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Minireview

Cell–cell fusion

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Abstract **Cell–cell fusion is a highly regulated and dramatic cellular event that is required for development and homeostasis. Fusion may also play a role in the development of cancer and in tissue repair by stem cells. While virus–cell fusion and the fusion of intracellular membranes have been the subject of intense investigation during the past decade, cell–cell fusion remains poorly understood. Given the importance of this cell-biological phenomenon, a number of investigators have begun analyses of the molecular mechanisms that mediate the specialized fusion events of a variety of cell types and species. We discuss recent genetic and biochemical studies that are beginning to yield exciting insights into the fusion mechanisms of *Saccharomyces cerevisiae* mating pairs, *Caenorhabditis elegans* epithelial cells and gametes, *Drosophila melanogaster* and mammalian myoblasts, and mammalian macrophages.**

Keywords: Cell fusion; Yeast; Myoblast; Macrophage; *Caenorhabditis elegans*; *Drosophila melanogaster*

1. Cell–cell fusion: a specialized form of membrane fusion

A cell, the basic unit of life, is defined by a plasma membrane. Membranes are central to the origin, the differentiation and the function of all cells. Membranes are lipid bilayers that also form intracellular compartments, which undergo constant fusion and fission to regulate molecular trafficking between organelles, between cells and their extracellular milieu, and between neighboring cells. To fuse with one another, membranes incorporate proteins as intrinsic recognition devices that secure the specificity and the efficacy of fusion. While intracellular membrane fusion depends on α-helical bundle structures similar to those used by many viruses to fuse with cells during infection, the mechanisms that mediate fusion of pairs and groups of cells remain poorly understood. Here we discuss our current knowledge about the mechanisms in a variety of species (yeast, nematodes, arthropods, and mammals) and cell types (gametes, epithelia, myoblasts, and macrophages). We have learned that although cell–cell fusion events are cell-type specific, they may share some mechanistic similarities.

2. Yeast mating

Yeast cells fuse when they mate (Fig. 1). Genetic studies of mating in the yeast *Saccharomyces cerevisiae* began over 30 years ago with the isolation of sterile (*ste*) mutants [1,2], placing yeast mating among the most intensively studied cell fusion processes.

S. cerevisiae has two mating types (sexes), *MAT**a*** and *MAT*α. Mating initiates with an exchange of pheromone signals between cells of the opposite mating type. MATα cells release the α-factor pheromone, which binds to a G-protein coupled receptor expressed exclusively on *MAT**a*** cells, and vice versa. The pheromone receptors activate a common MAP kinase signaling pathway resulting in three key responses: (1) the cell cycle arrests in G1 to insure that both cells have exactly one copy of each chromosome before they fuse; (2) cells reorient their growth axis to form a mating projection in the direction of a potential mate, and (3) transcription of mating genes is induced. This signal transduction pathway has served as a paradigm for signaling studies in diverse systems, and has been the subject of several comprehensive reviews [3,4]. In brief, the pheromone receptors Ste2 and Ste3 are linked to a G-protein complex containing Gpa1 (Gα), Ste4 (Gβ) and Ste18 (Gγ). The βγ subunits released upon pheromone binding activate the Ste20 PAK kinase and initiate the recruitment of a signaling complex that includes the MAP kinase scaffold Ste5 [5]. Ste5 recruits Ste11, Ste7 and Fus3, the three kinases of the pheromone signaling MAP kinase cascade, and brings the MAPKKK Ste11 into contact with the Ste20 kinase to promote Ste11 phosphorylation. Among the targets of the MAPK Fus3 are the transcription factor Ste12, which binds to pheromone response elements localized at the 5′ of pheromone-induced genes, the formin Bni1, which stimulates polarized assembly of actin cables within mating projections, and the cyclin-dependent kinase inhibitor Far1, which acts within the nucleus to arrest the cell cycle and is also translocated to the plasma membrane to aid in polarity establishment [6,7].

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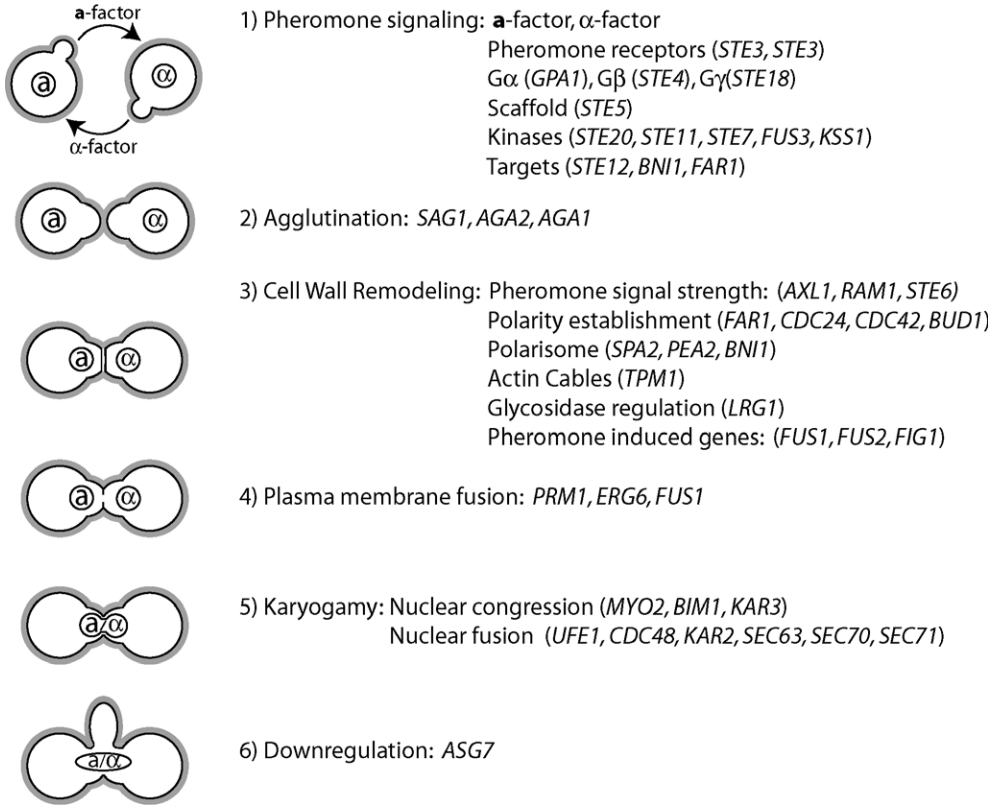

Fig. 1. Stages of the yeast mating process and genes that participate in each stage.

