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Review

Tethering Membrane Fusion: Common and Different Players in Myoblasts and at the Synapse

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Abstract: Membrane fusion is essential for the communication of membrane-defined compartments, development of multicellular organisms and tissue homeostasis. Although membrane fusion has been studied extensively, still little is known about the molecular mechanisms. Especially the intercellular fusion of cells during development and tissue homeostasis is poorly understood. Somatic muscle formation in *Drosophila* depends on the intercellular fusion of myoblasts. In this process, myoblasts recognize each other and adhere, thereby triggering a protein machinery that leads to electron-dense plaques, vesicles and F-actin formation at apposing membranes. Two models of how local membrane stress is achieved to induce the merging of the myoblast membranes have been proposed: the electron-dense vesicles transport and release a fusogen and F-actin bends the plasma membrane. In this review, we highlight cell-adhesion molecules and intracellular proteins known to be involved in myoblast fusion. The cell-adhesion proteins also mediate the recognition and adhesion of other cell types, such as neurons that communicate with each other via special intercellular junctions, termed chemical synapses. At these synapses, neurotransmitters are released through the intracellular fusion of synaptic vesicles with the plasma membrane. As the targeting of electron-dense vesicles in myoblasts shares some similarities with the targeting of synaptic vesicle fusion, we compare molecules required for synaptic vesicle fusion to recently identified molecules involved in myoblast fusion.

Keywords: cadherins, electron-dense plaques, F-actin, FuRMAS, IgSF, intercellular junctions, myogenesis, vesicle exocytosis

INTRODUCTION

Membranes define the borders of a cell and of intracellular organelles. Yet membranes must be remodelled via membrane fusion events during organogenesis or when molecules are transported between and within cells. Intercellular fusion of single cells occurs during the development of an organism to form different organs, such as placenta and muscles. Intracellular fusion of internal membranes occurs during exocytosis and protein trafficking. One common aspect of any type of membrane fusion is that the membranes first need to be brought into close contact, from the natural distance of 10–20 nm or more down to 1 nm at the site of ultimate fusion. To achieve this, integral transmembrane proteins involved in transport or cell communication must be removed from this site. Furthermore, fusion proteins are used to reduce the energy barrier for fusion by locally disrupting the membrane structure. This leads to hemifusion and stalk formation. Finally, a fusion pore opens and expands, and the content of the fused cells or organelles is mixed.

Relative to the mechanism of intracellular fusion of organelles, only rather little is known about the mechanism of intercellular fusion. Recent progress in understanding the intercellular fusion of myoblasts, which is essential for building muscles, has been achieved by using the powerful genetic analyses of *Drosophila* (Önel and Renkawitz-Pohl, 2009; Rochlin et al., 2010; Önel et al., 2011; Abmayr & Pavlath, 2012). Genes encoding proteins involved in myoblast fusion have been identified. Among these proteins are cell-adhesion proteins that not only mediate the recognition and adhesion of myoblasts but also play a conserved role in establishing trans-synaptic adhesions. Also identified are intracellular proteins involved in electron-dense vesicle targeting/exocytosis and actin cytoskeleton rearrangement. The vesicles might transport a fusogen that reduces the energy barrier for membrane fusion. Proteins involved in the targeting/

exocytosis of these vesicles are related to proteins supporting synaptic vesicle exocytosis. Recent models also propose that F-actin formation leads to membrane curvature and fusion pore formation. In this review, we will introduce the cellular and molecular biology of myoblast fusion in *Drosophila* and then highlight the function of conserved proteins during the formation of neuronal connections and discuss synaptic vesicle exocytosis in comparison to electron-dense vesicle exocytosis.

CELLULAR AND MOLECULAR BIOLOGY OF MYOBLAST FUSION

Cellular aspects

The larval body wall musculature of *Drosophila* consists of a stereotypic pattern of 30 abdominal muscles per hemisegment (Bate, 1990). The muscles arise during embryogenesis from the somatic mesoderm. Within the somatic mesoderm, two different myoblast populations are produced: fusion-competent myoblasts (FCMs) and founder cells (FCs; Ruiz-Gómez, 1998; Duan et al., 2001). Following myoblast specification, one FC fuses with several FCMs to build a mature myotube, which can contain 4–24 nuclei. The FC dictates the identity of the muscle, and fused FCMs adopt the transcriptional profile of the FC. Somatic muscle formation in *Drosophila* is completed within 5.5 h (Beckett & Baylies, 2007). The fusion of an individual FCM with the FC/growing myotube is even faster and is completed after approximately 12 min (Richardson et al., 2007).

