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Evolution of Programmed Cell Fusion: Common Mechanisms and Distinct Functions

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Eukaryotic cells have evolved diverged mechanisms to merge cells. Here, we discuss three types of cell fusion: (1) Non-self-fusion, cells with different genetic contents fuse to start a new organism and fusion between enveloped viruses and host cells; (2) Self-fusion, genetically identical cells fuse to form a multinucleated cell; and (3) Auto-fusion, a single cell fuses with itself by bringing specialized cell membrane domains into contact and transforming itself into a ring-shaped cell. This is a new type of selfish fusion discovered in *C. elegans*. We divide cell fusion into three stages: (1) Specification of the cell-fusion fate; (2) Cell attraction, attachment, and recognition; (3) Execution of plasma membrane fusion, cytoplasmic mixing and cytoskeletal rearrangements. We analyze cell fusion in diverse biological systems in development and disease emphasizing the mechanistic contributions of *C. elegans* to the understanding of programmed cell fusion, a genetically encoded pathway to merge specific cells. *Developmental Dynamics 239:1515–1528, 2010*.

Key words: cell fusion; non-self fusion; self-fusion; auto-fusion; sperm; egg; osteoclast; macrophage; placenta; epithelia; muscles; vulva; eye lens; tumor; stem cells; enveloped viruses; hybridoma; hybrid; syncytia; hemifusion; *Caenorhabditis elegans*; *Plasmodium*; *Chlamydomonas*; *Neurospora crassa*; *Drosophila*; yeast; sponges; plants; EFF-1; AFF-1; Syncytins; Prm1; CD9; IgSF; Izumo; SPE-9; SPE-38; SPE-41; SPE-42; EGG-1; EGG-2; HAP2/GCS1; FAST; Hox; FOS-1; Notch; Ras; Wnt; SCAR/WAVE; WASp; Actin; LIM2; MT1-MMP

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The only living units that seem to have no sense of privacy at all are the eukaryotic cells that have been detached from the parent organism and isolated in a laboratory dish. Given the opportunity, under the right conditions, two cells from widely different sources, a yeast cell, say, and a chicken erythrocyte, will touch, fuse, and the two nuclei will then fuse as well, and the new hybrid cell will now divide into monstrous progeny. Naked cells, lacking self-respect, do not seem to have any sense of self.

— Lewis Thomas, *The Medusa and the Snail* (Thomas, 1977)

INTRODUCTION

This review focuses on recent advances in the emerging field of cell fusion. From the beginning of the 21st century, there has been a revival of cell–cell-fusion research (Xiang et al., 2002; Abmayr et al., 2003; Blumenthal et al., 2003; Glass et al., 2004; Stein et al., 2004; Podbilewicz and Chernomordik, 2005; Vignery, 2005; Podbilewicz, 2006; Srinivas et al., 2007; Alper and Podbilewicz, 2008). Here, we will summarize some of the highlights of cell-fusion research with a special emphasis on work on the mechanisms and functions of cell fusion in *C. elegans* and other organisms including fungi, plants, sponges, flies, and mammals.

WHAT IS CELL FUSION?

Cell fusion is the process by which cells unite their membranes and cytoplasm to form a new organism via fertilization or mating. Cell fusion can also result in the formation of multinucleate cells (syncytia) with different shapes and functions, such as interconnected hyphae, muscle fibers, or

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giant macrophages (Shemer and Podbilewicz, 2003; Vignery, 2005; Chen et al., 2007; Oren-Suissa and Podbilewicz, 2007; Primakoff and Myles, 2007; Sapir et al., 2008; Read et al., 2009). Intracellular fusion of vesicles and tubes is a separate process, in which the cytoplasm and plasma membranes of individual cells do not merge. "Tissue fusion" is another process, different from cell fusion, in which multiple cells rearrange to change the shape of tissues such as in gastrulation, neurulation, trachea formation, and epithelial-mesenchymal transitions (Perez-Pomares and Foty, 2006). In tissue fusion, the membranes and cytoplasm of cells remain unmixed and no cell–cell fusion occurs.

WHAT ARE THE STAGES OF CELL FUSION?

Figure 1 summarizes the universal stages of cell fusion. We hypothesize that the stages of determination, specification, and differentiation of the cells that will fuse (stage I), as well as chemotaxis, cell migration, formation of tight adhesion complexes, changes in cell shape and signal transduction (stage II), are probably shared with other cellular processes. In contrast, the mechanisms that merge the membranes and that directly transform two cells into one are unique to cell membrane fusion pathways and, therefore, of special interest to cell and developmental biologists. This review will cover recent progress made in identifying key players of cell fusion (stage III).

WHAT ARE THE FUNCTIONS OF FUSED CELLS (SYNCYTIA)?

The fused cell can become a stable, permanent, and fully differentiated hybrid cell or it can form a new multicellular organism by cell proliferation, specification, and differentiation. Fused cells have functions in immunity, transport, locomotion, reproduction, growth, feeding, osmoregulation, sensory perception, and others. It was postulated that fusion drives organ formation and fused cells have useful shapes that function efficiently, forming stable structures such as rings or toroids (Podbilewicz, 2000; Shemer and Podbilewicz, 2000). Analyses of mutants that specifically block cell fusion have shown that cell fusion is genetically programmed and is required to determine cellular shapes and organ structures (Mohler et al., 2002; Shemer and Podbilewicz, 2002; Cassata et al., 2005; Podbilewicz and Chernomordik, 2005; Podbilewicz, 2006; Podbilewicz et al., 2006; Gattegno et al., 2007; Margalit et al., 2007; Sapir et al., 2007; Alper and Podbilewicz, 2008).

HOW DO CELLS FUSE?

Researchers have identified new paradigms of cell fusion based on what we have learned from enveloped viruses and intracellular membrane fusion processes. Different classes of enveloped viruses fuse to their target cells and can also fuse cells expressing their specialized membrane glycoproteins that induce membrane fusion (fusogens; for reviews see Chernomordik and Kozlov, 2005; Martens and McMahon, 2008; Sapir et al., 2008). Stage III of the cell-fusion process (Fig. 1) summarizes the current working model of how FF proteins (EFF-1 and AFF-1) from *C. elegans* fuse cell membranes resulting in cytoplasmic mixing and reorganization of the cytoskeleton (see Somatic Cell–Cell Fusion in *C. elegans* section).

WHAT ARE THE TYPES OF CELL FUSION KNOWN IN NATURE?

Figure 2 summarizes three types of cell fusion processes that have been found in different organisms. We will describe the three general classes of cell fusion and will give some examples in different biological systems.

NON-SELF FUSION (TYPE I)

Examples of this type of cell–cell fusion include most types of sex from yeast to humans, with the notable exception of conjugation in bacteria. It was hypothesized by Tatum and Lederberg that bacterial conjugation will involve cell fusion "we postulate a sexual phase in this strain of *E. coli*: a cell fusion which allows the segregation of genes in new combinations into a single cell" (Tatum and Lederberg, 1947). However, it has been established that type IV secretion and the formation of a proteinaceous tube connecting conjugating bacteria is the mechanism responsible for conjugation in *E. coli* (Lawley et al., 2003). Sexual fusion in protists, fungi, plants, and animals is a heterotypic cell–cell fusion process in which cells of different sexes or mating types unite their membranes to share and combine their genetic materials. Currently, there are a number of systems in which non-self cell-fusion pathways have been studied and different components required for sexual fusion have been identified (Oren-Suissa and Podbilewicz, 2007).

Yeast Mating

In budding yeast haploid cells following meiosis have two mating types: a and α.