Binding of *MAT**a*** cells to *MAT*α cells is initiated by an interaction between cell surface glycoproteins whose expression is induced by mating pheromones [8]. The α-agglutinin Sag1 contains three immunoglobulin-like domains. The a-agglutinin has two subunits: the smaller subunit Aga2 contains a Sag1 binding site, while the larger subunit Aga1 anchors the complex to the cell wall and stabilizes Aga2 in a binding conformation. The mating agglutinins are initially synthesized as GPI-anchored proteins and are then transferred to the cell wall by a transglycosylation reaction. However, an engineered form of Aga1 retained some activity after replacing its transmembrane domain with a GPI-anchor [9]. The agglutinins are essential for mating in aerated liquid cultures, where agitation produces sheer forces that oppose mating pair assembly, but are unnecessary for mating on a solid surface [10]. Thus, unknown low-affinity interactions are likely to complement agglutinin-mediated binding.

The cell walls of the two cells in a mating pair must be remodeled before the underlying plasma membranes can come into contact and fuse. Cell wall assembly defects typically lead to osmotic lysis. Yeast avoid osmotic lysis during mating by first assembling a unifying wall surrounding the junction and then selectively degrading the cell wall at the contact site. This carefully orchestrated process depends upon robust pheromone signaling and a set of cell polarity regulators and pheromone-induced genes (Fig. 1). The common phenotype found when these genes are mutated is an accumulation of arrested prezygotes that have cell wall separating the two plasma membranes [11,12]. In the *fus2* and *rvs161* mutants, small vesicles accumulate on either side of the intercellular junction [12]. These presumptive secretory vesicles are thought to deliver hydrolytic enzymes for cell wall remodeling and might also contain membrane proteins required for cell fusion.

Once cell wall remodeling is complete, osmotic gradients across the two plasma membranes drive them into tight apposition. If cytoplasmic osmolarity differs between the two cells, the cell with higher osmolarity can extend a finger of membrane bound cytoplasm into its mating partner [13,14]. Cytoplasmic fingers are rarely observed prior to fusion in wild-type mating, suggesting that plasma membrane fusion typically occurs shortly after membrane contact is achieved. Mitotic yeast cells treated with lyticase to remove their cell walls almost never fuse when their plasma membranes are manipulated into contact. Thus, the membrane fusion machinery is likely to be induced during mating.

Plasma membrane fusion is regulated by *PRM1*, a gene discovered in a bioinformatic screen for pheromone-inducible membrane proteins [13]. *PRM1* is not essential for mating, since 25% of Δ*prm1* mating pairs are able to fuse. Furthermore, cell fusion defects are only observed when *PRM1* is mutated in both mating partners. The defining phenotype of *prm1* mutants is an accumulation of arrested mating pairs with plasma membranes that are in contact, but unfused [13]. A second informative phenotype is simultaneous lysis of the two cells in a mating pair immediately after their plasma membranes come into contact [14]. In the absence of Prm1, uncoordinated fusion protein activity is thought to rupture the two plasma membranes instead of fusing them. Mutations in *FIG1*, a gene encoding another pheromone-induced membrane protein, lead to a lesser degree of membrane fusion arrest and

lysis [15]. Interestingly, the amount of lysis compared to fusion is increased in the absence of extracellular Ca^{2+} or by mutation of Tcb3, a membrane protein with three cytoplasmicly oriented C2 Ca^{2+} binding domains, suggesting that Ca^{2+} influx through a pre-lytic pore can activate a plasma membrane repair pathway. A similar mechanism might underlie the contribution of myoferlin to mouse myoblast fusion [16].

A fusion pore is the first aqueous connection between two membranes. Fusion pore opening and expansion can be measured by following the rate of GFP transfer between cells [17]. The fusion pore of a typical yeast mating pair opens suddenly and then gradually expands, but the initial opening is reversible. The size and expansion rate of the pore is regulated by Fus1, a membrane protein concentrated at sites of intercellular interaction that had been previously implicated in cell wall remodeling [17]. Fus1 has both genetic and physical interactions with a web of proteins implicated in cell polarity and fusion, suggesting that it may be an integrative regulator of the cell fusion process [18].

Once the plasma membranes of two yeast cells have fused, mating is completed by merger of the two nuclei in a process termed karyogamy [19]. Karyogamy does not occur in most developmental fusions, but it has been observed in syncytia resulting from HIV infections [20]. Nuclear congression, the first stage of karyogamy, is microtubule-dependent. The dynamic plus ends of microtubules emanating from the spindle pole body are transported by Myo2 along actin filaments and maintained at sites of intercellular contact by Bim1 and Kar3 [21]. After plasma membrane fusion, oppositely oriented microtubules from the two cells interact at their plus ends through Kar3 and Bik1 and depolymerize to pull the nuclei together [22]. The nuclear envelopes each have two lipid bilayers. The outer layer is contiguous with the ER, and its fusion is likely to involve ER localized SNAREs including Ufe1. However, the atypical SNARE disassembly factor Cdc48 is required in place of Sec18/NSF [23]. Furthermore, mutations in luminal ER proteins including the chaperone Kar2 and the translocon-associated proteins Sec63 and Sec70/71 also inhibit fusion of the outer nuclear envelope [24].

The final product of yeast cell fusion is a peanut-shaped zygote with a diploid nucleus. The cell cycle then resumes and diploid daughter cells bud off from the neck connecting the two parent cells. This transition is facilitated by Asg7, a MATa-specific cytoplasmic protein that enters the MATα cell after fusion to trigger Ste4 down regulation, thereby terminating the pheromone response and limiting cell fusion to a single pair of cells [25].

Although the preceding section provides only a broad overview of such thoroughly investigated processes as pheromone signaling and polarized morphogenesis, much remains to be learned about other aspects of mating. For example, little is known about how yeast recognize that they have formed a mating pair and signal that it is safe to proceed further on the mating pathway. Given the variety of screens that have been conducted for mating defective yeast, perhaps the biggest surprise is that the underlying mechanism of plasma membrane fusion remains largely unknown. Since fusion proteins in other systems typically form complexes with other proteins that participate in the fusion process, the best hope for identifying core components of the cell fusion machinery may be to screen for genes and proteins that interact with the three known components Prm1, Fig1 and Fus1.

3. Somatic cell and gamete fusion in nematodes

Syncytial cells form in a variety of tissues within nematodes, by cell–cell fusions that occur during progressive stages of development from the embryo to the mature adult. More than 30% of the 959 somatic nuclei in an adult hermaphrodite of *Caenorhabditis elegans* reside in multinucleate cells, in tissues ranging from neuronal support (glial) cells, to epidermal and internal epithelia, to contractile muscle [26]. Interestingly, although skeletal muscle is consistently syncytial in arthropods and vertebrates, the body-wall musculature responsible for motility of *C. elegans* is made up of only mononucleate cells. The pumping-swallowing muscles of the pharynx, however, comprise 13 coherent and precisely arranged syncytia [27]. Some evidence suggests that at least one mononucleate neuron may even form a ring-shaped process by fusion of distinct axons (W. Mohler and D. Hall, unpublished observations from original data of Albertson and Thomson [27]). Many cell–cell fusions yield binucleate cells, but several larger syncytia also form. One prominent case is an epidermal giant cell that grows via sequential waves of new fusions with mononucleate partners, from 2 nuclei (in mid-embryogenesis), to 23, to 46, to 74, to 112, to a final total 138 nuclei. In all instances, the ancestries and identities of each pair of fusion partner cells are invariant from specimen to specimen, and the timing of the fusion events is predictable.

