Unveiling the Mechanisms of Cell-Cell Fusion

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Cell-cell fusion is fundamental to the development and physiology of multicellular organisms, but little is known of its mechanistic underpinnings. Recent studies have revealed that many proteins involved in cell-cell fusion are also required for seemingly unrelated cellular processes such as phagocytosis, cell migration, axon growth, and synaptogenesis. We review advances in understanding cell-cell fusion by contrasting it with virus-cell and intracellular vesicle fusion. We also consider how proteins involved in general aspects of membrane dynamics have been co-opted to control fusion of diverse cell types by coupling with specialized proteins involved in cell-cell recognition, adhesion, and signaling.

The advent of membranes during evolution heralded the appearance of life itself by providing the basic unit of cellular structure and allowing the compartmentalization of metabolites, ions, organelles, and genetic material. Inherent in the organization of phospholipid bilayers is the necessity of maintaining membrane integrity so as to prevent promiscuous membrane fusion. Conversely, the ordered fusion of intracellular membranes is essential for basic cellular functions, and the temporally and spatially regulated fusion of intercellular membranes is required for the formation of multicellular organisms.

Interest in cell-cell fusion was initially stimulated decades ago by the discovery that somatic cells can be induced to fuse by viruses in vitro (*1*). Since then, virus- or chemical-induced cell-cell fusion has become a powerful tool for analysis of gene expression, chromosomal mapping, antibody production, and cancer immunotherapy. The importance of cell-cell fusion during development and disease is underscored by its involvement in a wide range of biological processes, including fertilization; the development of muscle, bone, and placenta; the immune response; tumorigenesis; and aspects of stem cell–mediated tissue regeneration (*2*–*10*). In spite of the diversity of cell types that undergo fusion, the underlying cellular processes, including cell-cell adhesion, alignment, and membrane mixing, are similar irrespective of the cell type. These observations suggest that different cell-cell fusion events may share common mechanisms.

Despite the importance of cell-cell fusion in the development and physiology of multicellular organisms, little is known about the mechanisms underlying this process. How do cells destined to do so recognize and fuse with each other? What determines the specificity of different cell-cell fusion events? What are the minimal requirements for two cells to fuse? Is there a specific set of membrane proteins dedicated to the process of cell-cell fusion, or do the effectors of the process also perform other cellular functions? Does cell-cell fusion involve the same types of mechanisms as other membrane fusion events, such as intracellular vesicle fusion and virus-cell fusion? In this Review, we discuss recent insights into the process of cell-cell fusion. We propose that this type of membrane fusion employs mechanisms distinct from those involved in other membrane fusion events and that the biochemical machinery for cell-cell fusion plays multiple roles in the control of membrane dynamics and cytoskeletal organization as a consequence of its coupling to different upstream and downstream effectors.

Dependence of Virus Cell and Intracellular Vesicle Fusion on α-Helical Bundles

To understand the mechanisms for cell-cell fusion, it is instructive to consider virus-cell fusion and intracellular vesicle fusion, which have been extensively studied. Enveloped viruses use transmembrane viral proteins to mediate fusion with host cell membranes (*11*–*14*). Class I viral fusion proteins, such as the influenza hemagglutinin (HA) and human immunodeficiency virus type 1 (HIV-1) envelope protein (Env), contain a hydrophobic fusion peptide that is normally buried within the molecule. In response to the low pH environment in the endosome (for HA) or binding to its receptor at the cell surface (for Env), both proteins are proteolytically cleaved such that their hydrophobic fusion peptide is exposed and inserted into the target membranes (*11*, *13*, *15*). Concomitantly, the fusion protein undergoes a conformational change in which two well-separated α-helices fold upon each other to form a hairpin-like α-helical bundle, thereby bringing the viral and cell membranes into close proximity and allowing for membrane fusion (*11*–*13*) (Fig. 1A). Class II viral fusion proteins use a different mechanism, which we will discuss later.

Intracellular vesicle fusion that occurs in the secretory and endocytic pathways depends on a similar α-helical bundle structure to bring membranes together (*11*–*13*). After recognition of vesicle and target membranes by the Rab guanosine triphosphatases (GTPases) and their effectors, the SNARE family of membrane proteins, initiates membrane juxtaposition and fusion in a manner similar to class I viral fusion proteins (*12*, *13*). Specifically, vesicle-anchored v-SNAREs and target-anchored t-SNARES interact to form a bundle of α helices (the SNAREpin) that brings apposing membranes together and promotes their fusion (*16*) (Fig. 1B).

The observation that intracellular protein SNAREs use similar α-helical bundles as class I viral fusion proteins raises the question of whether such structural intermediates could be a common feature of all cellular fusogens, including those mediating cell-cell fusion. Indeed, although dispensable for cell-cell fusion in vivo, flipped SNAREs are capable of inducing cell-cell fusion in cultured cells (*17*), and viral fusogens are potent inducers of cell-cell fusion in vitro and in vivo. However, our analyses of different types of cell-cell fusion events suggest that intercellular fusion might use mechanisms distinct from those underlying other types of membrane fusion events.