Pheromone detection activates a cascade of events that lead to chemoattraction of the two mating types, changes in cell shape known as shmooing, signaling, cell wall degradation, and attachment (White and Rose, 2001). Genetic pathways have been studied that participate in these processes in stages I and II (Fig. 1). Regarding merger of the membranes, there are a few genes that affect this process (stage III) but none of them have been identified as fusogens causing a complete and specific fusion of the membranes. Prm1, a multispan membrane protein has been suggested to play a role in the yeast-fusion process (Heiman and Walter, 2000; Jin et al., 2004, 2008; Aguilar et al., 2007; Heiman et al., 2007). Prm1 is not absolutely essential for cell fusion, since a mutation in both mating types results in partial fusion failure. Recently, structural analysis revealed evolutionary conserved cysteins in the extracellular loops, which crosslink the Prm1 homodimer and are essential for the activity of Prm1 in cell fusion (Olmo and Grote, 2010). It is surprising that the awesome power of yeast genetics has not been able to identify non-self cell fusogens essential and sufficient to fuse cells.

Mammalian Fertilization

The fertilization process in mammals includes gamete migration, structural

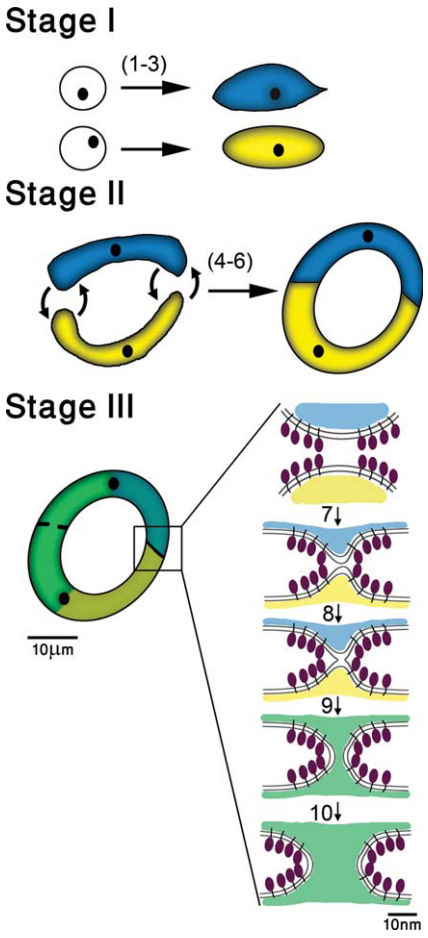

Fig. 1. Three universal stages in the cell-fusion pathway. The genetic and cellular process of cell fusion can be divided into three stages and each of these stages can be subdivided into additional steps. Stage I: Specification of the fusion fate. (1) Cells acquire specific fates. (2) Selected cells execute their fates and in some cases proliferate. (3) Cells undergo a differentiation program. Stage II: Cell attraction, attachment, and recognition. (4) Cells destined to fuse find partners using migration mechanisms. (5) Membrane microdomains interact and form cell-adhesion complexes. (6) Cell signaling result in close recognition. Stage III: Execution of cell membrane and cytoplasmic fusion. (7) Fusion-inducing proteins (FF proteins) localize to the cell membranes. (8) Fusogens bend membranes and drive hemifusion of the outer membranes. (9) Inner leaflets fuse, forming pores that expand and cytoplasm mix. (10) The cytoplasmic contents (e.g., cytoskeleton) rearrange into a syncytium.

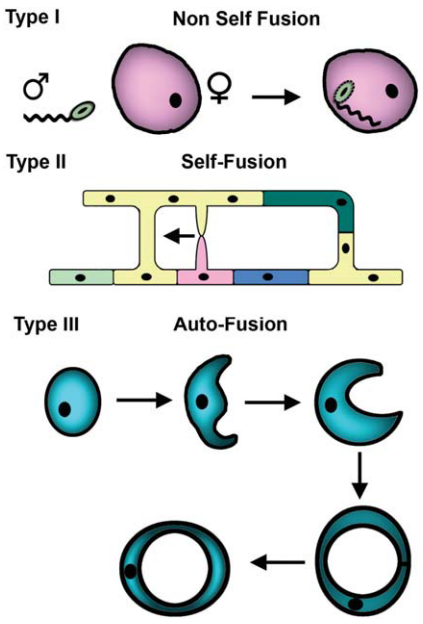

Fig. 2. Three types of cell fusion. Type I: Non-self-fusion. Two cells with different genetic information fuse during fertilization or mating in sexually reproducing organisms. Following this type I fusion, the new organism starts developing. Type II: Self-fusion. Genetically identical cells fuse within organisms as diverse as fungi, nematodes, and humans to form somatic tissues such as muscles, bones, placenta, lens of the eye, and hymen of a worm. Type III: Auto-fusion. A single selfish cell fuses with itself to form toroidal-shaped cells that create tubes. Three examples have been recently discovered in the digestive, reproductive, and excretory systems of *C. elegans*.

changes, adhesion, and fusion (Wassarman et al., 2001). For the zygote to form, the acrosomal membrane must fuse with the sperm membrane (exocytosis), the sperm must fuse with the egg, and the cortical granules must fuse with fertilized egg's membrane (exocytosis; Wassarman and Litscher, 2008). Using genetic approaches, two membrane proteins have been shown to be essential for sperm-egg fusion, Izumo in the sperm and CD9 in the egg. CD9 is a member of the tetraspanin super family of transmembrane proteins (Hemler, 2003). CD9 is required for fertilization, as CD9 knockout mice show severely reduced fertility (Kaji et al., 2000; Le Naour et al., 2000; Miyado et al., 2000). Several functions for CD9 in gamete fusion have been proposed, including (1) organization of membrane domains through cis or lateral interactions, to form the tetraspanin web (Primakoff and Myles, 2007; Lefèvre et al., 2010), (2) remodeling of the egg surface curvature (Runge et al., 2007), and (3) transfer of CD9 from the egg to the spermatozoon, suggesting a surprising model in which CD9 is also involved in membrane remodeling of the sperm (Barraud-Lange et al., 2007; Miyado et al., 2008). In addition, it was proposed that CD9-containing secreted vesicles (exosomes) have a fusion-facilitating activity, and sperm is competent for

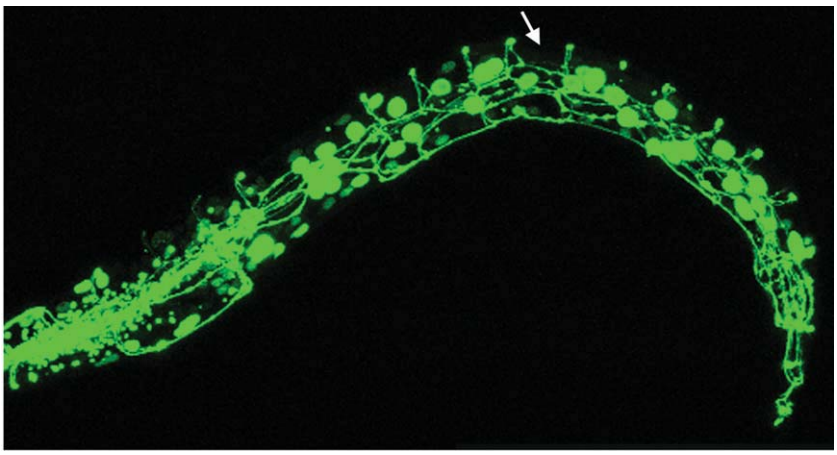

Fig. 3. EFF-1 is required in both fusing cells (genetic mosaic analysis). Image of a L1 stage *eff-1(–)* mutant larva expressing EFF-1 wild-type in cells containing the *sur-5::GFP* reporter (green nuclei). The *eff-1*, *sur-5::GFP* extrachromosomal array is lost in some lineages and mosaics are generated. For example, the arrow points to a cell in the dorsal hyp7 where the nucleus is not green and it failed to fuse. Green junctions are labeled with AJM-1::GFP expressed in the junctions between epithelial cells. Fusion is revealed by the loss of GFP fluorescence between two epithelial cells. Analyses of several genetic mosaics showed that EFF-1 is required in both cells for them to fuse (Shemer et al., 2004).

fusion only after association with these vesicles. However, this result has been challenged by recent studies that were unable to reproduce some of the original results (Gupta et al., 2009; Lefèvre et al., 2010). Thus, CD9 might be involved in various aspects of the fusion process, through overall organization of the plasma membrane, or through an involvement in a signaling pathway controlling sperm-egg fusion.