Multinucleate cells were first characterized by groups that determined the full cell lineage of *C. elegans*, using both light and transmission electron microscopy (TEM) [28,29]. The conclusion that syncytia form by fusion was supported by the observation that cells with quite distinct ancestries in the lineage (not simply sisters) could contribute nuclei to the same syncytium. Subsequently, the use of cell-junction markers – by monitoring their disappearance – permitted observation of the relative timing of individual fusion events [30–32]. Multidimensional imaging in combination with a plasma-membrane dye, GFP-labeled markers, and TEM has since revealed fusion events progressing via widening of a single aperture through the two cell membranes [33]. Membrane merger and cytoplasmic continuity between cells actually occur several minutes before intercellular junctions vanish.

Genetic screens for disruption of cell fusions in the development of the epidermis and vulva have repeatedly yielded recessive mutations in the gene *eff-1* [34]. Inactivation of *eff-1* leaves epithelial differentiation, migration, cell shape, and cell–cell contact unaffected, allowing embryonic morphogenesis to proceed almost entirely normally. However, most cell fusions in *eff-1* mutants are blocked in the initial step of membrane permeabilization; intact cell borders and intercellular junctions remain within fields of neighboring cells that would normally become completely fused. Yet, not all cell types are blocked in fusion by *eff-1* mutations, among them sperm and eggs [35,36]. Null mutants in *eff-1* ultimately acquire a severely abnormal morphology as postembryonic development progresses, but they remain fertile and viable in laboratory culture. This viability, combined with a penetrant phenotype, aided in the cloning of *eff-1*.

The *eff-1* gene encodes both single-pass integral membrane proteins and secreted isoforms, via alternative splicing, and *eff-1* expression is induced in fusion-fated cells shortly before fusion occurs [34]. Recent experiments have shown that forced expression of *eff-1* can induce fusion of normally non-fusing

cell-types from both nematodes and insects [35–38]. Fusogenicity appears to be specific to the integral-membrane protein isoforms EFF-1A and EFF-1B [36–38]. Yet, the rate of fusion can be enhanced *in vitro* by presence of a soluble extracellular fragment of EFF-1 [38], suggesting a physiological significance for the naturally occurring secreted isoforms encoded by the *eff-1* locus. Additionally, observation of both mixed cell cultures and genetic mosaic animals show that each cell in a pair of fusion partners must express membrane-bound EFF-1 in order to fuse [37,38]. In keeping with this requirement of mutual expression, observation of fluorescently tagged EFF-1::GFP in nematode embryos indicates that EFF-1 is predominantly retained in intracellular pools, accumulating at the plasma membrane only where there is direct contact between two EFF-1-expressing cells [36]. This suggests, among other possibilities, that EFF-1 may act as its own receptor allowing a simple homotypic interaction to define the propriety of a pair of neighboring cells for fusion.

EFF-1 currently stands as a prototype for developmentally regulated cellular fusogens; to date, no other membrane proteins have been shown to be both necessary and sufficient to induce fusion competence in development. Another *C. elegans* gene, C44B7.3, encodes a paralogue of EFF-1 [34]. However, although *eff-1* and C44B7.3 are very highly conserved among nematodes, recognizable homologues have not been found in the sequenced genomes of fungi, arthropods, or vertebrates. Interestingly, the strongest candidates for developmentally regulated cellular fusogens in mammals are the placental syncytins of primates and rodents, clear homologues of retroviral envelope fusogens [39,40]. Understanding the biophysical mechanism of syncytin fusogenicity will likely parallel the progress in understanding of membrane fusion in viral infection. In contrast, EFF-1 bears only a rough likeness (but no sequence similarity) to the overall structure of known viral fusion proteins, and it stands out as distinct from known viral fusogens by virtue of its homotypic mode of action [38].

Even though EFF-1 appears to act autonomously in inducing fusion, other gene products must regulate its action to yield such a reproducible pattern and sequence of fusion events. Genetic analysis has revealed that loss of function in components of the vacuolar H^+-ATPase (V-ATPase) complex causes ectopic fusions to occur beyond the limits of normal syncytia in the embryo [41]. This abnormal fusion depends upon *eff-1* activity, and the V0 complex is involved in secretion in other species [42,43]. This suggests that the V-ATPase complex may coordinate or focus EFF-1 function by targeting transport to specific fusion contacts. Another possibility for this interaction could be indirect: a defect in secretion of extra cellular matrix components may weaken fusion-blocking boundaries that appear to separate tissues during morphogenesis [36].

Whether to fuse or to remain an individual is a critical cell fate decision made by many cells during the development of the worm. Sister cells from a new cell division often opt for opposite choices shortly after they are born. Because cell fusion is a process integral to the development of several well-studied tissues and lineages (e.g. the vulva, the seam cells), a number of genes have been identified as critical to the decision to adopt a fusion fate; see a full review in ref [26]. Many of these are transcription factors that presumably regulate expression of fusogenic genes like *eff-1* or of fusion inhibitors like V-ATPase. In the case of *eff-1*, deletion analysis and reporter assays indicate that regulatory sequences comprise several separate enhancers distinctly tuned to activate transcription in specific tissues and at specific times in development [44].

As mentioned above, *eff-1* mutations do not block fertilization, so other mechanisms must drive sperm–egg fusion in the worm. Genetic studies of *C. elegans* fertility mutants suggest that they also differ from mammalian sperm in their mechanism of sperm–egg fusion. Three sperm-expressed proteins, SPE-9 (an EGF-repeat containing membrane protein), TRP-3/SPE-41 (a TRPC-type calcium channel), and SPE-38 (a novel tetraspan membrane protein), are required specifically for sperm

