of Neuroscience*, *31*, 9279–9288.

Toth, M. L., Melentijevic, I., Shah, L., Bhatia, A., Lu, K., Talwar, A., et al. (2012). Neurite sprouting and synapse deterioration in the aging *Caenorhabditis elegans* nervous system. *Journal of Neuroscience*, *32*, 8778–8790.

Valansi, C., Moi, D., Leikina, E., Matveev, E., Graña, M., Chernomordik, L. V., et al. (2017). Arabidopsis HAP2/GCS1 is a gamete fusion protein homologous to somatic and viral fusogens. *Journal of Cell Biology*, *216*, 1–11.

Vance, T. D. R., & Lee, J. E. (2020). Virus and eukaryote fusogen superfamilies. *Current Biology*, *30*, R750–R754.

Verma, S. K., Leikina, E., Melikov, K., & Chernomordik, L. V. (2014). Late stages of the synchronized macrophage fusion in osteoclast formation depend on dynamin. *Biochemical Journal*, *464*, 293–300.

von Besser, K., Frank, A. C., Johnson, M. A., & Preuss, D. (2006). Arabidopsis HAP2 (GCS1) is a sperm-specific gene required for pollen tube guidance and fertilization. *Development*, *133*, 4761–4769.

Weinstein, N., & Mendoza, L. (2013). A network model for the specification of vulval precursor cells and cell fusion control in *Caenorhabditis elegans*. *Frontiers in Genetics*, *4*, 1–24.

Weinstein, N., & Podbilewicz, B. (2016). Organogenesis of the *C. elegans* vulva and control of cell fusion. In *Organogenetic gene networks* (pp. 9–56). Springer International Publishing.

White, J. M. (1992). Membrane fusion. *Science*, *258*, 917–924.

White, J. G., Southgate, E., Thomson, J. N., & Brenner, S. (1986). The structure of the nervous system of the nematode *Caenorhabditis elegans*. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *314*, 1–340.

Wickner, W., & Schekman, R. (2008). Membrane fusion. *Nature Structural & Molecular Biology*, *5*, 658–664.

Wilson, K. L., Fitch, K. R., Bafus, B. T., & Wakimoto, B. T. (2006). Sperm plasma membrane breakdown during *Drosophila* fertilization requires Sneaky, an acrosomal membrane protein. *Development*, *133*, 4871–4879.

Wilson, L. D., Obakpolor, O. A., Jones, A. M., Richie, A. L., Mieczkowski, B. D., Fall, G. T., et al. (2018). The *Caenorhabditis elegans spe-49* gene is required for fertilization and encodes a sperm-specific transmembrane protein homologous to SPE-42. *Molecular Reproduction and Development*, *85*, 563–578.

Wood, W. B. (1988). Embryology. In W. B. Wood (Ed.), *The nematode C. elegans* (pp. 215–241). New York: Cold Spring Harbor Laboratory Press.

Wu, Z., Ghosh-Roy, A., Yanik, M. F., Zhang, J. Z., Jin, Y., & Chisholm, A. D. (2007). *Caenorhabditis elegans* neuronal regeneration is influenced by life stage, ephrin signaling, and synaptic branching. *Proceedings of the National Academy of Sciences of the United States of America*, *104*, 15132–15137.

Xiong, H., Mohler, W. A., & Soto, M. C. (2011). The branched actin nucleator Arp2/3 promotes nuclear migrations and cell polarity in the *C. elegans* zygote. *Developmental Biology*, *357*, 356–369.

Xu, S., & Chisholm, A. D. (2011). A Gαq-Ca2 + signaling pathway promotes actin-mediated epidermal wound closure in *C. elegans*. *Current Biology*, *21*, 1960–1967.

Yagi, M., Miyamoto, T., Sawatani, Y., Iwamoto, K., Hosogane, N., Fujita, N., et al. (2005). DC-STAMP is essential for cell-cell fusion in osteoclasts and foreign body giant cells. *Journal of Experimental Medicine*, *202*, 345–351.

Yang, Q., Roiz, D., Mereu, L., Daube, M., & Hajnal, A. (2017). The invading anchor cell induces lateral membrane constriction during vulval lumen morphogenesis in *C. elegans*. *Developmental Cell*, *42*, 271–285.e3.

Yang, Y., Zhang, Y., Li, W. J., Jiang, Y., Zhu, Z., Hu, H., et al. (2017). Spectraplakin induces positive feedback between fusogens and the actin cytoskeleton to promote cell-cell fusion. *Developmental Cell*, *41*, 107–120. e4.

Yanik, M., Cinar, H., Cinar, H. N., Chisholm, A., Jin, Y., & Ben-Yakar, A. (2004). Functional regeneration after laser axotomy. *Nature*, *432*, 822.

Yanik, M. F., Cinar, H., Cinar, H. N., Gibby, A., Chisholm, A. D., & Jin, Y. (2006). Nerve regeneration in *Caenorhabditis elegans* after femtosecond laser axotomy. *IEEE Journal on Selected Topics in Quantum Electronics*, *12*, 1283–1290.

Yochem, J., Gu, T., & Han, M. (1998). A new marker for mosaic analysis in *Caenorhabditis elegans* indicates a fusion between hyp6 and hyp7, two major components of the hypodermis. *Genetics*, *149*, 1323–1334.

Zhang, R., Lee, D. M., Jimah, J. R., Gerassimov, N., Yang, C., Kim, S., et al. (2020). Dynamin regulates the dynamics and mechanical strength of the actin cytoskeleton as a multifilament actin-bundling protein. *Nature Cell Biology*, *22*, 674–688.

Zhang, Y., Yang, Y., Zhu, Z., & Ou, G. (2017). WASP-Arp2/3-dependent actin polymerization influences fusogen localization during cell-cell fusion in *Caenorhabditis elegans* embryos. *Biology Open*, *6*, 1324–1328.

Zhu, T., Liang, X., Wang, X.-M., & Shen, K. (2017). Dynein and EFF-1 control dendrite morphology by regulating the localization pattern of SAX-7 in epidermal cells. *Journal of Cell Science*, *130*, 4063–4071.