Izumo is a sperm-specific Immunoglobulin Super Family (IgSF) membrane protein that localizes to the sperm plasma membrane after the acrosomal reaction. Using knockout mice, Izumo was shown to be essential for fertilization, and antibodies against human Izumo inhibit the fusion reaction in a xenospecies assay (Inoue et al., 2005). Recently, three proteins with homology in their N-terminus to Izumo were identified, defining a new protein family named "Izumo." Izumo was renamed Izumo 1 and was shown to form large complexes, associating with other sperm proteins (Ellerman et al., 2009). Thus, like CD9, Izumo might be involved in the organization or stabilization of protein complexes essential for the fusion reaction.

Sperm-Egg Fusion in *C. elegans*

In the self-fertilizing *C. elegans* hermaphrodites, fertilization occurs after the mature oocytes pass through the sperm-reservoir, the spermatheca, and come into contact with the sperm (Yamamoto et al., 2006; Singson et al., 2008; L'Hernault, 2009). Although *C. elegans* reproductive system differs from the mammalian one; the fundamental reproduction steps are similar: migration, adhesion, and gamete fusion (Fig. 1). *C. elegans* as a model to study fertilization has proven useful for isolating many mutants specifically affecting the three stages in the process of fertilization (Fig. 1), either through the sperm (spermatogenesis-defective genes, or *spe* genes) or through the oocyte. Most of these genes were isolated using forward genetic approaches, such as a group of spermatogenesis-defective genes encoding membrane proteins SPE-9, SPE-38, SPE-41/TRP-3, and SPE-42 (Singson et al., 1998; Chatterjee et al., 2005; Kroft et al., 2005). In these mutants, sperm are fully motile and able to migrate correctly to the site of fertilization, but are incapable of fertilizing the mature oocytes (Stage III). SPE-42 shares homology with the mammalian DC-STAMP protein, which is required for osteoclast and macrophage fusion (Yagi et al., 2005). SPE-42 is a predicted seven-pass transmembrane protein that has a DC-STAMP-like motif (Kroft et al., 2005). It will be interesting to learn whether the two homologues have similar functions in cell fusion.

Reverse genetic screens identified the mutually redundant membrane proteins EGG-1 and EGG-2, the first discovered egg components that appear to be required for fertilization (Kadandale et al., 2005). *egg-1egg-2* double mutant hermaphrodites are completely sterile and wild-type sperm contact the mutant egg, but cannot fertilize it (Kadandale et al., 2005). It is not clear what is the specific step in fertilization in which egg-1/2 act. Preliminary results suggest that EGG-1 and SPE-9 do not bind one another (Singson et al., 2008), but it will be interesting to identify possible interactions between egg and sperm membrane proteins.

Double Fertilization in Plants

In flowering plants (angiosperms) and in some gymnosperms, fertilization is fascinating because one non-self cell–cell fusion event is not enough. There are two independent but related fertilization events. One sperm fuses with an egg to form a zygote, and a second sperm fuses with a central cell to form the endosperm. Different genes required for different stages in the process of double fertilization in plants have been identified. One of them, *HAP2/GCS1*, is required for pollen tube guidance and fertilization (von Besser et al., 2006). *HAP2/GCS1* encodes a type-I membrane protein required in the sperm for fertilization. In *hap2/gcs1* Arabidopsis mutants, the sperm are deposited normally in the female gametophyte by a male pollen tube but both fertilization events fail and ovule targeting is reduced by twofold (von Besser et al., 2006). *HAP2/GCS1* related genes have been found in some, but not all, animals, plants, and protists (Steele and Dana, 2009). In two types of protists, this gene has been found to be required for fertilization (see below).

Fertilization in Protists (*Chlamydomonas* and *Plasmodium*)

While protists do not have double fertilization, they do have sex and both *Chlamydomonas* and *Plasmodium* have male gametes that have been found to require HAP2/GCS1 for mating (Hirai et al., 2008; Liu et al., 2008; Hirai and Mori, 2009). Mutations in this gene result in failure in mating and the male gamete requires HAP2/GCS1 for post-binding events during stage III of non-self cell fusion. HAP2 in *Plasmodium* may become a good target to prevent malaria (Blagborough and Sinden, 2009).

Enveloped Virus-Cell Fusion

Enveloped viruses from many different families must fuse their membranes to host cells or to the membranes of intracellular organelles to release their genome into the cytoplasm of the target cell and initiate infection (Earp et al., 2005; Kielian and Rey, 2006; Miyauchi et al., 2009a). The current paradigm of viral protein-mediated membrane fusion involves dramatic conformational changes in complexes of metastable fusion proteins. These changes in the structure of viral fusogens supply the forces necessary to overcome the energetic barriers preventing spontaneous fusion of biological membrane. This paradigm is largely based on structural and functional studies on membrane fusion mediated by enveloped virus spike glycoproteins (Earp et al., 2005; Kielian and Rey, 2006; Sapir et al., 2008; Miyauchi et al., 2009b). Based on the structure and conformational changes of these viral fusogens, the fusion pathways have been studied and three structural and functional classes of viral fusogens are known (Sapir et al., 2008). In all three classes of viral fusogens, the proteins that fuse the membranes are present in the viral membrane and not on the target membrane (Chernomordik and Kozlov, 2008). Usually, there are receptors on the target membrane that interact directly with the fusogen(s) or with associated viral proteins. The

trigger for virus-cell fusion can be low pH, processing of the fusogen precursor, binding to a receptor, or a combination of two or more of these events. Following the trigger, a conformational change in the structure of the fusogens deforms or bends the membranes and causes an approach of the membranes to distances closer than 10 nm. Often fusion peptides or loops participate in the approach of the membranes by inserting into and deforming the target membranes. A refolding or hairpin mechanism results in the approach of the membranes and the mixing of the outer leaflets via stalk and hemifusion intermediates. The lipid–protein interplay culminates in the formation of lipidic pores that expand probably with the help of oligomerization of the fusion proteins. This working model has variations according to the fusogens involved and has been extensively reviewed in the literature (Blumenthal et al., 2003; Chernomordik and Kozlov, 2005, 2008; Earp et al., 2005; Podbilewicz and Chernomordik, 2005; Kielian and Rey, 2006; Sapir et al., 2008).