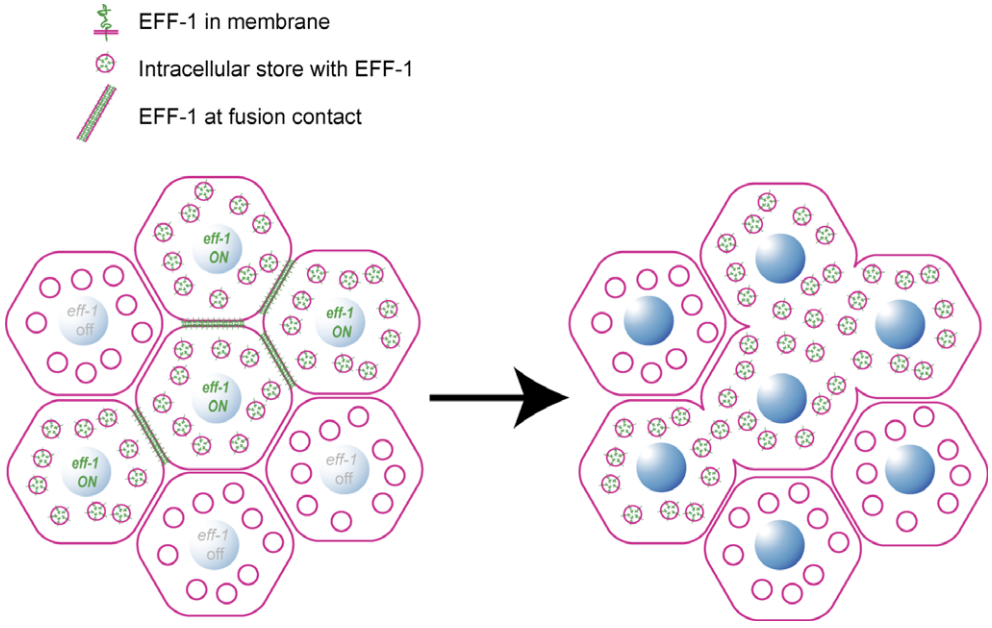

Fig. 2. Model portraying several aspects of EFF-1 function in patterning cell fusions. Homotypic contacts between *eff-1*-expressing cells induce fusion-partner-specific EFF-1 re-localization to the plasma membrane and subsequent cell membrane fusion. Neighboring cells not expressing *eff-1* remain unfused.

interaction and/or fusion with the egg [45–49]. Loss of function in any of the three genes yields sperm that activate and migrate normally but fail to fertilize eggs. TRP-3 is interesting, as its involvement seems to parallel the action of murine Trp2 channel in triggering the sperm acrosome reaction [50,47]. SPE-38, although containing four membrane spanning domains, does not encode a homologue of the mammalian CD9, and its structural similarity to yeast Prm1 is currently unclear [45].

Clearly much remains to be solved in understanding the fusion of somatic and gametic cells in nematodes, but some important lessons can already be inferred from the current knowledge. First, developmental fusogen mechanisms – e.g., the homotypic and sufficient case of EFF-1 – may be as simple as viral fusogen complexes, or possibly even simpler. Second, the deployment of a single simple fusion machine may be governed by complex regulatory inputs to induce precise fusions in many different tissues and different moments of development. Third, from EFF-1's lack of conservation between phyla, we can be fairly certain that more than one family of fusogenic proteins must drive cell fusions throughout Eukaryota. But how many families of cellular fusogens there are, and whether different clades of species or lineages of cells tend to employ unique proteins to drive cell fusion, can only be answered by finding the critical proteins in other model systems.

As to the physicochemical mechanism of action of EFF-1, much remains unknown. Is EFF-1 its own receptor? (see Fig. 2) Does it employ a virus-like fusion peptide to form membrane pores? The path to this level of understanding lies in a transition from largely genetic and cell-biological experiments to biochemistry, structural biology, and biophysics. Yet, the strengths of the nematode as a truly *in vivo* experimental system should remain critical in validating insights gained from experiments on the protein in isolation.

4. Myoblast fusion

Skeletal muscle is a unique organ that is composed of bundles of multinucleate muscle fibers. Each muscle fiber, or cylindrical muscle cell, is the product of fusion of hundreds, or even thousands, of myoblasts. Myoblast fusion during vertebrate embryogenesis occurs in two phases. Initially, myoblasts fuse with one another to form nascent myotubes with a small number of nuclei. This is followed by additional rounds of fusion between myoblasts and nascent myotubes, resulting in the formation of large, mature myotubes [51]. During late embryogenesis, a population of myoblasts, known as satellite cells, are set aside and will later become adult muscle stem cells. Satellite cells are able to proliferate, differentiate and fuse with existing muscle fibers or to form new fibers during postnatal growth, regeneration and maintenance of skeletal muscle [52].

A number of molecules have been implicated in the initial fusion between myoblasts by *in vitro* myoblast culture assays. Among these are cell adhesion molecules, metalloproteases, calcium and calcium-binding proteins, lipids and phospholipases, all of which have been the subject of excellent reviews and will not be further discussed here [51,53–55]. Recent studies using myoblast culture assays in combination with mouse knock-out models have begun to uncover molecules that regulate fusion of myoblasts with nascent myotubes (reviewed by [51]) (Table 1). In particular, studies of calcium signaling in mammalian muscle growth have revealed components of the NFATC2 pathway in the second phase of fusion. These include the transcription factor NFATC2, an activator of the pathway (prostaglandin $F_{2\alpha}$ or $PGF_{2\alpha}$) and a secreted molecule regulated by NFATC2 (interleukin-4 or IL-4) [56–58]. A potentially parallel pathway to that of NFATC2 is mediated by the mammalian target of rapamycin (mTOR), which in turn may regulate the secretion of another unknown factor that is essential for myoblast–myotube fusion [59]. Additional molecules that play a role in the second phase of fusion include the C2 domain-containing transmembrane protein, myoferlin, which is involved in binding calcium-sensitive phospholipids [16], and the secreted protein follistatin, which is activated by deacetylase inhibitors to induce muscle growth [60]. Recently, mannose receptor, another transmembrane protein, has been shown to be required in directed cell migration leading to myoblast–myotube fusion [61].

Compared to *in vitro* assays used to analyze mammalian myoblast fusion, the fruit fly *Drosophila* offers a great *in vivo* system to study this process. Unlike the mammalian skeletal muscle that takes days and weeks to generate, the somatic musculature of *Drosophila* develops within hours during embryogenesis, and each of the ~30 muscle fibers in a hemi segment of a fly embryo is a product of fusion between only 3 to 25 myoblasts [62]. In addition, the cellular events of recognition, adhesion, alignment and membrane merger are conserved during myoblast fusion in *Drosophila* embryos, making the fly somatic musculature an amenable system to dissect myoblast fusion under physiological conditions.