In *Drosophila,* electron-dense structures have been observed at apposing membranes during myoblast recognition and adhesion (Doberstein et al., 1997). These structures are both electron-dense vesicles (Figure 1A)
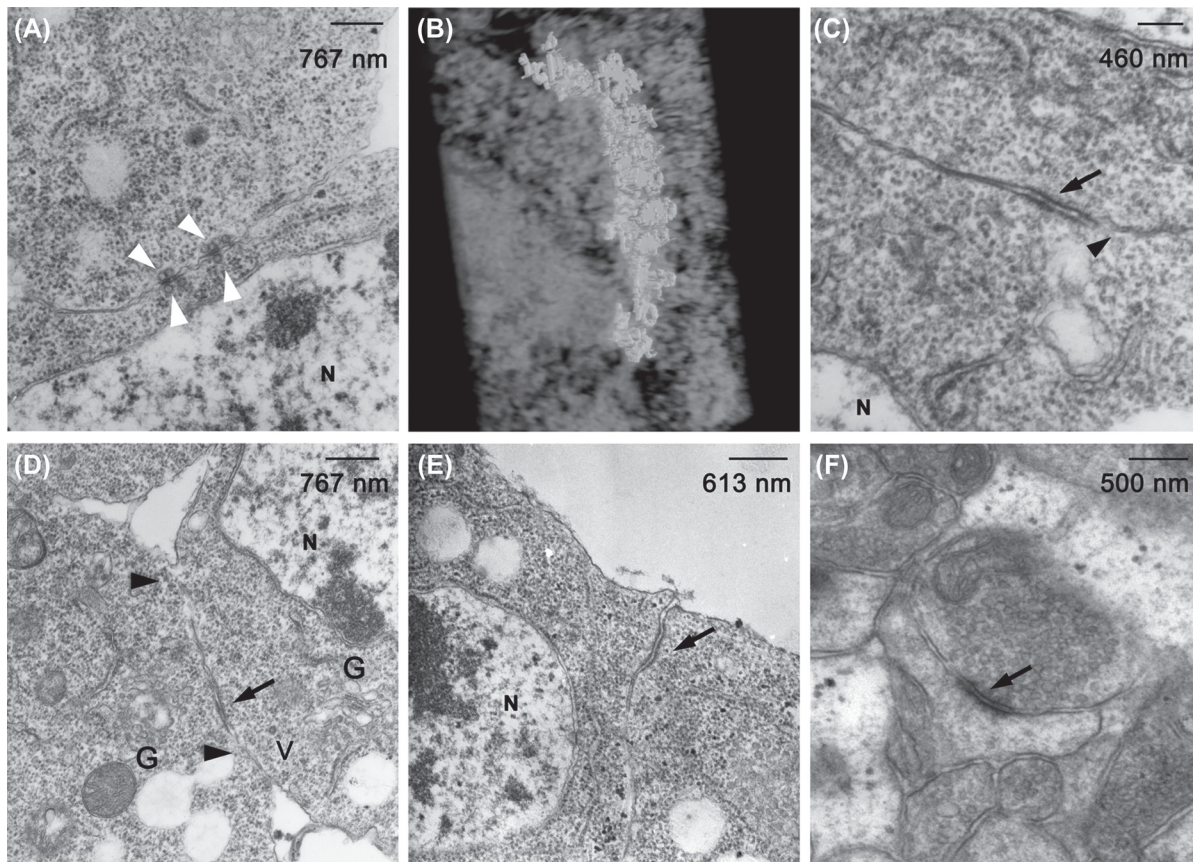

Figure 1. Intermediate steps of myoblast fusion and comparison to intercellular junctions. (A, C, D and E) Electron micrographs of *Drosophila* stage 14 wild-type embryos conventionally fixed as described in Berger *et al.* (2008). (A) A set of electron-dense vesicles that align at apposing membranes of adhering myoblasts (arrowheads). (B) Serial electron micrographs from Doberstein *et al.* (1997; Figure 3) are aligned using the Volocity v5.3 software from Perkin Elmer and show a cloud of vesicles termed the prefusion complex (orange). (C) and (D) Electron-dense plaques between apposing myoblasts (arrows). Doberstein *et al.* (1997) are the first to describe these structures in *Drosophila* myoblasts and observed them in areas of plasma membrane breakdown (C and D, arrowheads). (E) Adherence junction between *Drosophila* epithelial cells with electron-dense material on both sides of the cells (arrow). Epithelial junctions consist of E-cadherins, which form homotypic interactions and link the cells with the actin cytoskeleton. (F) Excitatory synapse in the hippocampal CA1 stratum radiatum from a mouse fixed in 1% formaldehyde/1% glutaraldehyde. The cellular junction (arrow) between two neurons contain proteins of the IgSF family and N-cadherin.