An Overview of Cell-Cell Fusion

Cell-cell fusion is a widespread phenomenon in organisms ranging from yeast to humans. A number of cell-cell fusion events have been studied in varying detail, including yeast mating, epidermal cell fusion in *Caenorhabditis elegans*, myoblast fusion, fertilization, trophoblast fusion in the placenta, macrophage fusion, and stem cell fusion. Although much remains to be learned about the underlying molecular mechanisms, the following sections provide an overview of our current understanding of these cell-cell fusion events.

Yeast fusion. In *Saccharomyces cerevisiae*, the mating of a- and α-type cells involves cell

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cycle arrest, cell wall degradation, and polarized cell growth, followed by membrane juxtaposition and fusion (*18*) (Fig. 2A). Despite extensive genetic screens for mating-defective mutants in yeast, no mutants have been found in which the membrane fusion process is directly disrupted, perhaps partly because of functional redundancy. To circumvent this problem, Heiman and Walter conducted a bioinformatic screen in search of previously uncharacterized pheromone-regulated membrane proteins (Prms), with the assumption that such proteins might serve as fusogens for pheromone-induced yeast fusion. Phenotypic analyses suggest that one of the Prms, Prm1p, might function in membrane fusion, because *prm1* mutant cells show normal membrane juxtaposition without fusion (*19*). However, it is not clear whether Prm1 is the long-sought fusogen for yeast fusion, because only 50% of *prm1* mutant cells are defective in mating (*19*). In addition, Prm1p lacks hydrophobic fusion peptides or coiled-coil domains capable of forming α-helical bundles, which characterize the SNAREs and class I viral fusion proteins. Thus, if Prm1 or a related protein is a fusogen for yeast mating, its molecular mechanisms are likely to be distinct from those employed by SNAREs and class I viral fusogens.

Epidermal cell fusion in C. elegans. The nematode *C. elegans* provides a unique system to study cell-cell fusion, because about a third of its 959 somatic cells fuse to form 44 multinucleated syncytia. Fusion occurs throughout development of the nematode and is required for the formation of multiple organs (*20*, *21*). Among them, the fusion of epidermal cells has been most well characterized (Fig. 2B). Genetic screens for fusion-defective mutants have identified a potential fusion gene named *epithelial fusion failure 1* (*eff-1*) (*22*), which is required for epidermal cell-cell fusion and can induce ectopic fusion in certain other tissues (*23*, *24*). *eff-1* encodes a protein containing a transmembrane domain and an extracellular hydrophobic peptide (EHP) that is required for fusion (*22*, *24*). Unlike viral fusion peptides, which are thought to be involved in driving fusion-pore formation after virus-host binding, the EHP has been shown to be required in the localization of EFF-1 to sites of cell-cell contact before the pore-forming reaction (*24*). The functional difference between the EHP and the viral fusion peptides, together with the lack of coiled-coil domains in EFF-1, suggest that the putative fusogen EFF-1 is likely to use a different mechanism to mediate fusion than that of class I viral fusogens and SNAREs.

Myoblast fusion in Drosophila. Fusion of mononucleated myoblasts to form multinucleated muscle fibers is an essential step in skeletal muscle differentiation. *Drosophila* embryos contain two populations of myoblasts that are destined to fuse: founder cells that serve as ''seeds'' for future muscle fibers and fusion-competent cells that are attracted to and fuse with founder cells (*2*, *3*). Genetic studies in *Drosophila* have identified two classes of proteins that are required for myoblast fusion. One class includes immunoglobulin (Ig) domain–containing transmembrane proteins, such as Dumbfounded (Duf, also known as Kirre), Roughest (Rst, also known as Irrec), Sticks and stones (Sns), and Hibris (Hbs) (*25*–*29*). Although Duf and Rst are required redundantly for myoblast fusion in founder cells, Sns and Hbs are specifically expressed—and in the case of Sns, required—in fusion-competent cells (Fig. 2C). These cell surface receptors are thought to mediate recognition and adhesion of the two types of muscle cells through direct interactions, as demonstrated for Duf and Sns (*30*). Notably, none of these cell surface proteins contains a hydrophobic fusion peptide or coiled-coil domain capable of forming α-helical bundles, as seen in SNAREs and class I viral fusion proteins. A number of intracellular signaling proteins have also been found to control myoblast fusion in *Drosophila*. These include the small GTPases, Drac and ARF6; their guanine nucleotide exchange factors, Myoblast city (Mbc) and Loner; and the adaptor protein Antisocial (Ants, also known as Rols7), which links the fusion receptor Duf to Mbc (*31*–*37*). It is unclear at present whether these intracellular proteins solely function in cytoskeleton remodeling, which is required for extensive alignment of apposing membranes, or if they are also directly involved in destabilization of the lipid bilayers during the fusion process.

Mammalian fertilization. Fertilization is perhaps the most well-known form of cell-cell fusion. As in yeast mating, membrane fusion between sperm and egg occurs after a series of prefusion events, including penetration of the outer layer of the oocyte by sperm, secretion of enzymes by the lysosome-like acrosome in the sperm head, and penetration of the egg's inner layer, the zona pellucida, by the sperm. Only after entry of the sperm into the egg's perivitelline space does fusion occur between the sperm and egg plasma membranes.