FAST Fusogens in Non-Enveloped Reoviruses

Reovirus fusion-associated small transmembrane FAST proteins are a unique group of non-structural viral membrane proteins that, when expressed in cells, induce cell–cell fusion and not virus–cell fusion (Shmulevitz and Duncan, 2000). The FAST proteins are the smallest known membrane fusion proteins. This group of membrane fusion proteins, with ectodomains of only ~20–40 residues, have 10- or 15-fold fewer amino acids in their ectodomain than the average viral membrane fusogens from enveloped viruses. The current model proposes that the FAST fusion machines are sufficient to promote lipid mixing and membrane fusion. FAST proteins have multiple motifs in their ectodomains including flickering fatty acid interactions with membranes, structurally dynamic amphipathic motifs, and fusion peptides. The endodomains appear to function both in cis and trans to mediate pore formation, via protein–membrane and protein–protein interactions (Barry and Duncan, 2009; Top et al., 2009). The structure of the transmembrane domain may promote membrane curvature and transition through unconventional stages of the fusion process (Clancy and Duncan, 2009). This working model proposes that mechanical energy provided by extensive conformational changes in complex, multimeric fusion machines may not be the only way to accomplish protein-mediated fusion of biological membranes. Some of the stages in cell fusion induced by FAST proteins appear to differ from the stages found in cell fusion induced by enveloped viruses (Clancy et al., 2010).

Useful Cell–Cell Fusion in a Lab Dish

Monoclonal antibodies are produced by hybrid cells obtained by fusing a cancerous myeloma cell with a B lymphocyte from the spleen of animals immunized with a specific antigen. Hybridomas producing monoclonal antibodies are then selected in vitro. The first hybridomas in the early 1970s were between rat and mouse cells (Cotton and Milstein, 1973). The selection and screening of fused cells that divide and produce high amounts of specific antibodies was a major breakthrough, and the Nobel prize was awarded to Kohler and Milstein (Kohler and Milstein, 1975). Fusion in a lab dish can be obtained using polyethylene glycol (PEG), electric fields, lasers, or viral fusogens such as Sendai viruses (Kohler and Milstein, 1975). Researchers have induced self- and non-self cell fusion in tissue culture, in whole *C. elegans* embryos, and between diploid differentiated cells and enucleated oocytes to address many diverse biological questions ranging from the cell cycle to intracellular trafficking and animal cloning (Johnson and Rao, 1970; Rothman et al., 1984; Schierenberg and Wood, 1985; Campbell et al., 1996). Thus, under the right conditions PEG, viral and FF fusogens induce cell–cell fusion (for reviews see Earp et al., 2005; Podbilewicz and Chernomordik, 2005; Zimmerberg and Kozlov, 2006).

SELF-FUSION (TYPE II)

Examples of this type of cell–cell fusion involve merger of cells that are genetically identical, such as cells that grow by vegetative fusion (e.g., hypha in fungi) and somatic cell–cell fusion in differentiated animal cells (e.g., mammalian muscles and nematode skin).

Fusion in Filamentous Fungi

Fusion between cells that grow and differentiate is found in hyphal self-fusion in *Neurospora crassa* (Glass et al., 2004). It is believed that the interconnected state of hypha may control homeostasis by the interchange of water, nutrients, nuclei, and organelles. Conidial Anastomosis Tube (CAT) fusion between identical cells involves a self-signaling oscillating mechanism between the tips of hyphal cells. CAT fusion is a model to study self-signaling and self-fusion (Read et al., 2009). CAT and hyphal fusion in the mature filamentous fungi colony may share the same mechanisms including self-signaling via MAP kinase pathways, osmosensing pathway, and Prm1-dependent membrane merger similar to yeast mating (see above; Fleissner et al., 2009a). Some of the components of self-fusion in filamentous fungi may be similar to pheromone-induced G protein–coupled receptors and MAP kinase pathways in budding yeast (Fleissner et al., 2009b). Thus, mating in yeast (non-self-fusion) and filamentous fungi hyphal fusion (self-fusion) may share some common mechanisms (Fleissner et al., 2009b). However, in *Neurospora* there is no known function for a sex pheromone-signaling pathway controlling MAP kinase activity (Read et al., 2009).

Blastomere Fusion in Sponges

Some sponges (Porifera, Hexactinellida) have a very unusual body plan consisting of a giant multinucleate cell. Embryos first generate 64 cells and then initiate self-fusion of blastomeres (Leys et al., 2006). In the glass sponge *Oopsacas minuta*, the 32-cell embryo undergoes asynchronous, asymmetric, and complete divisions. The sixth division is asymmetric and is followed by self-fusion between blastomeres of different sizes and functions. From gastrulation through

larval elongation, many cell fusion events form specialized polarized tissues, including internal flagellated chambers and a giant multiciliated epithelium. Later in the developing larva, mononucleate and multinucleate cells fuse to form a single giant syncytium connected by multiple cytoplasmic bridges (Leys et al., 2006). Since HAP2/GCS1 (see Double Fertilization in Plants section) has been recently identified in sponges (Steele and Dana, 2009), it is conceivable that this gene may have a conserved function in self-fusion of blastomeres. The transformation of a cellular embryo into a syncytial animal supports the hypothesis that early metazoans may have been cellular and not syncytial.

Mammalian Eye Lens Fusion

The lens of the eye in mice is similar to a transparent onion in which each layer is formed from outside to inside (Shi et al., 2009). The lens focuses the light and images to the retina and to accomplish this task efficiently the lens must be transparent and requires a high refractive index. The current model of the lens is that it is composed from several syncytial layers and that these layers are divided in three main regions: Region 1 is a central syncytium with a refractive index of 1.51. Region 2 is formed of concentric syncytial layers. The cells of each stratum partially fuse allowing free diffusion of contents within each syncytium (stratified syncytial epithelium; refractive index = 1.37). Region 3 is the external anterior surface or cellular layer that generates new layers during development. The stratified multilayered syncytia do not form in the claudin-like Lim2 mosaic mutant mice (Shi et al., 2009). The role of LIM2 protein in lens syncytium formation may have functions in the development and maintenance of other stratified multilayered syncytia (Shi et al., 2009). The new findings on the development of the rodent eye lens may have important implications for the treatment of cataracts and other lens-related diseases.

Macrophage Fusion Forms Osteoclasts and Giant Cells

Osteoclasts are giant multinucleate cells that originate from fusion between precursor macrophages. Osteoclasts are important for bone formation and resorption (Vignery, 2008). Macrophages also form multinucleate cells in response to chronic inflammation caused by foreign bodies or pathogens. Macrophages may also fuse to stem cells to repair and regenerate tissues and to tumor cells as part of a metastatic process (Vignery, 2008). Little is known about stage III in the pathway of macrophage fusion (Fig. 1). Recently, the membrane metalloprotease MT1-MMP has been shown to regulate macrophage migration before they undergo fusion to form osteoclasts (Stage II). MT1-MMP modulates locomotion of bone marrow osteoclast progenitor cells, spreading, and lamellipodia formation (Gonzalo et al., 2010). Wild-type cells can fuse with MT1-MMP mutant cells, showing that MT1-MMP expression is required in only one of the fusion partners. The working model proposes that MT1-MMP is expressed in the cell membrane of bone marrow myeloid cells and recruits p130Cas-Rac1 to the lamellopodia, stimulating cell migration (Gonzalo et al., 2010). Cell migration is required before binding and tight adhesion of myeloid cells and will eventually culminate in cell–cell fusion. Impaired migration of myeloid cells in MT1-MMP−/− cells can result in inefficient migration and, therefore, reduced fusion resulting in slightly smaller osteoclasts.