that accumulate as a cloud (Figure 1B) and electron-dense plaques (Figure 1C). It has been suggested that the vesicles emanate from the Golgi (Kim et al., 2007), and the plaques are often found close to areas of membrane breakdown (Figure 1C and E, arrowheads; Doberstein et al., 1997). Both electron-dense structures are rare to observe in wild-type myoblasts, and their function during myoblast fusion still remains nebulous. However, the plaques show striking similarities to intercellular junctions, such as the adherence junctions in *Drosophila* epithelial cells (Figure 1E, arrow) and the mammalian excitatory synapse (Figure 1F, arrow).

Myoblast recognition and adhesion

Cell-adhesion molecules of the immunoglobulin super family (IgSF) in *Drosophila* include Dumbfounded/Kin of Irre (Duf/Kirre), Hibris (Hbs), Roughest/Irregular Chiasm C (Rst/IrreC), and Sticks and Stones (Sns), which are also known as the irre cell recognition module (IRM) proteins (Fischbach et al., 2009; Figure 2A; Table 1). Duf/Kirre is expressed specifically on the surface of FCs, where it attracts FCMs (Ruiz-Gómez et al., 2000) via heterophilic interaction with the FCM-specific IgSF protein Sns (Bour et al., 2000; Galletta et al., 2004). The paralog of Duf/Kirre, Rst/IrreC, is expressed on the surface of both myoblast types and mediates homophilic interactions (Galletta et al., 2004). However, Duf/Kirre can substitute for Rst/IrreC in FCs and vice versa (Strünkelnberg et al., 2001). The paralog of Sns is Hbs, which, like Sns, is exclusively expressed in FCMs (Artero et al., 2001; Dworak et al., 2001) and can partially substitute for Sns (Shelton et al., 2009), but its function during fusion remains unknown.

In adhering FCs and FCMs, Duf/Kirre, Rst/IrreC and Sns are expressed in a ring-like structure at cell–cell contact points (Kesper et al., 2007; Sens et al., 2010; Önel et al., 2011). The rings expand during the fusion process, which suggests that fusion takes place within the area restricted by these IgSFs (Önel & Renkawitz-Pohl, 2009; Önel et al., 2011). Furthermore, Duf/Kirre, Hbs, Rst/IrreC and Sns are involved in the formation of a cell-communication structure that recruits further proteins, for example, Swiprosin-1 (DSwip-1; Figure 2B; Table 1) and Blown fuse (Blow; Figure 2C), to the site of cell–cell contact. The structure that forms when cells adhere has been termed fusion-restricted myogenic-adhesive structure (FuRMAS; Kesper et al., 2007). Homologs of Duf/Kirre and Sns are also important for vertebrate myoblast fusion (Srinivas et al., 2007; Sohn et al., 2009; Durcan et al., 2013), which indicates that the role of IgSF proteins in myoblast fusion is evolutionarily conserved.

Another cell-adhesion molecule that has been recently described to mediate the adhesion of FCs and FCMs during myoblast fusion is neuronal (N-)cadherin (Figure 2A;

Table 1). N-cadherin is a classical member of the Ca^{2+}-dependent cadherin superfamily. In contrast to the IgSFs that form a ring-like structure at cell–cell contact points, N-cadherin is uniformly distributed around the plasma membrane of FCs and FCMs (Dottermusch-Heidel et al., 2012). As in mammals, the loss of N-cadherin in *Drosophila* does not disturb myoblast fusion (Charlton et al., 1997; Dottermusch-Heidel et al., 2012), which points towards compensatory mechanisms. In mammals, N-cadherin is replaced by other members of the classical cadherin family (Krauss, 2010). Thus, *Drosophila* N-cadherin could share redundant functions with another member of the cadherin family. Genetic interaction data suggest that *Drosophila* N-cadherin is regulated at the site of fusion by the guanine-nucleotide exchange factor Schizo/Loner (Siz), which binds to the intracellular domain of N-cadherin and activates the small Arf1-GTPase during myoblast fusion (Dottermusch-Heidel et al., 2012; Figure 2A). The cadherin extracellular region is 22 nm in length (Nagar et al., 1996), whereas the hemifusion of membranes occurs at an intermembrane distance of 1 nm (Kozlovsky et al., 2004). Hence, it seems that N-cadherin needs to be displaced from the ultimate site of fusion to reduce membrane distance. In contrast, it is believed that the IgSF molecules are redistributed from the site of fusion by the expansion of the ring structure.