Historically, a number of proteins have been hypothesized to mediate membrane fusion during fertilization. Most prominent among these are A Disintegrin and Metalloprotease (ADAM) family transmembrane proteins on the sperm, such as fertilin α, fertilin β, and cyritestin, and integrins on the egg surface (*6*). However, genetic studies in mice have demonstrated that these proteins are dispensable for membrane fusion (*38*, *39*). To date, the only protein that has been shown to be required for sperm-egg fusion is CD9, a tetraspanin family protein on the egg surface containing four transmembrane domains (*6*, *40*) (Fig. 2D). CD9 mutant eggs bind to sperm normally, but are defective in

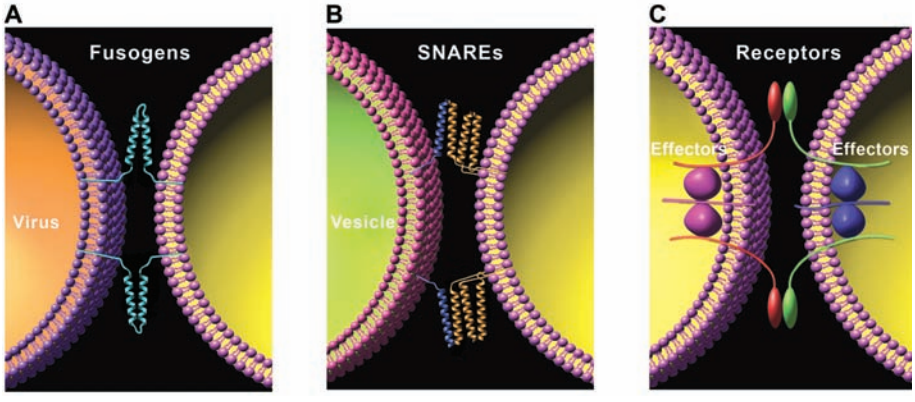

Fig. 1. Models of membrane fusion. (**A**) Enveloped viruses use a single fusion protein to mediate all steps of membrane fusion. This schematic shows the action of the influenza HA upon activation by low pH. The trimeric HA is simplified as a single polypeptide. HA contains a hydrophobic fusion peptide that is inserted into the target membrane. The HA protein forms a hairpin-like structure, in which coiled α helices near the viral membrane fold back and pair with another α helix adjacent to the fusion peptide. Thus, the hairpins bring the viral and cell membranes into close proximity, allowing the initiation of lipid mixing and fusion. (**B**) Fusion between an intracellular vesicle and the target membrane is mediated by SNAREs, which are membrane-embedded receptors localized on the vesicle and target membranes. SNAREs form a core complex that contains a stable coil of four α helices (the SNAREpin). The SNAREpins bring the apposing lipid bilayers together, allowing membrane fusion. (**C**) A two-component system may be used to mediate some cell-cell fusion events. The first component consists of transmembrane receptors (red and green). Their extracellular domains mediate close juxtaposition of the two cell membranes, whereas their cytoplasmic domains organize multiprotein complexes in the two adhering cells. The second component consists of the multiprotein fusion complexes (purple and blue). Protein(s) in the fusion complex (cytoplasmic or membrane proteins) function to destabilize the lipid bilayer, leading to the formation of fusion pores and cytoplasmic mixing.

sperm-egg membrane fusion (*41*–*43*). It remains unclear how CD9, which lacks any hydrophobic fusion peptide or coiled-coil domains, exerts its function during fusion. Because tetraspanin proteins often form multiprotein complexes in the cell membrane (*44*), CD9 could potentially mediate fusion by organizing a multiprotein fusion complex at the site of sperm-egg binding.

Fusion of placenta trophoblasts. In the mammalian placenta, trophoblasts fuse to form a syncytial layer of cells (the syncytiotrophoblast) that functions as a barrier between maternal and fetal blood vessels (*5*). Little is known about the proteins required for trophoblast fusion except for syncytin, a single-pass transmembrane protein that can induce ectopic cell-cell fusion in transfected cells (*45*, *46*) (Fig. 2E). Syncytin is nearly identical to the envelope protein of the human endogenous retrovirus HERV-W, prompting the hypothesis that placental trophoblasts may use a captured viral protein for fusion (*45*). Syncytin functions as a class I fusion protein like HA. However, this mechanism of trophoblast fusion is unlikely to be universal, because syncytin is only present in primates and not in other mammals in which placental trophoblasts also undergo cell fusion to form the layer of syncytiotrophoblasts (*47*).

Macrophage fusion: Osteoclasts and giant cells. Macrophages can differentiate and fuse to form two types of multinucleated cells, osteoclasts and giant cells, that are important for bone resorption and the immune response, respectively (*7*, *8*). Several studies using in vitro cell-cell fusion assays have implicated transmembrane proteins in macrophage fusion (*8*) (Fig. 2F), including CD44, macrophage fusion receptor (MFR), and CD47. Among them, MFR and CD47 both contain Ig domains and act as a receptor-ligand pair on apposing cells to effect macrophage fusion. Thus, MRF and CD47 appear to function in a manner analogous to that of cell surface proteins such as Duf and Sns in *Drosophila* myoblast fusion.