Trophoblast Fusion in the Placenta

A healthy and complete pregnancy depends on the formation and maintenance of the placenta. During cleavage, the embryo divides into the inner cell mass, which gives rise to the embryo proper, and the outer cell mass, which gives rise to the epithelial trophoblasts, the major constituents of the placenta. In primates and rodents, trophoblasts fuse to form the syncytiotrophoblast that can then invaginate into the uterine wall and allow for the blastocyst implantation in the uterine wall. The syncytiotrophoblast will serve as a barrier between maternal and fetal blood (Potgens et al., 2002). Later on throughout the pregnancy, the mononucleate cytotrophoblasts will fuse with the syncytiotrophoblast for its growth and maintenance (Huppertz et al., 2006; Huppertz and Borges, 2008). Several genes have been implicated in the process of trophoblast fusion, among them connexin 43, cadherin 11, CD98, and the best-characterized candidate fusogens Syncytin 1 and 2 (Blond et al., 2000; Mi et al., 2000; Cronier et al., 2002; Blaise et al., 2003; Getsios and MacCalman, 2003; Kudo and Boyd, 2004; Malassine et al., 2007). Syncytin-1 is encoded by the HERV-W retroviral element, and belongs to the class I viral fusogens. In vitro expression in tissue culture cells of Syncytin 1 or 2 results in the formation of multinucleate cells (Blond et al., 2000; Mi et al., 2000; Blaise et al., 2003), suggesting that Syncytin 1 can function as a fusogen. For extensive reviews on cell fusion in the placenta see (Huppertz et al., 2006; Huppertz and Borges, 2008; Larsson et al., 2008).

Myoblast Fusion in Flies and Vertebrates

Embryonic myoblast fusion events in *Drosophila* occur over a 5.5-hr period, and include fusion of mononucleate myoblasts (the founder cell with the fusion-competent myoblasts, FCM) (Bate, 1990). The founder cell is the organizer of muscle formation that attracts and fuses with two FCMs, generating a muscle precursor. Later on, the FCMs fuse with the muscle precursors, to generate mature myotubes. Recent reviews on Drosophila myogenesis describe this field (Abmayr et al., 2008; Richardson et al., 2008). Multiple experiments uncovered a link between myoblast fusion and cytoskeleton remodeling. The actin nucleation–promoting factors SCAR/WAVE and Wiskott-Aldrich syndrome protein (WASp) were shown to have roles at the point of contact between the FCMs and the enlarging myotube. SCAR and WASp are required for the fusion process but it is not clear whether they function before the initiation of stage III targeting exocytic vesicles or during pore

expansion and completion of the fusion process (Kim et al., 2007; Massarwa et al., 2007; Richardson et al., 2007; Berger et al., 2008; Guerin and Kramer, 2009). Recent results support the model in which SCAR functions before the formation of fusion pores, whereas WASp acts in the expansion of pores and completion of myoblast fusion (Gildor et al., 2009).

Similarly to Drosophila myoblast fusion, mammalian muscle cells also undergo two molecularly distinct phases of cell fusion. In the first, myoblasts fuse with one another to generate a multinucleate myotube. Additional myoblasts fuse with the myotube in the second phase, increasing the size of the myofiber (Bate, 1990). However, populations equivalent to FMCs and founder cells have not been clearly identified outside Drosophila. Several conserved genes in flies and vertebrates have been found to play functions in myoblast fusion, such as β1-integrin, the tetraspanin CD9, IgSF proteins, and M-cadherin (Strunkelnberg et al., 2001; Chen and Olson, 2004; Richardson et al., 2008; Menon et al., 2005; Krauss, 2007; Moore et al., 2007; Srinivas et al., 2007; Jansen and Pavlath, 2008). Actin-cytoskeleton remodeling was found to have a crucial role also in mammalian myoblast fusion, influencing cell-shape changes. Inhibition of a member of the WAVE actin remodeling complex, Nap1, resulted in a decrease in myoblast fusion, and actin-depolymerizing agents impair the formation of lamellipodial and filopodial extensions, and as a result myobalst fusion is compromised (Nowak et al., 2009).

Cell Fusion and Cancer

Diverse enveloped viruses, some of which are normally carried by different organisms including humans, may cause self-fusion of cells. The resulting hybrids may then become a source of aneuploid cells that may contribute to cell transformation and tumorous growth (Duelli and Lazebnik, 2003; Duelli et al., 2007). Thus, non-self-fusion induced by viral fusogens may induce self-fusion between normal cells with the potential of transforming them into neoplastic growth. Giant cells have been observed in association to various cancers, but it is not clear whether these multinucleate cells originated by cell fusion or by failure of cytokinesis (Sutton and Weiss, 1966; Huang et al., 1993; Sitar et al., 1994). The use of cell fusion as a tool to make vaccines against cancer has been explored and is in the early stages of research and development (Lundqvist et al., 2004).

Stem Cell Fusion to Differentiated Cells Is Not Ideal for Therapies

Several reports suggest that stem cells can fuse to fully differentiated cells ranging from hepatocytes to cerebellar neurons (Wurmser and Gage, 2002; Medvinsky and Smith, 2003; Vassilopoulos et al., 2003; Wang et al., 2003; Shi et al., 2004; Johansson et al., 2008; Nygren et al., 2008; Flohr et al., 2009). However, it is not clear how frequent these events occur in nature, what is the function of these self-fusion events, what is the mechanism of these fusion processes, and whether they give rise to useful hybrids or to precursors of cancerous cells. The potential use of stem cell–cell fusion for therapies in regenerative medicine or gene therapy is not ideal since polyploidy in the resulting hybrids may result in chromosomal defects, uncontrolled growth, aneuploidy, and cancer.

Somatic Cell–Cell Fusion in *C. elegans*

One third of all somatic nuclei in *C. elegans* reside in syncytia generated by self-cell fusion (Podbilewicz and White, 1994; Shemer and Podbilewicz, 2000). Multinucleate cells in *C. elegans* have high diversity in cell lineage origin, structure, and function (Nguyen et al., 1999; Mohler et al., 1998; Podbilewicz, 2006). For example, in terms of size, the syncytial cells contain from 2 to 139 nuclei. In terms of shape, multinucleate cells in the worm can be flat, thin, and tubular such as the giant epidermal cell hyp7 with 139 nuclei, and many are small binucleate cells in the pharynx, vulva, or in the spike of the embryonic tail. Syncytial cells in the vulva and uterus form rings or toroids forming epithelial tubes, and the hymen has an "H" shape with 9 nuclei. In terms of function, there are syncytia in the epidermis, pharynx, vulva, hymen, uterus, glands, and tail. Some of these cells are secretory, others have muscular contractile functions, epidermal, or reproductive functions. Often syncytial cells form tubes that interconnect forming stacks of rings with functions as diverse as copulation, egg-laying, and feeding. In the adult hermaphrodite, there are a total of 300 nuclei contained in the 44 syncytia generated by self-cell–cell fusion compared to 659 mononucleate cells (Shemer and Podbilewicz, 2000; Oren-Suissa and Podbilewicz, 2007; Alper and Podbilewicz, 2008).

Numerous transcription factors have been identified that specify the fates of cells destined to fuse (Stage I; Fig. 1). These genes encode for Zinc finger–containing proteins, GATA factors, Hox factors, homeobox-containing transcription factors, and transcriptional repressors (Alper and Kenyon, 2001, 2002; Koh and Rothman, 2001; Koh et al., 2002, 2004; Shemer and Podbilewicz, 2002; Cassata et al., 2005; Margalit et al., 2007; Mason et al., 2008). Downstream of the transcription factors responsible for the specification of fusion fates, there are many effectors necessary to bring the fusion-fated cells to precise positions and at appropriate distances that will allow cell fusion to proceed (Stage II).