Signalling fusion and F-actin regulation

Recently identified players in myoblast fusion are the MARVEL domain protein Singles Bar (Sing; Estrada et al., 2007) and the EF-hand domain protein DSwip-1 (Hornbruch-Freitag et al., 2011). MARVEL (MAL and related proteins for vesicle trafficking and membrane link) domain proteins are characterized by four transmembrane helix regions. In mammals, proteins containing this domain signature are involved in membrane apposition and vesicle trafficking (Sanchez-Pulido et al., 2002). *sing* is transcribed in both FCs and FCMs (Estrada et al., 2007; Table 1). *sing* mutant embryos possess a significantly higher number of prefusion complexes. It is therefore hypothesized that Sing is involved in the targeting or in the exocytosis of the electron-dense vesicles to the plasma membrane (Estrada et al., 2007). Support for this idea comes from studies on the Ca^{2+}-binding protein DSwip-1. This protein was identified from a databank search for Ca^{2+}-binding proteins that are expressed in the somatic mesoderm. DSwip-1 contains two Ca^{2+}-binding EF-hand domains and one coiled-coil domain. Its mammalian homolog Swiprosin-1/EFHD2 was first discovered in human lymphocytes (Vuadens et al., 2004) and in B cells where it associates with lipid rafts (Mielenz et al., 2005). In myoblasts, DSwip-1 is only present in FCMs at cell–cell contact points during adhesion, as is Sns. DSwip-1
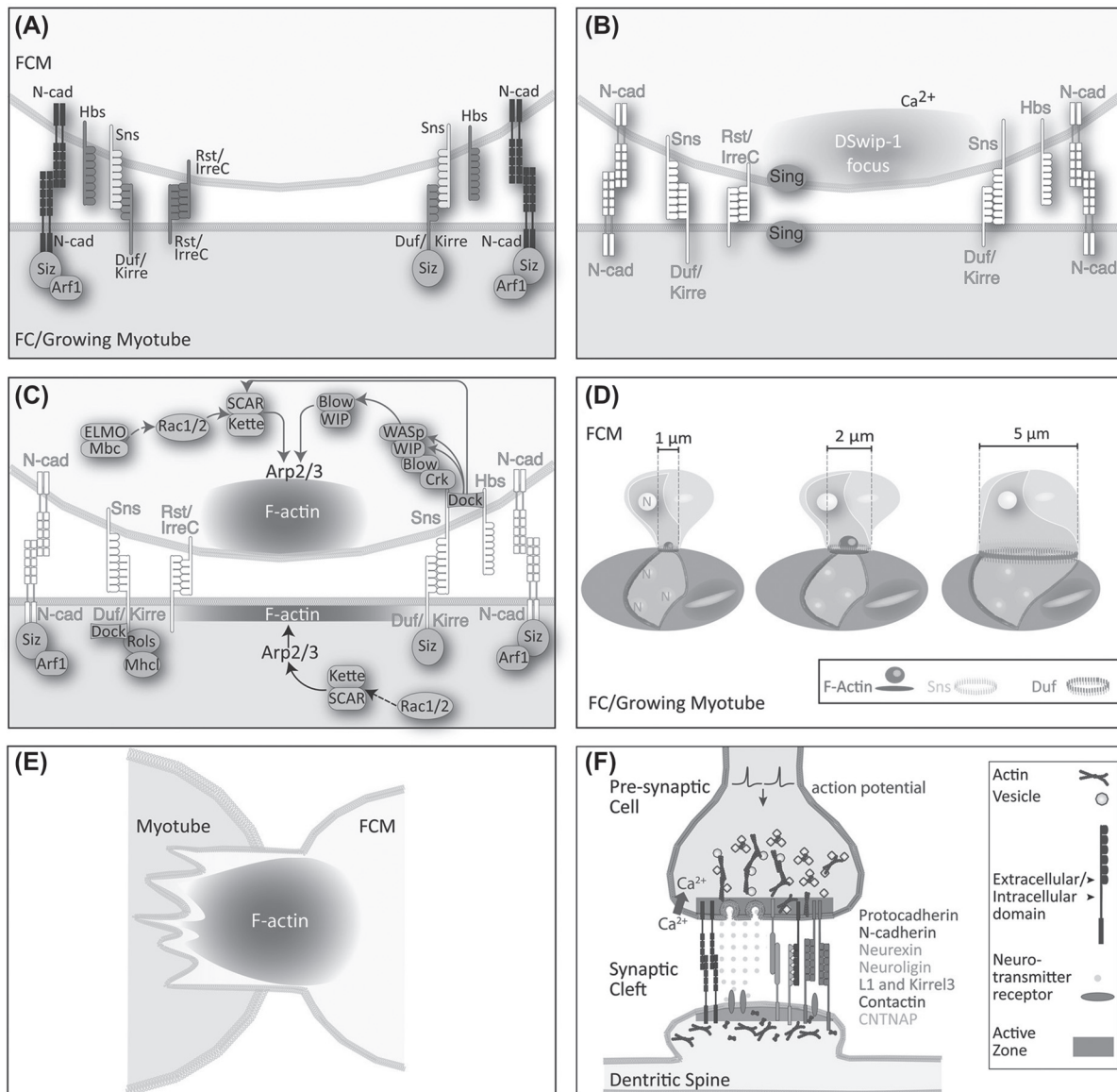
Figure 2. Overview of proteins involved in myoblast fusion and in mediating trans-synaptic adhesions. (A) *Adhesion molecules.* The IgSF proteins Duf/Kirre and Sns interact heterophilically on the surface of FCs and FCMs. Rst/IrreC interact homophilically. Whether the FCM-specific IgSF Hbs interacts heterophilically with Duf/Kirre or Rst/IrreC on the surface of FCs is not yet clear. The Ca^{2+}-dependent adhesion molecule N-cadherin is additionally involved in the recognition and adhesion of FCs and FCMs and forms homodimers. The GTPase Arf1 and Schizo/Loner (Siz) are involved in the displacement of N-cadherin, probably to reduce the distance between apposing membranes. Siz also binds to the intracellular domain of Duf/Kirre (Bulchand et al., 2010). (B) *Signalling molecules.