Stem cell fusion. Cell-cell fusion has emerged as an unexpected and complicating mechanism in tissue regeneration by stem cells (*9*, *48*, *49*). Classical experiments demonstrated the potential for genetic reprogramming in heterokaryons under experimental conditions (*50*), but it was not appreciated until recently that this type of cell-cell fusion could occur in vivo (*10*). Circulating hematopoietic stem cells (HSCs) have been shown to fuse with a wide variety of target cells, including cardiac myocytes, hepatocytes, Purkinje cells, and oligodendrocytes, with consequent modification of the gene expression profile of the stem cell (*9*, *48*, *49*) (Fig. 2G). Relatively little is known about the mechanisms of stem cell fusion. It is also unclear whether this is a regulated process or a "random" event that occurs at low frequency or whether it is the HSCs themselves or their descendents that actually fuse with the target cells. Studies of liver regeneration suggest that macrophages derived from HSCs, instead of HSCs themselves, have the capacity to fuse with hepatocytes (*51*–*54*), although these studies cannot formally exclude the possibility of transdetermination of HSCs. If macrophages are indeed the source of hepatocyte fusion partners, it is tempting to speculate that proteins involved in macrophage fusion might also function in stem cell–based fusion, at least in the context of liver regeneration.

Insights from Studies of Cell-Cell Fusion

Cell-cell fusion does not appear to be mediated by α-helical bundles. Despite their seemingly different biological functions, the intracellular fusogen SNAREs and class I viral fusion proteins both use an α-helical bundle structure to promote membrane juxtaposition and fusion. Could other cellular fusogens, such as those governing cell-cell fusion, adopt a similar mechanism to bring lipid bilayers together? So far, this does not appear to be the case. With the exception of syncytin, a retroviral envelope protein that might have been captured by the human genome, none of the cell surface proteins identified so far in the various cell-cell fusion processes resemble SNAREs or class I viral fusion proteins. Although it is formally possible that such proteins are yet to be identified, it seems more likely that cell-cell fusion uses different mechanisms. Fusion of class II viruses with host cells does not involve an α-helical bundle conformation (*55*), which supports the idea that such a conformation is not an absolute requirement for membrane fusion.

An unexpected aspect of *Drosophila* myoblast fusion is that intracellular signaling proteins, which are recruited to the sites of fusion by and function downstream of the cell surface receptors, are required for fusion (*2*, *3*). We speculate that other cell-cell fusion events may involve a similar intracellular signaling input. Such cell-cell fusion events might use a "bipartite" fusion system rather

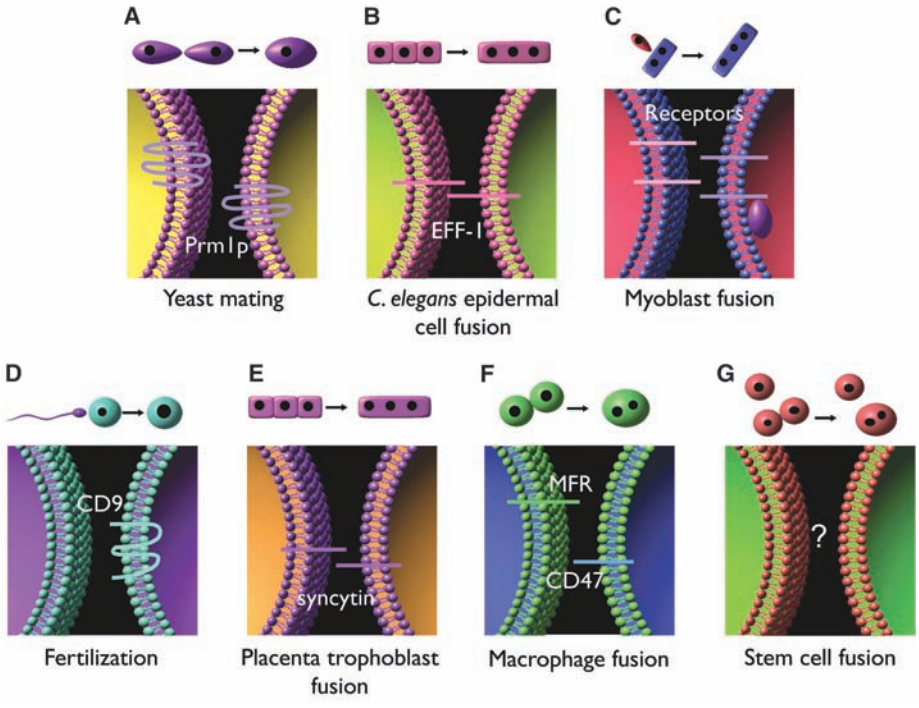

Fig. 2. Simplified versions of various types of cell-cell fusion events. (**A**) Membrane fusion between yeast a and α cells. A five-pass transmembrane protein, Prm1p, functions in both types of cells and is partially required for fusion. (**B**) Epidermal cell fusion in *C. elegans*. A putative single-pass transmembrane protein, EFF-1, is necessary and sufficient for fusion of this type of cell. (**C**) Myoblast fusion in *Drosophila*. Ig domain–containing proteins are localized in founder (purple) and fusion-competent (pink) cells. The purple oval in the founder cell represents the multiprotein complex organized by the fusion receptor. Such a complex has yet to be identified in fusion-competent cells. (**D**) Sperm-egg fusion during mammalian fertilization. A tetraspanin on the egg surface, CD9, is required for the membrane fusion process. (**E**) Trophoblast fusion during syncytiotrophoblast formation in the placenta. Syncytin, the envelope protein of a human retrovirus, is proposed to mediate trophoblast fusion. (**F**) Macrophage fusion to form osteoclasts in the bone or giant cells during immune response. Ig domain–containing proteins are implicated, including a receptor-ligand pair of MFR and CD47. (**G**) Stem cell fusion. Proteins mediating this type of fusion are completely unknown.