In *Drosophila*, myoblast fusion occurs between two types of muscle cells, muscle founder cells and fusion competent myoblasts [63]. Muscle founder cells reside in a mesodermal layer that is close to the ectoderm, whereas the pool of fusion competent myoblasts occupies several deeper cell layers in the embryo, close to the endoderm. The identities of the two cell types are specified by a cascade of transcription factors (reviewed in [64]). While subsets of muscle founder cells express different "selector genes", all fusion competent myoblasts are specified by a single transcription factor, Lame duck (also know as Myoblast incompetent and Gleeful) [65–67]. During myoblast fusion, muscle founder cells attract fusion competent myoblasts, which migrate and extend filopodia toward founder cells, followed by adhesion and fusion between the two populations of cells. Analogous to vertebrate myogenesis, myoblast fusion in *Drosophila* also occurs in two phases. While the initial phase of fusion yields bi- or tri-nucleate muscle precursors, the second phase of fusion gives rise to multinucleate muscle fibers with distinct position, orientation and size [68].

Ultrastructural analyses have revealed fascinating details of *Drosophila* myoblast fusion [69]. Paired vesicles with electron dense margins are observed along the apposed membranes between founder and fusion competent myoblasts. Little is known about the origin of the vesicles, their biochemical composition and their function during myoblast fusion. These vesicles presumably resolve into elongated electron-dense plaques along the two membranes. Subsequently, small membrane discontinuities (fusion pores) form, which lead to the mixing of the cytoplasm and fusion of the two cells. It is worth noting that the presence of multiple fusion pores along apposing myoblast membranes is in contrast to the formation and expansion of a single fusion pore between yeast and *C. elegans* fusion

Table 1
Molecular players in fusion of various species and cell types

	Saccharomyces cerevisiae	*Caenorhabditis elegans*		*Drosophila melanogaster*	*Mus musculus*[a]	*Rattus norvegicus*/*Mus musculus*
Process	Mating	Epithelial development	Fertilization	Myogenesis		Osteoclast and giant cell formation
Cell/tissue type	MATa, MATα	Epidermis, vulva, pharynx, etc.	Sperm–egg	Muscle founder cells and fusion competent myoblasts	Myoblasts and myotubes	Monocyte/macrophage lineage
Surface receptor	Ste2, Ste3					DC-STAMP
Secreted factors	a and α factor				IL-4, Follistatin, $PGF_{2\alpha}$	RANKL, IL-4, MCP-1
Migration					Mannose receptor	
Adhesion	Aga1, Aga2, Sag1			Duf, Rst, Sns, Hbs		MFR/SIRPα, CD47, Mannose receptor, cadherins, CD9, CD81, P2X7, beta1 and beta2 integrins
Intracellular signaling, morphogenesis and vesicular transport	Gpa1, Ste5, Fus3, Far1, Cdc42, Tpm1, Fus1, Fus2, etc.	V-ATPase	TRP-3, SPE-38, SPE-9	Ants, Mbc, Loner, Arf6, Rac, Blow, Crk, Kette	Myoferlin mTOR NFATC2	CD44 V-ATPase Vo d2
Fusion	Prm1, Fus1	EFF-1				
Nuclei	Karyogamy	Polykaryon	Nuclear congression, breakdown, and mitosis	Polykaryon	Polykaryon	Polykaryon
Downregulation	Asg7					

[a]Only showing molecules implicated in the second phase of fusion between myoblasts and myotubes.

partners [17,33]. Nevertheless, the significance of these fusion intermediates (vesicle, plaque, pore) in *Drosophila* myoblast fusion is underscored by their absence in various fusion mutants [69].

Genetic and molecular studies in the last decade have yielded significant insights into the mechanisms underlying myoblast fusion in *Drosophila* (reviewed in [69,70]) (Fig. 3). An important early discovery is that recognition and adhesion between muscle founder cells and fusion competent myoblasts are mediated by immunoglobulin (Ig)-domain containing transmembrane proteins. In founder cells, two such proteins, Dumbfounded (Duf; Also known as Kirre) and Roughest (Rst; Also know as Irre-C), are both expressed and play redundant functions to attract fusion competent myoblasts [71,72]. Fusion competent myoblasts also express two Ig-domain containing transmembrane proteins, Sticks and stones (Sns) and Hibris (Hbs), with Sns required for fusion and Hbs modifying the activity of Sns [73–75]. Expression of full length or membrane-anchored forms of these transmembrane proteins in *Drosophila* cultured cells results in cell adhesion without membrane fusion, suggesting that these proteins are not sufficient to induce cell fusion in a heterologous system [76]. In contrast, expression of the membrane anchored Duf extracellular domain (Duf-TM-EC) in the developing mesoderm enables the first phase of fusion in *duf*, *rst* double mutant embryos, resulting in bi-nucleate muscle precursors [77]. It remains to be determined if the discrepancy between *in vivo* and cell culture studies is due to the specific spatial arrangement of myoblasts *in vivo*, or ectopic expression of a founder cell-specific adhesion molecule (Duf-TM-EC) in fusion competent myoblasts, or other unknown fusion regulator(s) specifically present *in vivo*.

While the extracellular domains of the fusion receptors are required for myoblast recognition and adhesion, the cytoplasmic domains of these proteins recruit multiprotein complexes to the membrane, in order to induce additional rounds of fusion and, eventually, generate multinucleate muscle fibers. In founder cells, the Duf receptor recruits an adaptor protein Antisocial (Ants; Also known as Rols7) to sites of fusion [78,79]. Ants contains several potential protein–protein interaction motifs, including ankyrin repeats, tetratricopeptide repeats (or TPRs) and a coiled-coil domain, making it a likely candidate as a scaffolding protein. In support of this, Ants interacts with a cytoskeleton-associated protein, Myoblast city (Mbc) [80], thus linking the Duf receptor with downstream signaling components [78]. Mbc is the *Drosophila* homologue of the mammalian protein, DOCK180, which was first identified as a major binding partner for the SH2/SH3 domain-containing adaptor protein Crk [81]. Although loss-of-function mutations in *Drosophila* Crk are not yet available, overexpressing a membrane-targeted form of Crk caused a fusion defect, implying a function of Crk during myoblast fusion [69]. However, recent studies show that the interaction between Mbc and Crk is not required to bring Mbc to sites of fusion, nor is it required for Mbc's function in vivo, suggesting that Crk may affect fusion by interacting with other proteins [82]. Interestingly, DOCK180 is an unconventional guanine nucleotide exchange factor (GEF) [83] for the small GTPase, Rac, which is an important regulator of the actin cytoskeleton and is also required for myoblast fusion [84,85]. Thus, the Duf → Ants → Mbc → Rac pathway is required in founder cells to transduce fusion signal from the membrane to the actin cytoskeleton.