* Sing, which consists of seven-transmembrane spanning helix regions, is present in FCs and FCMs and acts in FCMs together with the Ca^{2+}-binding EF-hand domain protein DSwip-1 to target or exocytose the vesicles of the prefusion complex. (C) *Adaptor proteins and F-actin regulators.* The multidomain protein Rols7 transfers the fusion signal from the membrane into the FC by binding to the intracellular domain of Duf/Kirre. MhcI is a downstream partner of Rols7. Furthermore, the SH2-SH3 adaptor proteins Crk and Dock transfer the fusion signal into FCs and FCMs. Crk binds to the intracellular domain of Sns. Dock binds to the intracellular domain of Duf/Kirre, Hbs and Sns. Crk and Dock link cell adhesion with Scar- and WASp-dependent Arp2/3 regulation. The regulatory Scar complex, to which Kette belongs to, regulates Arp2/3-dependent actin polymerization in FCs and FCMs. In FCMs, the Scar complex is activated by the GEF Mbc that catalyses the GDP-to-GTP exchange on the small GTPases Rac1 and Rac2 (Haralalka et al., 2011). The GEF that activates Rac1 and Rac2 in FCs is unknown. Arp2/3-dependent F-actin focus formation in FCMs is additionally controlled by the WASp/WIP complex. Competition between WASp and Blow for WIP binding leads to the formation of new actin filaments, which promote the formation of finger-like protrusions, as shown in E. (D) *Model of F-actin regulation and FuRMAS expansion.* One possible function of F-actin formation during myoblast fusion is possibly the expansion of the ring-like structure formed by the IgSF proteins from 1 to 5 μm in diameter. (E) *Model of F-actin formation and membrane stress.* Another possible role for F-action could be to induce membrane stress at the FCM membrane, which subsequently induces fusion pore formation. (F) *Chemical synapse.* Cell-adhesion molecules of the cadherin, protocadherin, IgSF, neuroligin, neurexin and CNTNAP families connect presynaptic and postsynaptic

accumulates in *sing* mutant myoblasts (Hornbruch-Freitag et al., 2011). Thus, successful cell adhesion leads to the targeting or exocytosis of the prefusion complex in FCMs, which involves the Ca^{2+}-binding protein, DSwip-1, and the MARVEL domain protein, Sing (Figure 2B). A corresponding Ca^{2+}-binding protein in FCs that mediates Sing-dependent prefusion complex targeting or exocytosis has not yet been identified.