Two Genes Execute Programmed Cell Fusion in *C. elegans*

The wild-type functions of the genes *aff-1* and *eff-1* are required for the initiation and execution of programmed cell fusions in the nematode *C. elegans* (Stage III) (Mohler et al., 2002; Sapir et al., 2007). The reduction or loss of *eff-1* or *aff-1* function results in a transformation in the fates of specific cells that normally fuse. In *eff-1* or *aff-1* mutants, such cells fail to fuse. *eff-1* and *aff-1* mutant cells in the embryos appear normal in morphology and behavior, indicating that programmed cell fusion is not an essential aspect of nematode embryonic development (Mohler et al., 2002; Sapir et al., 2007). However, postembryonic failure in cell

fusion results in numerous behavioral and morphological defects (Shemer and Podbilewicz, 2002; Koh et al., 2004; Cassata et al., 2005; Margalit et al., 2007; Mason et al., 2008). Failure of embryonic or postembryonic cell fusion results in ectopic cell migrations of unfused epithelial cells during larval development. Unfused cells do not proliferate and only rarely can be induced to change their fates (Mohler et al., 2002; Sapir et al., 2007). For example, vulval precursor cells (VPCs) that fail to fuse in *eff-1* mutants can be induced to proliferate and change their fate from epidermal to vulval (Shemer and Podbilewicz, 2002). However, this ectopic induction of unfused cells has a low penetrance [e.g., only 6% of the *eff-1*(*hy21*) mutant animals have the multivulva phenotype] (Mohler et al., 2002). Cell fusion is a mechanism to change and fix the shapes of cells and organs by preventing ectopic migrations but is not generally required to prevent proliferation or changes in cell fates (Cassata et al., 2005; Gattegno et al., 2007). In contrast, ectopic embryonic cell fusions result in embryonic lethality (Shemer et al., 2004; Cassata et al., 2005; del Campo et al., 2005; Sapir et al., 2007). Thus, too much cell fusion kills embryos and fusion failure has no apparent embryonic phenotypes.

The genes *eff-1* and *aff-1* define the first known step of a developmental pathway for genetically programmed cell fusion, suggesting that these genes may be involved in the execution of cell–cell fusion during *C. elegans* development (Shemer et al., 2004; Podbilewicz et al., 2006; Sapir et al., 2007). The double mutant *eff-1aff-1* is unviable, has many deformities linked to a general fusion failure in different organs, and has very low fertility (Mohler et al., 2002; Sapir et al., 2007). Genetic mosaic analysis of *eff-1* has shown that this gene is required in both cells in order for them to fuse (Fig. 3; (Shemer et al., 2004). *eff-1* and *aff-1* act independently to fuse different cells during *C. elegans* development (Sapir et al., 2007). While *eff-1* fuses most hypodermal precursors including all the 139 cells that form the largest cell in the worm, the hypodermal giant cell hyp7, *aff-1* fuses some pharyngeal cells, hyp5, the anchor cell to the utse hymen (Fig. 4C) and the terminal fusion of the seam cells in the L4 to adult transition. *eff-1* fuses the rings vulF, vulE, and vulC while *aff-1* fuses vulA and vulD (Sapir et al., 2007, 2008). *eff-1* is also responsible for the fusion of the 3ry VPCs progeny and fusion of some, but not all, the pharyngeal myoepithelial cells (Shemer and Podbilewicz, 2002). The activities of *eff-1* and *aff-1* are regulated in a sex-specific manner (Podbilewicz, 2006). For example, *eff-1* and *aff-1* are activated in specific cells during vulva morphogenesis, uterus formation, and hymen development in the hermaphrodite (Shemer and Podbilewicz, 2002; Sapir et al., 2007). EFF-1 is also involved in sex-specific morphogenesis of the male tail in a process regulated by the master sexual regulator *tra-1* via *mab-3* and *dmd-3* (doublesex) in a genetic pathway that also involves Wnt signaling (Mason et al., 2008).

Cell fusion failure in *eff-1* or *aff-1* mutants prevents the initiation of the fusion process (Mohler et al., 2002; Sapir et al., 2007). The unfused cell membranes remain at a distance of around 10 nm, demonstrating that *eff-1* activity is required to initiate the cell-fusion process (Shemer et al., 2004; Gattegno et al., 2007). In temperature-sensitive mutants of *eff-1*, cells can initiate fusion at the semipermissive temperature and microfusion pores of up to 250 nm form but fail to expand, suggesting that EFF-1 is required not only to initiate but also to expand fusion pores (Shemer et al., 2004; Gattegno et al., 2007).

EFF-1 and AFF-1 Proteins Act as Cell Fusogens

eff-1 and *aff-1* encode novel type I membrane glycoproteins conserved within nematodes (Sapir et al., 2008). Ectopic expression of EFF-1 or AFF-1 in cells that normally do not fuse or at times when cell fusion does not occur is enough to fuse cells but only if both membranes express the FF fusogen proteins simultaneously and in sufficient amounts (Podbilewicz, 2006; Sapir et al., 2007). In addition, expression of FF proteins in heterologous tissue culture insect cells results in their fusion (Fig. 4; (Podbilewicz, 2006; Sapir et al., 2007). Thus, the FF family of proteins are eukaryotic cell–cell fusogens that have been expressed in cells in culture and caused them to fuse (Fig. 5) (White, 2007). The only other proteins able to fuse cells are membrane glycoproteins from some enveloped viruses, FAST proteins, syncytins, and flipped SNAREs (Blaise et al., 2003; Hu et al., 2003; Sapir et al., 2008). The characterization of EFF-1 and AFF-1 fusion in tissue culture has demonstrated that membrane fusion is via a hemifusion intermediate as was shown for other membrane fusogens including intracellular SNARE-mediated fusion, and viral-induced fusion (Podbilewicz, 2006; Sapir et al., 2008). Hemifusion appears to be a universal intermediate shared by all well-characterized membrane-fusion processes (Fig. 1) (Chernomordik and Kozlov, 2005; Martens and McMahon, 2008; Sapir et al., 2008).

There is a single isoform of AFF-1 and *eff-1* encodes for at least four isoforms generated by alternative splicing (Mohler et al., 2002; Sapir et al., 2007). The two transmembrane proteins EFF-1A and EFF-1B differ in their cytoplasmic tails. It has been proposed that the cytoplasmic tail of EFF-1A may interact with 14-3-3 proteins and that these interactions may be important for fusion (Balla et al., 2006). However, the putative motif that is present in EFF-1A and not in EFF-1B and that is proposed to bind 14-3-3 is not essential for cell fusion in vitro. Both EFF-1A and EFF-1B can fuse heterologous Sf9 cells (Podbilewicz, 2006). Similar surface expression of AFF-1 results in much more potent fusogenic activity than EFF-1A, suggesting that AFF-1 may be a stronger fusogen than EFF-1 (Fig. 4A and B) (Podbilewicz, 2006; Sapir et al., 2007). In addition, AFF-1 expression also results in cell lysis probably as a result of auto-fusion (see Auto-Fusion (Type III) section). A chimera having the AFF-1 extracellular domain (AFF-1EC) with EFF-1A transmembrane and cytoplasmic tails results in higher surface expression with an increase in the percentage of cells that fuse in the heterologous cell culture assay (Sapir et al., 2007). These experiments show that three different cytoplasmic tails can support cell–cell fusion in heterologous