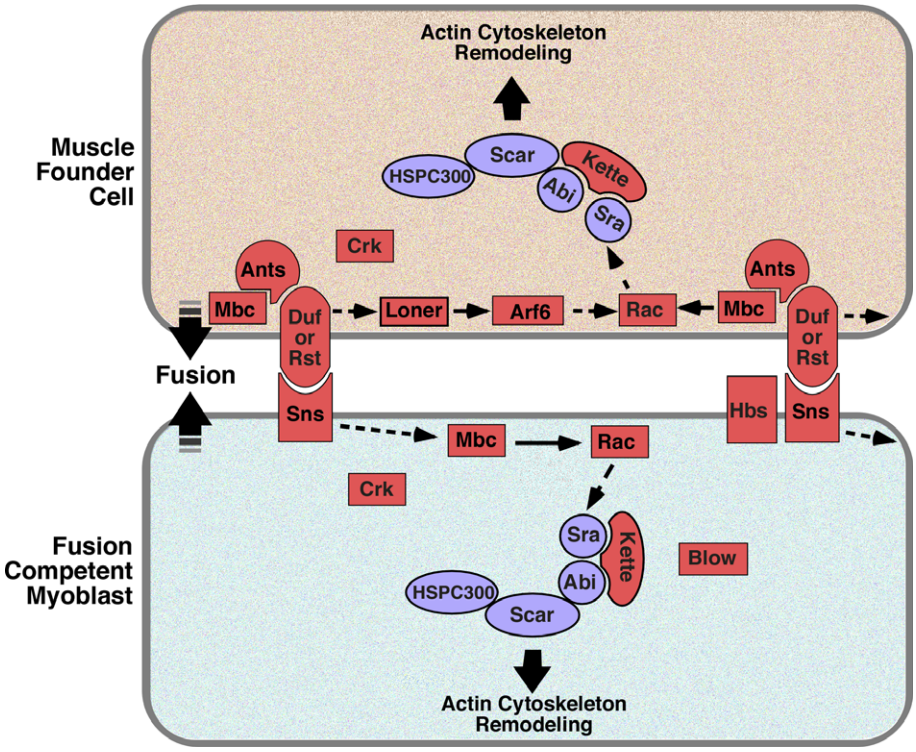

Fig. 3. A model describing signal transduction during myoblast fusion. Proteins that have been characterized in myoblast fusion are shown in red, and others are in purple. Solid arrows indicate demonstrated interactions and dashed ones indicate the existence of potential intermediary proteins.

How does Rac regulate the actin cytoskeleton during myoblast fusion? Previous studies have shown that Rac acts as a positive regulator of actin polymerization through a Wiskott-Aldrich syndrome protein (WASP) family member, WASP-family verprolin-homologues (WAVE) (also know as Scar in *Drosophila*) [86,87]. WAVE is present in a five-protein complex (WAVE complex) that include four other proteins, Nap1 (also know as Kette in *Drosophila*), Sra1/PIR121, Abi and HSPC300, each of which has been associated with cytoskeletal function [88]. The WAVE complex could be involved in WAVE localization or inhibition of WAVE's activity, the latter of which is antagonized by Rac activation [89]. Interestingly, one of the components of the WAVE complex, Kette, is also required for myoblast fusion [90] suggesting that Rac may act through the WAVE complex to regulate Scar activity during the fusion process. Kette has also been shown to genetically interact with a PH-domain containing protein, Blown fuse (Blow) which is necessary for fusion [69]. However, the specific function of Blow in myoblast fusion remains unknown.

Besides the Duf → Ants → Mbc → Rac → Scar pathway, Duf also recruits Loner, a Sec7 domain containing GEF for the small GTPase Arf6, through an unknown intermediary protein(s) to sites of fusion [91]. The Loner → Arf6 module is independent of the Ants → Mbc → Rac → Scar pathway, but is required for the proper localization of Rac [91]. Thus the two pathways converge at the small GTPase Rac and are both involved in transducing the fusion signal to the actin cytoskeleton. It is formally possible that the Loner → Arf6 module performs additional functions other than localizing Rac, given that Arf6 also plays a role in lipid modification and vesicle trafficking [92].

Less is known about the signal transduction events in fusion competent myoblasts. Several proteins that are required for fusion are expressed and/or required in both cell populations, including Mbc and Kette [82,90], suggesting that the actin cytoskeleton in fusion competent myoblasts also undergoes rearrangements during fusion. It remains to be determined if signal transduction from the membrane receptor Sns to the actin cytoskeleton is mediated by fusion competent cell-specific protein(s), as in founder cells.

It appears that all signaling events in myoblast fusion uncovered to date lead to remodeling of the actin cytoskeleton. Based on the cellular phenotypes of myoblasts in various fusion mutants, it is likely that the actin cytoskeleton may perform multiple functions during myoblast fusion. First, actin cytoskeletal rearrangement is likely to be involved in myoblast migration and filopodia formation. This is supported by the presence of a large number of round-shaped fusion competent myoblasts in deeper layers of embryos mutated for either *mbc* or *rac* [63,84]. Second, the actin cytoskeleton may play a role in a later step during myoblast fusion, after cell recognition and adhesion, since mutations in certain upstream regulators of the actin cytoskeleton do not affect myoblast attachment [78,79,91]. What is the precise function of the actin cytoskeleton following myoblast adhesion? Could it be involved in transporting fusion-related vesicles, if they are of exocytic origin, to sites of fusion? Could it serve as a scaffold to stabilize plasma membrane interactions? Or could it directly impact lipid mixing by producing mechanical strain, or even inducing/expanding breaks, on the lipid bilayers? Answers to these questions await future investigations that will provide unprecedented insights into the mechanisms of myoblast fusion in flies and in human.

5. Macrophage fusion in mammals

In contrast with most fusing cell types, which undergo fusion as a required part of their developmental program, macrophages fuse rarely and reside in tissues as mononucleate cells. Macrophages fuse in specific and rare instances to form new cells, which are osteoclasts and giant cells. This indicates that the fusion of macrophages is a tightly controlled event. Like most other fused cells, except for sperm–oocyte fusion and yeast mating, their nuclei keep their integrity within a shared cytoplasm.

Macrophages are mononucleate cells that belong to the myeloid lineage. They are ubiquitously present in tissues, in which they adjust to local tissue environment and physiology to secure homeostasis and repair. So macrophages "wear many hats" and are true "cells without borders", characterized by mobility, plasticity and adaptability. In some respects, macrophages appear as primitive nurturing cells that have maintained their "independence" and refined their "social support". While macrophages have long been regarded as the "tissue cleaners", they are now recognized as highly sophisticated entities, many of whose functions remain to be discovered. In specific and rare instances, macrophages are attracted to one another and fuse to form multinucleate osteoclasts in bone, or giant cells in chronic inflammatory sites. Macrophages have evolved a mechanism to augment their size, but why would macrophages increase their number of nuclei? Why cannot macrophages work together as a team without having to fuse? This is a question that is central to the evolution of the skeleton and of the innate/adaptive immune system, via osteoclasts and giant cells, respectively.