DSwip-1 is not the only protein whose localization depends on successful cell adhesion. Also F-actin accumulation is dependent. The recognition and adhesion of myoblasts results in the formation of a dense and dynamic F-actin focus in FCMs and a thin F-actin sheath in FCs (Kesper et al., 2007; Richardson et al., 2007; Sens et al., 2010). The F-actin focus in FCMs co-localizes with DSwip-1, but F-actin formation does not depend on DSwip-1 (Hornbruch-Freitag et al., 2011). F-actin formation during myoblast fusion involves the evolutionarily conserved actin-related protein (Arp)2/3 complex (Figure 2C; Massarwa et al., 2007; Richardson et al., 2007; Berger et al., 2008). The activity of the Arp2/3 complex in cells is generally controlled by nucleation-promoting factors (NPFs) of the Wiskott-Aldrich Syndrome protein (WASp) family (Pollard, 2007; Takenawa & Suetsugu, 2007; Rotty et al., 2013). Both the NPF suppressor of the cyclic AMP receptor (Scar; also known as WAVE) and WASp (Massarwa et al., 2007; Schäfer et al., 2007; Richardson et al., 2007; Berger et al., 2008; Gildor et al., 2009) control Arp2/3-dependent F-actin formation during myoblast fusion (Figure 2C). Scar solely controls F-actin formation in FCs, but cooperates together with WASp in F-actin focus formation (Figure 2C; Berger et al, 2008; Sens et al., 2010; Haralalka et al., 2011). The WASp-interacting partner WIP (also known as Verprolin1 and Solitary in *Drosophila*) and WASp localize in FCMs at cell–cell contact points (Kim et al., 2007; Massarwa et al., 2007; Schäfer et al., 2007; Berger et al., 2008). Recent studies suggest that the stability of the WIP–WASp complex is modulated by the FCM-specific protein, Blow, which competes with WASp for WIP binding (Figure 2C; Jin et al., 2011). The localization of Blow to the site of cell–cell contact is Sns-dependent (Kesper et al., 2007; Jin et al., 2011).

Scaffold and adaptor proteins bind to the intracellular domain of the cell-adhesion molecules to transfer the signal in FCs and FCMs to the actin cytoskeleton (Kim et al., 2007; Bonn et al., 2013; Kaipa et al., 2013).

The multidomain protein Rols7 and the SH2-SH3 adaptor protein Dock (known as Nck in vertebrates) are able to bind to the intracellular domain of Duf/Kirre in FCs (Kreisköther et al., 2006; Kaipa et al., 2013). In contrast, both the SH2-SH3 adaptor protein Crk and Dock bind to the intracellular domain of Sns in FCMs (Kim et al., 2007; Kaipa et al., 2013). Dock binds additionally to the intracellular domain of Hbs (Kaipa et al., 2013). To connect cell adhesion with F-actin formation, Crk interacts with Blow and WIP (Figure 2C; Kim et al., 2007; Jin et al., 2011), whereas Dock interacts with Scar, WIP and WASp (Figure 2C arrows; Kaipa et al., 2013).

At the ultrastructural level, *blow* mutants stop fusion after prefusion complex formation (Doberstein et al., 1997). *wasp* and *wip* mutants, in contrast, arrest fusion during membrane breakdown (Massarwa et al., 2007, Berger et al., 2008). However, *Arp3* mutants stop fusion at a later state, after the formation of a fusion pore. Although a fusion pore forms in *Arp3* mutants, the FCM fails to integrate into the growing myotube. Thus, Arp2/3-dependent actin polymerization seems to affect different steps of myoblast fusion.