than a single fusogen such as viral fusion proteins (Fig. 1C). We propose that fusion receptors such as Duf and Sns not only function in tethering cells together for fusion but also contribute to bringing the apposing membranes into close proximity, in a manner distinct from that of α-helical fusogenic proteins. In a subsequent step, these receptors recruit intracellular proteins such as Ants and Loner to form multiprotein complexes at the sites of fusion. These multiprotein complexes in turn destabilize the lipid bilayer either directly or indirectly by modifying the activity of additional membrane proteins, thus leading to the formation of fusion pores and fusion of the two apposing cells.

The actin cytoskeleton and cell-cell fusion. Several intracellular proteins involved in *Drosophila* myoblast fusion have well-established roles in regulating the actin cytoskeleton. These include Drac, which controls actin polymerization; Mbc, an upstream regulator of Drac; and Kette, a regulator of Wiskott-Aldrich syndrome protein (WASP)–dependent actin cytoskeleton rearrangement (*31*, *36*, *37*, *56*). Constitutively active Drac or a loss of function in *kette* blocks the formation of fusion pores, a late step during myoblast fusion, without affecting early stages of the fusion process (*56*, *57*). These studies raise the issue of the role of the actin cytoskeleton in the formation of fusion pores. The actin cytoskeleton could be involved in transporting essential proteins to sites of fusion, or perhaps it serves as a scaffold to stabilize membrane-membrane interactions. Alternatively, or in addition, the cytoskeleton might directly affect lipid mixing by producing mechanical strain on the lipid bilayer.

We speculate that the actin cytoskeleton could be a general requirement for cell-cell fusion. Furthermore, the cytoskeleton might play a widespread role in membrane fusion events beyond cell-cell fusion. For example, virus-induced cell-cell fusion is blocked when the actin cytoskeleton of the host cells is perturbed by expressing a dominant negative Rac GTPase (*58*). In addition, both the actin- and microtubule-based cytoskeleton are implicated in intracellular vesicle fusion because they participate in transporting vesicles from their sites of synthesis to the sites of membrane fusion (*59*).