Multinucleation has two main effects on macrophages: it increases their size and, consequently, it endows them with the ability to resorb large components that cannot otherwise be internalized by a single cell. The number of nuclei that multinucleate macrophages contain appears to be proportional, hence adapted, to the size of the target/foreign body they resorb. Instead of internalizing a target, such as a bacterium, and routing it to lysosomes for degradation, multinucleation allows macrophages to degrade components extracellularly. Multinucleate macrophages attach firmly to their target via a sealing zone, a ring that seals off an extracellular compartment (reviewed by [93]). The content of this compartment has a low pH that facilitates the dissolution (e.g. bone), or killing (e.g. pathogens) of the target, and the activation of lysosomal enzymes. Hence it is considered an "extracellular lysosome". Multinucleation endows macrophages with an enhanced capacity, meaning that two macrophages cannot do what one binucleate macrophage does. Hence, multinucleate macrophages are more than the sum of their parts. This capacity is best illustrated by the vast array of genes that are differently regulated during osteoclastogenesis (reviewed by [94]). Indeed, multinucleation is an essential step in the differentiation of osteoclasts as mononucleate macrophages cannot resorb bone efficiently. This happens in diseases in which macrophages cannot fuse, such as in some forms of osteopetrosis where bones are thick and brittle.

As to the fate of osteoclasts, it is sad to say that their half-life is about three days, hence considerably shorter than that of monocytes and macrophages, which can be measured in months. Osteoclasts are therefore potent destroyers that have a full-blown life, but at a high price. However, the half-life of giant cells might be longer, if we assume that they remain alive within granulomas, which are long-lived entities.

It is now well accepted that bone marrow-derived macrophages and monocytes be activated by the cytokines RANKL to differentiate into osteoclasts, and IL-4 to form giant cells (reviewed by [95,96]). This is in addition to the growth factors M-CSF and GM-CSF, which secure their growth and their survival. It is interesting to note that IL-4 promotes the differentiation of both multinucleate giant cells and myoblasts, which occurs via an autocrine mechanism [56]. Surprisingly, IL-4 prevents the differentiation of multinucleate macrophages into osteoclasts, which suggests that IL-4 activates specific sets of genes that might be adapted to chronic inflammation [97]. Nevertheless, while RANKL is required for the formation of osteoclasts *in vivo*, the requirement for IL-4 to form giant cells *in vivo* remains open. In addition, the chemokine MCP-1, which promotes the migration of macrophages, stimulates the formation of both mouse giant cells *in vivo* and human osteoclasts *in vitro* [98,99].

Components of the putative machinery that mediates the fusion of macrophages were identified initially using monoclonal antibodies that both recognized cell surface determinants and altered fusion in tissue culture. The first protein identified by antibodies that blocked fusion of macrophages *in vitro* was designated macrophage fusion receptor (MFR), now called SIRPα [100–104] because of its structural resemblance to CD4, the cell surface receptor for HIV infection. Like CD4, MFR/SIRPα is a plasma membrane protein that belongs to the superfamily of immunoglobulins (IgSF) and contains three extracellular Ig loops. Subsequently, MFR/SIRPα was shown to bind CD47, which also belongs to the IgSF, and the recombinant soluble extracellular domains of both MFR/SIRPα and CD47 were reported to block fusion in culture. While CD47 expression is ubiquitous, that of MFR is restricted to myeloid cells and neurons. In addition, MFR/SIRPα expression is strongly but transiently induced at the onset of fusion in macrophages while that of CD47 remains constant, further suggesting that fusion is a regulated event. CD47 contains one extracellular Ig variable domain (IgV) followed by five predicted transmembrane segments terminating in a cytoplasmic tail. MFR/SIRPα contains one extracellular amino-terminal IgV domain and two adjacent immunoglobulin constant (IgC1) domains. A lower-molecular-weight form of MFR/SIRPα (MFR-s) lacks the C1 domains and contains only the V domain (Fig. 4). The IgV loop of CD47 binds to the IgV domain of both forms of MFR/SIRPα, an interaction that is likely blocked by monoclonal antibodies to MFR/SIRPα. An interesting hypothesis is that during fusion, CD47 binds first to the long form of MFR/SIRPα, to secure the recognition/attachment of macrophages, and then switches to the short form of MFR to bring apposed plasma membranes closer to one another. Upon binding of CD47 to the short form of MFR/SIRPα and possible bending of CD47-MFR's IgV domains, the distance between the plasma membranes of adjacent cells could be reduced to 5–10 nm, which might increase the probability of spontaneous fusion.

Another interesting hypothesis is that CD47, which is related to proteins expressed by Vaccinia and Variola viruses [105], promotes Ca^{2+} entry into fusing partners, like A38L does, possibly by forming a pore [106]. Indeed, as discussed earlier, pore formation is used by yeast cells to mate and myoblasts to form multinucleate muscle cells [17,69]. Likewise, the overexpression