Possible roles for F-actin during myoblast fusion

F-actin foci formation during myoblast fusion is highly dynamic (Richardson et al., 2007) and essential for fusion. Different F-actin regulation mutants stop fusion at different steps. So what is the function of F-actin during myoblast fusion? In wild-type myoblasts, the ring-like structure formed by Duf/Kirre, Sns and Rst/IrreC expands from 1 µm to 5 µm (Figure 2D, Kesper et al., 2007; Önel et al., 2011). This structure fails to expand in mutants defective in F-actin formation, for example, *kette* and *blow* (Kesper et al., 2007). Thus, one possible role for F-actin formation in FCs and FCMs is the expansion of the FuRMAS (Kepser et al., 2007; Figure 2D). The finding that fusion pores remain small in *Arp3* mutant myoblasts indicates that F-actin is also required for the integration of the FCM into the FC/growing myotube (Berger et al., 2008). FCMs extend finger-like protrusions towards the FC/growing myotube (Figure 2E; Sens et al., 2010). *blow, wip* and *wasp* mutants fail to form these protrusions (Sens et al., 2010; Jin et al., 2011). Therefore, it has been suggested that F-actin is required to form finger-like protrusions, which induce membrane stress and lead to fusion pore formation.

Figure 2. (Continued).

structures. Presynaptically, neurotransmitters are stored in synaptic vesicles. Upon the arrival of an action potential and local increase in the Ca^{2+} concentration, synaptic vesicles fuse with the plasma membrane at the active zone and release their content into the synaptic cleft. Released neurotransmitters bind to receptors located in the postsynaptic membrane, thereby initiating or modulating signal transduction pathways and/or inducing changes in the membrane potential. Presynaptically, actin filaments (F-actin) are involved in clustering synaptic vesicles and presumably in recruiting synaptic vesicles to the active zone. In excitatory synapses, F-actin determines the morphology of their postsynaptic compartments, termed dendritic spines.

Table 1. Proteins involved in myoblast fusion in *Drosophila*, in synapse formation and synaptic vesicle exocytosis.

Myoblast adhesion			Trans-synaptic adhesion				
Drosophila	Cell type	Protein class	*Drosophila*	*C. elegans*	Mammals	Cell type	Protein class
Duf/Kirre[1]	FC	IgSF			KIRREL3[2]	Presynaptiic? Postsynaptic?	IgSF
Rst/IrrC[3]	FC, FCM	IgSF	Rst/IrreC[4]			Presynaptic	IgSF
Sns[5]	FCM	IgSF					
Hbs[6]	FCM	IgSF	Hbs[4]			Postsynaptic	IgSF
				SYG-1[7]		Presynaptic	IgSF
				SYG-2[8]		Postsynaptic	IgSF
N-cadherin[9]	FC, FCM	Cadherin	N-cadherin[10]		N-cadherin[11]		Cadherin
			Flamingo[12]	Fmi-1[13]		Presynaptic	Atypical cadherin (7TM)

Tethering factors: Myoblast fusion	Synaptic vesicle exocytosis			
Drosophila	*Drosophila*	*C. elegans*	*Mammals*	
None	Unc13[14]	Unc-13[15]	Munc13-1[16]	
			Munc13-2[16]	
			Munc13-3[16]	
			Sec8[17]	

MARVEL domain proteins: Myoblast fusion		Synaptic vesicle exocytosis		
Drosophila	Cell type	*Drosophila*	*C. elegans*	*Mammals*	Expression
Sing[18]	FC, FCM	Synaptogyrin[19]	Unc-11[20]	Synaptogyrin[21]	Abundant expression on the vesicle membrane
				Synaptophysin[22,23]	Abundant expression on the vesicle membrane.
				Synaptoporin[24]	Vesicle membrane

Intracellular Ca^{2+} binding proteins: Myoblast fusion		Synaptic vesicle exocytosis		
Drosophila	Cell Type	*Drosophila*	*C. elegans*	*Mammals*
DSwip-1[25]		Unc13[14]	Unc-13[15]	Munc13-1,2,3[16]
		Synaptotagmin[26,27]	Synaptotagmin[28]	Synaptotagmin[26]