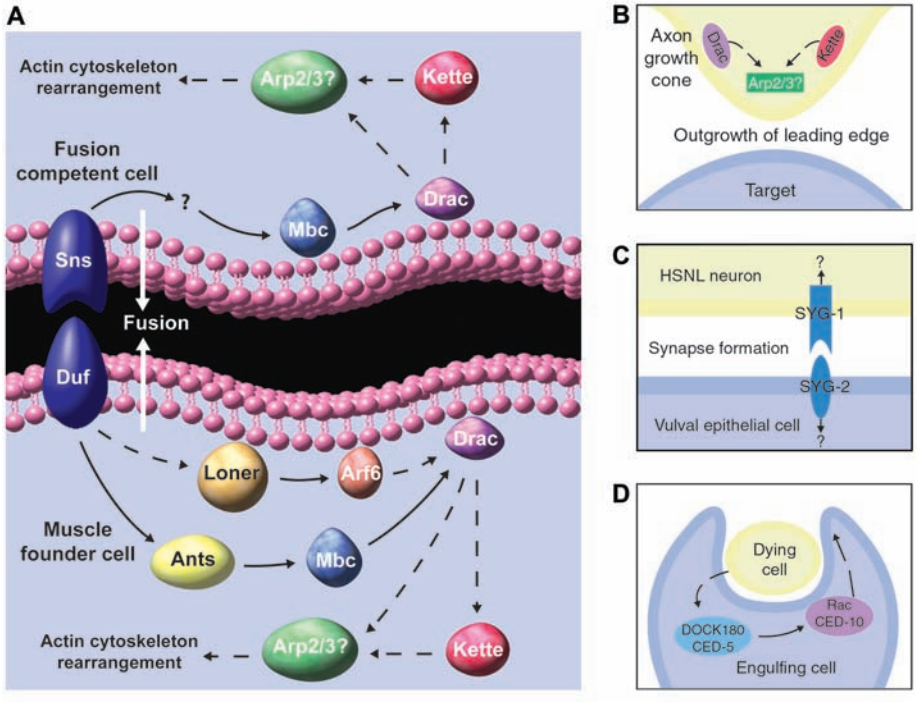

Fig. 3. Components of cell-cell fusion events are implicated in other fusion-independent cellular processes. (**A**) Major components in *Drosophila* myoblast fusion. Transmembrane receptors (dark blue) mediate the juxtaposition of the plasma membranes of a founder cell and a fusion-competent cell. For simplicity, only Duf in the founder cell and Sns in the fusion-competent cell are shown. Two independent signaling pathways, Ants → Mbc → Drac and Loner → ARF6 → Drac, are required to transduce the fusion signal from the membrane receptor to the cytoskeleton. Kette may function downstream or in parallel with Drac to regulate actin cytoskeleton rearrangement, perhaps through the Arp2/3 complex. (**B**) Drac and Kette may function through Arp2/3 to regulate neurite outgrowth and axon pathfinding during nervous system development. (**C**) The *C. elegans* homologs of Duf and Sns, SYG-1 and SYG-2, are required for proper synapse formation between the HSNL neuron and vulval epithelial cells. (**D**) The mammalian and *C. elegans* Mbc and Drac homologs, DOCK180 (CED-5 in *C. elegans*) and Rac (CED-10 in *C. elegans*), are required for phagocytes to engulf dying cells. Solid arrows indicate direct protein interactions.

The specificity and evolution of cell-cell fusion. Studies of various cell-cell fusion processes have identified specialized proteins that are required for specific fusion events. For example, syncytin, a protein involved in trophoblast fusion, differs from EFF-1, the putative fusogen in *C. elegans* epidermal fusion. Furthermore, the function of these fusion proteins is limited to certain species. For example, syncytin is only found in primates and EFF-1 is only present in several closely related nematode species (*22*, *45*, *46*).

The existence of species- and cell type–specific fusion proteins does not exclude a possible involvement of common molecular machinery for fusion. For example, Ig domain–containing proteins are involved in both myoblast and macrophage fusion, suggesting that this type of cell surface protein might have a more general function in multiple fusion events. It is also conceivable that intracellular proteins regulating the actin cytoskeleton might be involved in cell-cell fusion events beyond myoblast fusion.

Cell-cell fusion and other fusion-independent cellular processes. Many components of cell-cell fusion processes have been implicated in other aspects of membrane or cytoskeletal reorganization (Fig. 3). For example, besides their function in myoblast fusion, Drac and Kette are required for axonal growth and guidance by modulating the activity of downstream actin-regulatory proteins (*60*, *61*). The *C. elegans* homologs of Duf and Sns, SYG-1 and SYG-2, are required in certain motor neurons for correct synapse formation (*62*, *63*). Homologs of Mbc and Drac in mammals and *C. elegans* are required for phagocytosis and cell migration, which require extensive cytoskeletal rearrangements (*37*, *64*). Thus, proteins involved in cell-cell fusion are likely to play pleiotropic roles in other cellular processes involving changes in membrane or cytoskeletal structures, with specificity being achieved through the coupling of these proteins to different upstream and downstream effectors. These specific effectors, in turn, determine whether a cell will become part of a syncytium or remain solitary. For example, in cells destined to fuse, the upstream membrane receptors mediate recognition and adhesion of plasma membranes between specific populations of cells, whereas the downstream intracellular proteins transduce fusion signals to the cytoskeleton. The absence of any component in the pathway results in a failure of fusion. Therefore, cell-cell fusion is delicately controlled by a cascade of specific effectors, which ensures the successful fusion between proper partners and protects cells from aberrant fusion.

Cell-cell fusion and human disease. Given the indispensable role of cell-cell fusion in development, it is expected that abnormalities of cell-cell fusion might contribute to certain

human diseases. Indeed, defects in sperm-egg fusion represent a major cause of infertility. Certain muscle diseases, such as centronuclear myopathy and myotonic dystrophy, characterized by minute myofibers, may be due partly to defects in myoblast fusion (*65*, *66*). Furthermore, defects of placental trophoblast fusion result in pregnancy complications such as preeclampsia (*67*), and defects in osteoclast fusion can cause bone abnormalities such as osteopetrosis (*68*).

Although failure of cell-cell fusion can contribute to human pathology, unregulated cell-cell fusion may also promote diseases, especially cancer (*69*). Many tumor cells, for unknown reasons, are particularly fusogenic. Fusion between tumor and normal somatic cells generates hybrid cells that are often more malignant than parental cells, perhaps because of their increased growth rate, resistance to drugs and apoptosis, or ability to metastasize (*70*–*72*). Moreover, these hybrids may facilitate the production of a diversity of malignant cell types (*73*). To some extent, tumor-somatic cell fusion is similar to stem cell fusion, in that both types of fusion result in diverse progeny in various tissues (*9*, *48*, *69*). The analogy between tumor and stem cell fusion is consistent with the hypothesis that tumor cells are of stem cell origin (*74*, *75*).

Therapeutic applications of cell-cell fusion. Perhaps the most well-known application of cell-cell fusion is the production of monoclonal antibodies using hybridomas, the fusion products of antibody-secreting and immortal B cells (*76*). Cell-cell fusion has also been explored to develop more effective cancer immunotherapy. Conventional cancer immunotherapy involves vaccination using dendritic cells that express specific tumor antigens (*77*, *78*). Fusion of tumor cells with intact dendritic cells produces hybrids that express the complete spectrum of tumor-associated antigens. Vaccination with such hybrids is currently being tested as a more effective immunotherapy against cancer (*79*).