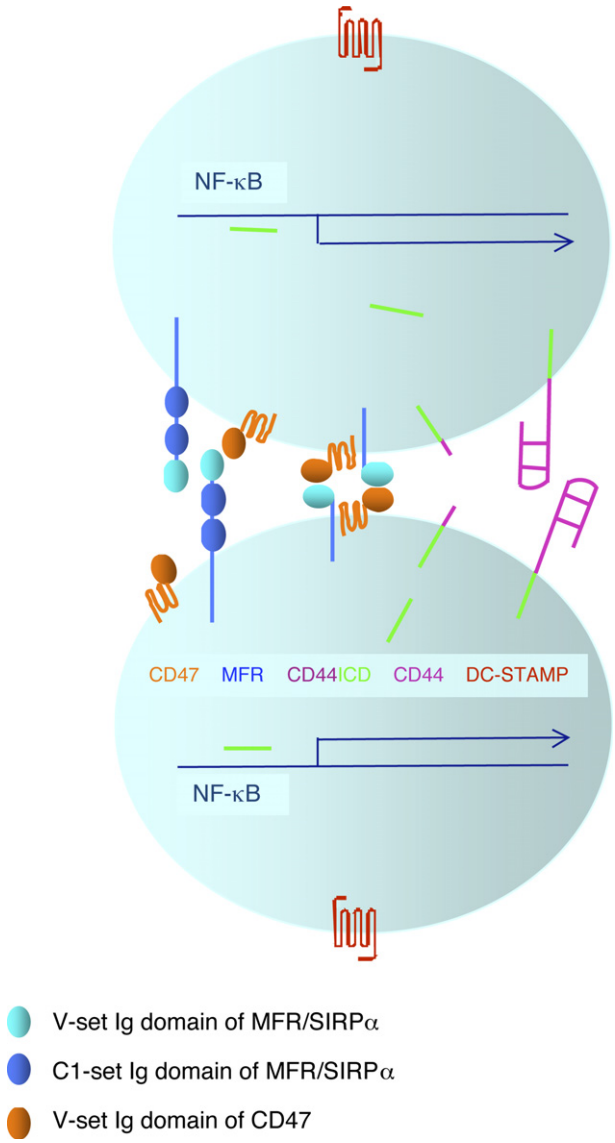

Fig. 4. Hypothetical mechanism for fusion of macrophages. Macrophage–macrophage recognition/adhesion is achieved by binding of MFR/SIRPα to CD47. The stepwise association of the long form of MFR and then the short form of MFR (MFR-s) with CD47 reduces the distance between the plasma membranes. The shedding of the extracellular domain of MFR might facilitate this association (Cui and Vignery, unpublished observation). The distance between macrophage plasma membranes could be reduced to 5–10 nm if MFR-s and CD47 bend upon binding. Meanwhile, the extracellular domain of CD44 also sheds, further facilitating plasma membranes from opposite cells to get closer, and fuse. In addition, the intracellular domain of CD44 is cleaved by a gamma secretase complex and translocates to the nucleus to promote the activation of NF-κB. NF-κB is a transcription factor that is indispensable for osteoclastogenesis. DC-STAMP, upon activation by its (unknown) ligand, regulates fusion.

of CD47 or A38L leads to cell death [107]. This raises the possibility that once the membranes from opposite cells are closely apposed and stable, CD47 molecules may create a pore that triggers cell–cell fusion. Although this last possibility is highly speculative, it opens an interesting avenue of research. In support of the MFR/SIRPα-CD47 hypothesis, a recent report indicates that osteoclast formation is strongly reduced in the absence of CD47-MFR/SIRPα-interaction [108].

Similarly to MFR/SIRPα, the expression of CD44, the receptor for hyaluronan, is strongly and transiently induced at the onset of macrophage fusion, which suggests a role in fusion [109]. While no cell surface ligand for CD44 has been identified, the fate of CD44 during the fusion of macrophages has recently been elucidated. It was known that the extracellular domain of CD44, which is cleaved by MT1-MMP, sheds from the plasma membrane. The extracellular domain of CD44 shedding from the plasma membrane might allow opposite macrophage plasma membranes to entertain a closer interaction, and to facilitate their fusion [110]. In addition, following the shedding of the extracellular domain of CD44, its intracellular domain is cleaved by presenilin, which belongs to a large enzymatic complex called ''gamma secretase''. CD44 intracellular domain translocates to the nucleus to promote the activation of the transcription factor NF-κB, which is required for the differentiation of osteoclasts. Interestingly, the extracellular domain of MFR/SIRPα also sheds from the plasma membrane of macrophages during fusion (Cui and Vignery, unpublished observation).

Additional cell surface molecules that might also play a role in macrophage attachment leading to fusion include CD9 and CD81, which, like *C. elegans* SPE-38, are tetraspan membrane proteins [111]. Cadherin and the purigenic receptor P2X7 appear to facilitate the fusion of macrophages into osteoclasts and giant cells, respectively [112,113], although P2X7 receptor knockout mice show normal osteoclasts [114]. Inhibitors of mannose receptor expression prevent macrophage fusion *in vitro* [115], and beta1 and beta2 integrins mediate the adhesion of macrophages at the onset of fusion [116]. Although each one of these molecules might participate at some level in cell–cell recognition and/or attachment, none is required for fusion.

The most dramatic observation has been DC-STAMP, which was reported recently to be required for the fusion of macrophages [117]. Mice that lack DC-STAMP lack multinucleate osteoclasts and giant cells, and develop a mild form of osteopetrosis. Because DC-STAMP is a seven-transmembrane receptor, it is reminiscent of CXCR4, the co-receptor for HIV that is required for fusion, and of yeast Ste2 and Ste3, G-protein coupled receptors responsible for the initiation of fusion. Ligation of DC-STAMP, by a yet unknown ligand, might regulate rather than mediate fusion. While a larger number of unknowns surround DC-STAMP, the question of whether DC-STAMP interacts with, or regulates the expression of MFR/SIRPα-CD47 and CD44 remains open.

Most recently, mice that lack the V-ATPase Vo subunit d2 were reported to exhibit impaired osteoclast fusion [118]. Hence, in contrast with *C.elegans*, the V-ATPase favors fusion in macrophages such that mice that lack V-ATPase Vo subunit d2 develop a mild form of osteopetrosis.

While the question of whether macrophages fuse with somatic cells for repair, and cancer cells for metastasis has been recently discussed, and remains open [103,119], the actual molecular mechanics of macrophage fusion remain poorly understood. Indeed, unlike viruses, which often contain one protein in their coat, plasma membranes from cells are rich in proteins, integral and membrane-associated, which are themselves modified post-translationally and decorated by lipids and sugar moieties. The level of complexity of plasma membrane proteins, complicated by their intracellular domain, which transduces signals downstream, suggests that the cell–cell fusion machinery is more

complex than originally anticipated and that its members might act in a sequential manner to secure fusion. The need for cell–cell recognition, then attachment, and finally fusion, in addition to regulatory mechanisms like DC-STAMP, leads us to believe that we are at the very beginning of understanding the mechanics of macrophage fusion.

6. Conclusion

Cell–cell fusion emerged as a new field of research subsequent to major advances in our understanding of the molecular mechanisms of membrane fusion. It has become clear that fusion of viruses with host cells and of intracellular membranes during trafficking is mediated by a set of proteins that are specific to each type of fusing cell and each type of membrane, respectively. However, the molecular mechanisms that mediate cell–cell fusion remain largely unknown, and the fusion machinery remains to be characterized. Nonetheless, some common aspects have become apparent as cells must follow a well-ordered ritual in order to fuse. First, cells that are destined to fuse send off signals to enter a "prefusion" state. These prefusion signals can be reciprocal, like the ***a***- and α- mating pheromones in yeast, or asymmetric, as in the case of DC-STAMP in macrophages. Next, adhesive interactions form between the plasma membranes of the two fusion partners. In yeast, membrane contact is promoted by turgor pressure within a pair of cells held in place by a common cell wall. In *Drosophila* myoblast fusion and mammalian macrophage fusion, members of the IgSF mediate adhesion and also initiate extensive intracellular signaling events. Ultimately, fusion partners actively engage in interactions via specialized transmembrane proteins. These interactions can be homotypic and directly trigger fusion, exemplified by the *C. elegans* fusogen EFF-1, or, as in the case of yeast Prm-1, they can stabilize the apposing membranes as they fuse.

We anticipate that future studies will uncover new factors that participate in various cell–cell fusion processes and lead to a richer understanding of membrane fusion mechanisms. This work promises to provide new insights into diseases, such as osteopetrosis, in which normal cell fusion is disrupted, and lead to potential treatment of degenerative diseases like muscular dystrophy through myoblast fusion-based cell therapy.

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