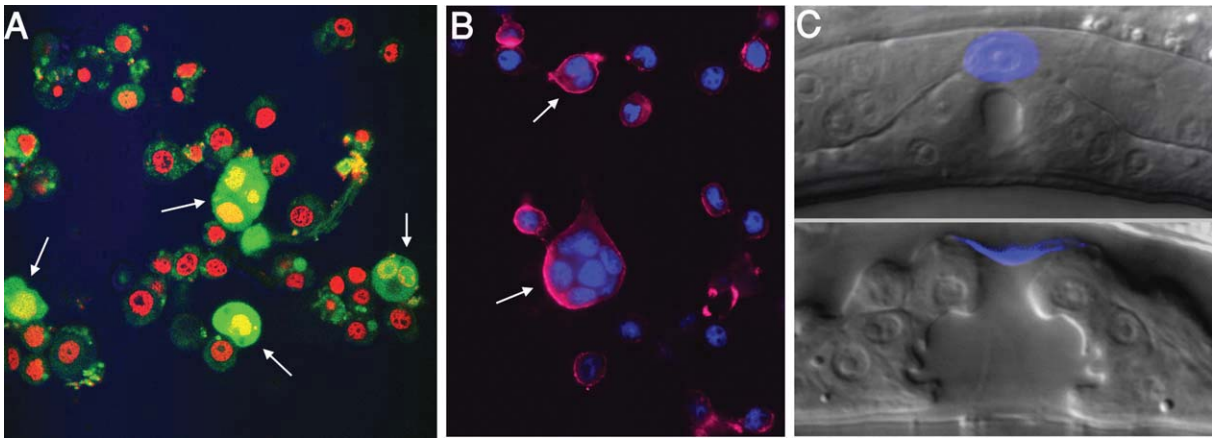

Fig. 4. EFF-1 and AFF-1 act in both fusing cells in vitro and in vivo. **A:** Sf9 insect cells expressing EFF-1A form multinucleate syncytial cells by cell fusion (arrows). Green, cytoplasmic GFP reporter show cells expressing EFF-1A. Red, nuclei Hoechst staining. Arrows, syncytial cells (Podbilewicz et al., 2006). **B:** Sf9 insect cells expressing AFF-1-V5. Red, Immunofluorescence using antiV5 tag antibody shows surface expression of AFF-1. Blue, DAPI nuclear stain. Arrows, multinucleate cells (Sapir et al., 2007). **C:** Anchor cell (blue) on the dorsal side of the vulva invagination at the early-mid L4 stage before fusion to the utse (top). After fusion to the utse (hymen; bottom), the GFP reporter (blue pseudocolor) is transferred to the hymen (blue) from the anchor cell at the L4 Christmas tree stage. This fusion event is mediated by AFF-1 (Sapir et al., 2007).

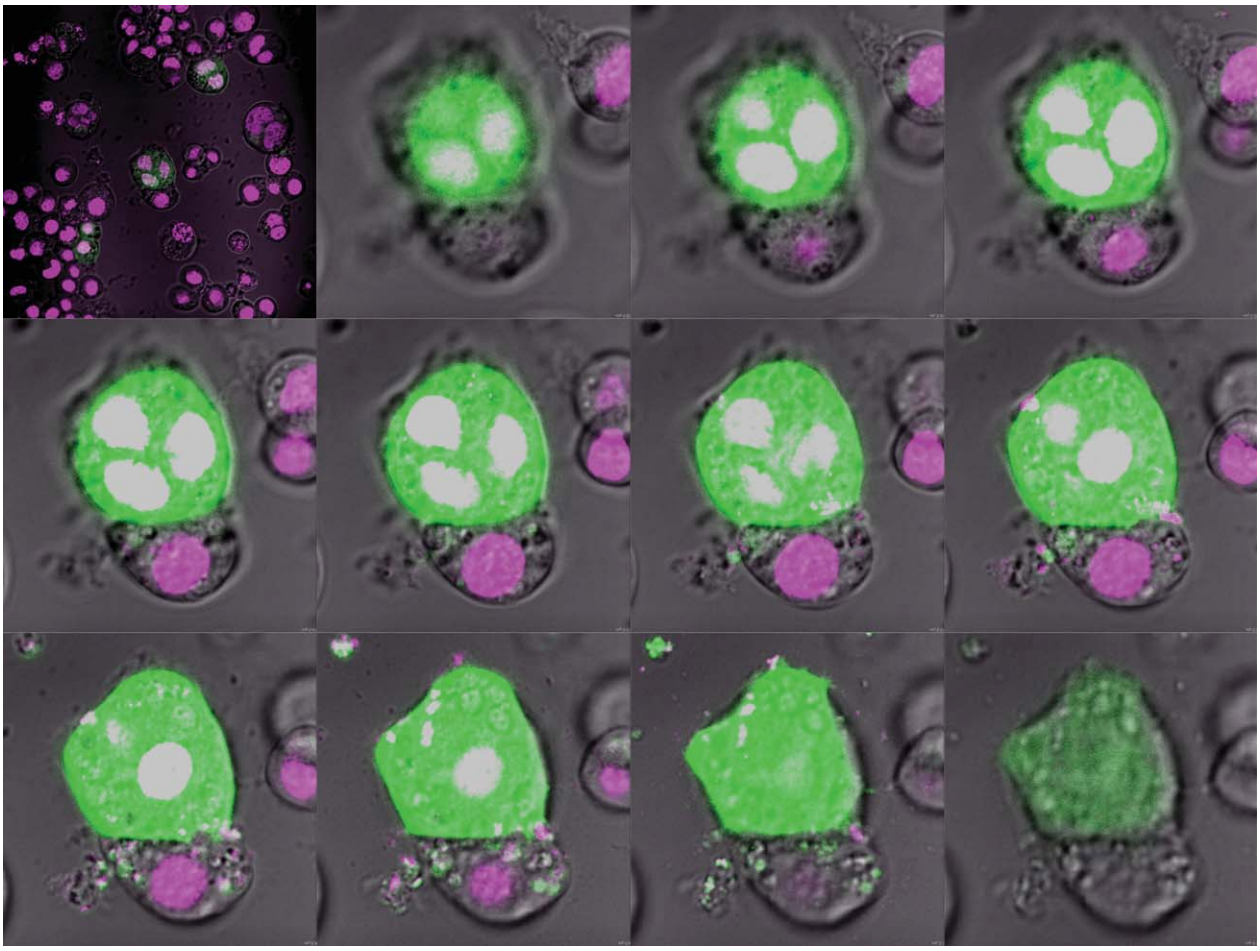

Fig. 5. EFF-1 can fuse heterologous insect cells in vitro. Top left image shows a low-magnification confocal image of a field of Sf9 insect cells transiently expressing EFF-1A from *C. elegans*. Several multinucleate cells are shown in the field. Under optimal conditions, 65% of the cells expressing EFF-1 become multinucleate by cell fusion (Podbilewicz et al., 2006). A central multinucleate cell was imaged with the two-photon confocal microscope and Z-series were generated. Eleven confocal serial sections show that the green cell in the center has 4 nuclei in a shared cytoplasm and the mononucleate cell in the bottom is tightly attached to the green cell but did not fuse probably because it does not express EFF-1A on the surface (the reporter cytoplasmic GFP is not expressed; Podbilewicz et al., 2006).

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Sf9 insect cells (Podbilewicz, 2006; Sapir et al., 2007). The functions of the EFF-1 isoforms in vivo have not been determined and the secreted isoforms EFF-1C and EFF-1D do not appear to have fusogenic activities in a cell culture system (Podbilewicz, 2006). It is conceivable that these secreted isoforms may be regulators of the fusion-inducing transmembrane isoforms. Alternatively, EFF-1C and EFF-1D may function in other processes independently of the fusogenic activities of the transmembrane fusogens.