The realization that multiple components of the cellular machinery for cell-cell fusion also function in intracellular signaling suggests opportunities for therapeutically modulating the fusion process. Enhancement of cell-cell fusion will bring about increased efficacy of gene therapy for target tissues in vivo. As a hint of things to come, the fusogenic potential of muscle satellite cells has been explored as an approach for cell-based gene delivery to skeletal muscle. For example, intramuscular injection of normal myoblasts into dystrophic mice has been shown to restore dystrophin expression to dystrophin-negative muscles (*80*).

Concluding Remarks

Despite recent advances in our understanding of cell-cell fusion, a central question remains: What is the mechanism underlying plasma membrane merger? The apparent lack of cell-cell fusogens that resemble class I viral fusion proteins and SNAREs indicates the involvement of a distinct mechanism. Although studies of *Drosophila* myoblast fusion suggest a two-component system involving receptor-mediated membrane juxtaposition followed by membrane destabilization, the molecular details of this process are yet to be elucidated. It also remains to be determined whether a universal principle can be extrapolated from the diverse array of cell-cell fusion events currently under investigation. Understanding the basic mechanisms of cell-cell fusion promises to yield insight into related biological processes such as apoptosis, neurogenesis, tumorigenesis, and stem cell biology and, ultimately, to allow the process to be therapeutically manipulated in the setting of human disease.

Note added in proof: An Ig domain–containing transmembrane on sperm, Izumo, has recently been reported to mediate fertilization (*81*), which supports our hypothesis that this type of cell surface protein may have a general function in multiple cell-cell fusion events.

References and Notes

1. Y. Okada, *Exp. Cell Res.* **26**, 98 (1962).
2. S. M. Abmayr, L. Balagopalan, B. J. Galletta, S. J. Hong, *Int. Rev. Cytol.* **225**, 33 (2003).
3. E. H. Chen, E. N. Olson, *Trends Cell Biol.* **14**, 452 (2004).
4. V. Horsley, G. K. Pavlath, *Cells Tissues Organs* **176**, 67 (2004).
5. A. J. Potgens *et al.*, *Placenta* **23** (suppl. A), S107 (2002).
6. P. Primakoff, D. G. Myles, *Science* **296**, 2183 (2002).
7. J. M. Anderson, *Curr. Opin. Hematol.* **7**, 40 (2000).
8. A. Vignery, *Int. J. Exp. Pathol.* **81**, 291 (2000).
9. A. J. Wagers, I. L. Weissman, *Cell* **116**, 639 (2004).
10. J. Pomerantz, H. M. Blau, *Nat. Cell Biol.* **6**, 810 (2004).
11. D. M. Eckert, P. S. Kim, *Annu. Rev. Biochem.* **70**, 777 (2001).
12. R. Blumenthal, M. J. Clague, S. R. Durell, R. M. Epand, *Chem. Rev.* **103**, 53 (2003).
13. R. Jahn, T. Lang, T. C. Sudhof, *Cell* **112**, 519 (2003).
14. L. D. Hernandez, L. R. Hoffman, T. G. Wolfsberg, J. M. White, *Annu. Rev. Cell Dev. Biol.* **12**, 627 (1996).
15. J. J. Skehel, D. C. Wiley, *Annu. Rev. Biochem.* **69**, 531 (2000).
16. T. Weber *et al.*, *Cell* **92**, 759 (1998).
17. C. Hu *et al.*, *Science* **300**, 1745 (2003).
18. J. M. White, M. D. Rose, *Curr. Biol.* **11**, R16 (2001).
19. M. G. Heiman, P. Walter, *J. Cell Biol.* **151**, 719 (2000).
20. G. Shemer, B. Podbilewicz, *Dev. Dyn.* **218**, 30 (2000).
21. G. Shemer, B. Podbilewicz, *Bioessays* **25**, 672 (2003).
22. W. A. Mohler *et al.*, *Dev. Cell* **2**, 355 (2002).
23. G. Shemer *et al.*, *Curr. Biol.* **14**, 1587 (2004).
24. J. J. del Campo *et al.*, *Curr. Biol.* **15**, 413 (2005).
25. M. Ruiz-Gomez, N. Coutts, A. Price, M. V. Taylor, M. Bate, *Cell* **102**, 189 (2000).
26. M. Strunkelnberg *et al.*, *Development* **128**, 4229 (2001).
27. B. A. Bour, M. Chakravarti, J. M. West, S. M. Abmayr, *Genes Dev.* **14**, 1498 (2000).
28. H. A. Dworak, M. A. Charles, L. B. Pellerano, H. Sink, *Development* **128**, 4265 (2001).
29. R. D. Artero, I. Castanon, M. K. Baylies, *Development* **128**, 4251 (2001).
30. B. J. Galletta, M. Chakravarti, R. Banerjee, S. M. Abmayr, *Mech. Dev.* **121**, 1455 (2004).
31. S. Hakeda-Suzuki *et al.*, *Nature* **416**, 438 (2002).
32. E. H. Chen, B. A. Pryce, J. A. Tzeng, G. A. Gonzalez, E. N. Olson, *Cell* **114**, 751 (2003).
33. E. H. Chen, E. N. Olson, *Dev. Cell* **1**, 705 (2001).
34. S. D. Menon, W. Chia, *Dev. Cell* **1**, 671 (2001).
35. A. Rau *et al.*, *Development* **128**, 5061 (2001).
36. M. R. Erickson, B. J. Galletta, S. M. Abmayr, *J. Cell Biol.* **138**, 589 (1997).
37. E. Brugnera *et al.*, *Nat. Cell Biol.* **4**, 574 (2002).
38. Z. Y. He *et al.*, *Dev. Biol.* **254**, 226 (2003).
39. C. Cho, H. Ge, D. Branciforte, P. Primakoff, D. G. Myles, *Dev. Biol.* **222**, 289 (2000).
40. K. Kaji, A. Kudo, *Reproduction* **127**, 423 (2004).
41. F. Le Naour, E. Rubinstein, C. Jasmin, M. Prenant, C. Boucheix, *Science* **287**, 319 (2000).
42. K. Miyado *et al.*, *Science* **287**, 321 (2000).
43. K. Kaji, S. Oda, S. Miyazaki, A. Kudo, *Dev. Biol.* **247**, 327 (2002).
44. J. M. Tarrant, L. Robb, A. B. van Spriel, M. D. Wright, *Trends Immunol.* **24**, 610 (2003).
45. S. Mi *et al.*, *Nature* **403**, 785 (2000).
46. J. L. Blond *et al.*, *J. Virol.* **74**, 3321 (2000).
47. R. Pijnenborg, W. B. Robertson, I. Brosens, G. Dixon, *Placenta* **2**, 71 (1981).
48. F. D. Camargo, S. M. Chambers, M. A. Goodell, *Cell Prolif.* **37**, 55 (2004).
49. K. O'Malley, E. W. Scott, *Exp. Hematol.* **32**, 131 (2004).
50. H. M. Blau, B. T. Blakely, *Semin. Cell Dev. Biol.* **10**, 267 (1999).
51. F. D. Camargo, M. Finegold, M. A. Goodell, *J. Clin. Invest.* **113**, 1266 (2004).
52. H. Willenbring *et al.*, *Nat. Med.* **10**, 744 (2004).
53. G. Q. Daley, *Nat. Med.* **10**, 671 (2004).
54. F. D. Camargo, R. Green, Y. Capetanaki, K. A. Jackson, M. A. Goodell, *Nat. Med.* **9**, 1520 (2003).
55. F. X. Heinz, S. L. Allison, *Curr. Opin. Microbiol.* **4**, 450 (2001).
56. R. H. Schroter *et al.*, *Development* **131**, 4501 (2004).
57. S. K. Doberstein, R. D. Fetter, A. Y. Mehta, C. S. Goodman, *J. Cell Biol.* **136**, 1249 (1997).
58. S. E. Pontow, N. V. Heyden, S. Wei, L. Ratner, *J. Virol.* **78**, 7138 (2004).
59. J. A. Hammer III, X. S. Wu, *Curr. Opin. Cell Biol.* **14**, 69 (2002).
60. T. Hummel, K. Leifker, C. Klambt, *Genes Dev.* **14**, 863 (2000).
61. J. Ng *et al.*, *Nature* **416**, 442 (2002).
62. K. Shen, C. I. Bargmann, *Cell* **112**, 619 (2003).
63. K. Shen, R. D. Fetter, C. I. Bargmann, *Cell* **116**, 869 (2004).
64. P. W. Reddien, H. R. Horvitz, *Annu. Rev. Cell Dev. Biol.* (2004).
65. E. Farkas-Bargeton *et al.*, *J. Neurol. Sci.* **83**, 145 (1988).
66. L. Wockel *et al.*, *Acta Neuropathol. (Berlin)* **95**, 547 (1998).
67. C. W. Redman, I. L. Sargent, *Placenta* **21**, 597 (2000).
68. T. Miyamoto, T. Suda, *Keio J. Med.* **52**, 1 (2003).
69. D. Duelli, Y. Lazebnik, *Cancer Cell* **3**, 445 (2003).
70. F. R. Miller, A. N. Mohamed, D. McEachern, *Cancer Res.* **49**, 4316 (1989).
71. D. M. Duelli, Y. A. Lazebnik, *Nat. Cell Biol.* **2**, 859 (2000).
72. J. M. Pawelek, *Melanoma Res.* **10**, 507 (2000).
73. L. Larizza, V. Schirrmacher, *Cancer Metastas. Rev.* **3**, 193 (1984).
74. D. R. Bell, G. Van Zant, *Oncogene* **23**, 7290 (2004).
75. P. A. Beachy, S. S. Karhadkar, D. M. Berman, *Nature* **432**, 324 (2004).
76. G. Kohler, C. Milstein, *Nature* **256**, 495 (1975).
77. U. Trefzer, P. Walden, *Mol. Biotechnol.* **25**, 63 (2003).
78. J. N. Blattman, P. D. Greenberg, *Science* **305**, 200 (2004).
79. U. Trefzer *et al.*, *Int. J. Cancer* **110**, 730 (2004).
80. T. Partridge, *Neuromuscul. Disord.* **12** (suppl. 1), S3 (2002).
81. N. Inoue *et al.*, *Nature* **434**, 234 (2005).

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