The purified ectodomain of EFF-1A and EFF-1B (EFF-1EC) stimulates cell–cell fusion when added to Sf9 cells expressing EFF-1A (Podbilewicz, 2006). This suggests that EFF-1EC can help in the oligomerization of EFF-1 proteins in cis and trans and that these complexes are required for efficient cell–cell fusion through bending of membranes and formation of lipidic fusion pores via hemifusion (Fig. 1, Stage III). Alternatively, large complexes of EFF-1 may be stabilized by EFF-1EC and may be necessary for the expansion of nanometric lipidic pores into large macrofusion areas of several microns in diameter (Steps 9–10 in Fig. 1; Podbilewicz and Chernomordik, 2005). In summary, in vivo and in vitro studies on EFF-1A, EFF-1B, and AFF-1 are beginning to shed light on the mechanism of biological self-fusion of cells via hemifusion, pore formation, and expansion of nano-lipidic pores to macrofused areas of several micrometers.

Repression of Cell Fusion in Vulva, Seams, and Tail

Since both EFF-1 and AFF-1 are such potent fusogens, and ectopic fusion results in embryonic lethality and numerous postembryonic defects, there are a number of mechanisms that prevent expression of FF proteins in *C. elegans*. This is usually accomplished through transcriptional repression of *eff-1* or *aff-1* expression (Alper and Podbilewicz, 2008). Different genetic pathways at specific sites and times during development directly or indirectly control FF fusogens activities and these include sex determination, homeotic genes, Barrier-to-Autointegration Factor (BAF-1), Engrailed/CEH-16, Notch signaling, Wnt signaling, bHLH REF-1 factor, Zn-finger REF-2, FOS-1, and GATA factors (Alper and Kenyon, 2001, 2002; Koh and Rothman, 2001; Koh et al., 2002, 2004; Shemer and Podbilewicz, 2002; Cassata et al., 2005; Margalit et al., 2007; Alper and Podbilewicz, 2008; Mason et al., 2008). In addition, vATPases have been reported to repress *eff-1* activities probably through posttranslational mechanisms (Kontani et al., 2005; Kontani and Rothman, 2005).

AUTO-FUSION (TYPE III)

Plasma-membrane fusion between different membrane regions of the same cell occurs during endocytosis, phagocytosis, lysis, and ring-cell formation (Sharma-Kishore et al., 1999; Kozlovsky and Kozlov, 2003; Rasmussen et al., 2008; Stone et al., 2009). Auto-fusion can be a form of lysis usually resulting in cell death, and appears to be controlled by fusogens such as the Env glycoprotein of HIV (Cao et al., 1996) and Prm1 during yeast mating (Heiman and Walter, 2000; Jin et al., 2004, 2008; Aguilar et al., 2007; Grote, 2008).

John White discovered auto-fusion in *C. elegans* when he ablated with a laser half of the vulva primordium and found that the remaining half vulva precursors can form a complete although smaller vulva (Sharma-Kishore et al., 1999). Specifically, he observed that cells can autonomously migrate, and in the absence of a fusion partner form an auto-apical junction (Fig. 2). This auto-junction between two domains of the plasma membrane of the same cell can eventually fuse and then disassemble the junctions, forming a continuous mononucleate ring (Sharma-Kishore et al., 1999). Similar mononucleate vulval rings form in non-operated mutant worms that have a multivulva (Muv) phenotype. In *let-60/Ras* gain-of-function mutants and in other mutants in *C. elegans* and in different nematode species, auto-fusion of vulval rings has been reported (Shemer et al., 2000; Louvet-Vallee et al., 2003; Broday et al., 2004; Kolotuev and Podbilewicz, 2004, 2008; Kiontke et al., 2007).

Transformation of a Single Cell Into a Tube by Auto-Epithelial Cell Fusion

Two adjacent cells in the digestive tract of wild-type *C. elegans* embryos invariantly undergo a conformational change to form single-cell toroids/rings (Rasmussen et al., 2008). These cells independently remodel their shapes wrapping around cell processes and forming rings. Each of these cells forms epithelial auto-apical junctions attaching to each other (Rasmussen et al., 2008). Auto-fusion of each ring at the apical junction results in merging of the membranes, mixing of the cytoplasm, remodeling of the epithelial junctions forming a stable polarized cell with a lumen and a single diploid nucleus. Thus, the toroidal rings fix their morphology into ring structures through auto-fusion (Rasmussen et al., 2008).

To prevent cross cell–cell self-fusion between different rings, it appears that each of these adjacent cells autofuse using distinct homotypic fusogens (EFF-1 or AFF-1). Moreover, the Notch pathway is positively activated in one of the rings (pharyngeal myoepithelial cell pm8) to express AFF-1 allowing auto-fusion. Simultaneously, Notch negatively regulates EFF-1 expression in pm8 preventing it from fusing with vpi1 intestinal valve EFF-1-expressing cell (Rasmussen et al., 2008).

Another auto-fusion that has recently been discovered in *C. elegans* is in the excretory duct cell (Stone et al., 2009). This single epithelial cell wraps forming a tube with an apical junction. In this case, AFF-1 is required to fuse the membranes of this auto-junction resulting in disassembly of the apical junction and the formation of a tube with a single nucleus required for the normal function of the excretory (renal) system of the worm.

Interestingly, in all these cases, the normal digestive and excretory system tubes, and in the operated or mutated reproductive rings, auto-fusion is mediated by AFF-1 or EFF-1 fusogens, probably preventing cross-fusion. Single-cell tubes are also found in mammalian endothelial capillaries, *Drosophila* tracheal tubes, and in mammalian lungs. But, to our knowledge, auto-fusion of ring-shaped cells has not been described outside

C. elegans. We hypothesize that numerous undiscovered cell autofusion events exist in different organisms from fungi to humans.

FUTURE DIRECTIONS

Ten years ago, it was already known that nearly one-third of all the somatic cells born in *C. elegans* fuse to form diverse tissues and organs. It was hypothesized that one of the functions of cell fusion is to form cells with different shapes and functions (Podbilewicz, 2000; Shemer and Podbilewicz, 2000). Fusomorphogenesis explained that cell fusion drives organ formation and predicted that specific failure in cell fusion will result in deformations and defective organogenesis (Podbilewicz, 2000; Shemer and Podbilewicz, 2000).

The next step was the isolation of mutations that result in a complete and specific failure of cell fusion in *C. elegans* (Mohler et al., 2002; Shemer and Podbilewicz, 2002; Cassata et al., 2005; Podbilewicz and Chernomordik, 2005; Podbilewicz, 2006; Margalit et al., 2007; Sapir et al., 2007; Alper and Podbilewicz, 2008). Phenotypic analyses demonstrated that cells fuse to sculpt organs (Witze and Rothman, 2002; Oren-Suissa and Podbilewicz, 2007; Sapir et al., 2008). Recent studies are focusing on the biophysical mechanisms of FF-mediated cell–cell fusion in vivo and in vitro (Podbilewicz et al., 2006; Gattegno et al., 2007; Sapir et al., 2007). Future investigations will reveal whether FF proteins can fuse liposomes; what is the 3D structure of these fusogens; whether there is a trigger that initiates the fusion reaction; whether there are conformational changes in these proteins required for the formation of lipidic fusion pores via hemifusion; and whether other proteins interact with FF proteins to expand the fusion pores. The challenge for the next decade is to identify and characterize the missing fusogens that fuse cells during self-, non-self, and auto-fusion in all kingdoms of life and to decipher how cells fuse at a mechanistic level.

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