[1]Ruiz-Gómez et al. (2000), Strünkelnberg et al. (2001).
[2]Komori et al. (2008).
[3]Strünkelnberg et al. (2001).
[4]Sugie et al. (2010).
[5]Bour et al. (2000).
[6]Artero et al. (2001), Dworak et al. (2001).
[7]Shen and Bargmann (2003).
[8]Shen et al. (2004).
[9]Dottermusch-Heidel et al. (2012).
[10]Prakash et al. (2005).
[11]Fannon and Colman (1996), Uchida et al. (1996).
[12]Bao et al. (2007).
[13]Najarro et al. (2012).
[14]Aravamudan et al. (1999).
[15]Maruyama and Brenner (1991).
[16]Brose et al. (1995).
[17]Takamori et al. (2006).
[18]Estrada et al. (2007).
[19]Stevens et al. (2012).
[20]Nonet et al. (1999).
[21]Stenius et al. (1995).
[22]Jahn et al. (1985).
[23]Wiedenmann and Franke (1985).
[24]Knaus et al. (1990).
[25]Hornbruch-Freitag et al. (2011).
[26]Perin et al. (1991)
[27]DiAntonio et al. (1993).
[28]Nonet et al. (1993).

CONSERVED PLAYERS INVOLVED IN SYNAPSE FORMATION AND MAINTENANCE

To form functional networks, neurons are interconnected via chemical or electrical synapses. In chemical synapses, action potentials arriving at presynaptic terminals induce neurotransmitter release via Ca^{2+}-dependent synaptic vesicle exocytosis (Figure 2F). The released neurotransmitters diffuse through the synaptic cleft and bind to receptors on the postsynaptic membrane, thereby depolarizing the postsynaptic membrane potential and activating or modulating signal transduction pathways. A prerequisite for a functional chemical synapse is the establishment and maintenance of trans-synaptic adhesions. This involves the function of cell-adhesion molecules that link presynaptic and postsynaptic structures. A number of cell-adhesion molecules have been implicated in synapse maturation and maintenance including neurexins/neuroligins; leucine-rich repeat proteins, such as leucine-rich repeat transmembrane neuronal proteins, netrin-G ligands and signalling lymphocyte activation molecules; tyrosine kinase receptor proteins, such as EphrinB and their ligands; and integrins (Figure 2F). As these protein classes have been reviewed recently in detail (Togashi et al., 2009; Missler et al., 2012; Thalhammer & Cingolani, 2013) and are not involved in myoblast fusion, we focus here only on the cell-adhesion molecules cadherins and Ig-domain proteins.

The most ancient Ig-domain proteins that constitute trans-synaptic adhesions in mammals and invertebrates are the evolutionarily conserved Nephrin and Neph proteins (Giagtzoglou et al., 2009; Fischbach et al., 2009). In *Caenorhabditis elegans,* synapse formation of the hermaphrodite-specific motor neuron (HSNL) is controlled by the Neph1 homolog SYG-1 and the Nephrin homolog SYG-2 (Shen & Bargmann, 2003; Shen et al., 2004; Table 1). SYG-2 is expressed on vulva epithelial cells, where it interacts with SYG-1 on the HSNL neuron. SYG-2 thereby recruits SYG-1 to the site, where the presynapse is formed.

The *Drosophila* orthologs of Neph1 and Nephrin are Duf/Kirre and Rst/IrreC, and Sns and Hbs, respectively. Hbs and Rst/IrreC are also essential for the communication between retinal axons and their postsynaptic partners in eye development. During the development of the visual centre in *Drosophila*, presynaptic photoreceptor cells (R cells) extend their axons to the lamina layer. The R axons express the segment polarity gene *hedgehog*, which induces the differentiation of lamina precursor cells into postsynaptic lamina precursor cells (pLPCs). As a consequence, pLPCs express Hbs, which interacts with Rst/IrreC and enables pLPCs to associate with R axon bundles (Sugie et al., 2010; Table 1).

IRMs have also been identified in vertebrates. In mouse embryos, the Duf/Kirre homolog Neph2/Kirrel3 is expressed in the central nervous system and in the dorsal root ganglia of the peripheral nervous system (Komori et al., 2008; Völker et al., 2012). Furthermore, Neph1 and Neph2/Kirrel3 are expressed in the nervous system of mouse and chick embryos (Völker et al., 2012). However, Neph3/Kirrel2 is transcribed in the cerebellar anlage and the spinal cord of mice embryos. Furthermore, Neph2/Kirrel3 possibly plays a role in axonal pathfinding, cell recognition and synapse formation between dorsal root ganglia neurons and their target cells (Komori et al., 2008; Table 1).

Another conserved protein involved in trans-synaptic adhesion is N-cadherin (Table 1). It controls synaptic morphology and function (Hansen et al., 2008; Sheng et al., 2013). Furthermore, N-cadherin acts as a trans-synaptic sensor for synaptic activity, which promotes N-cadherin dimer formation (Tanaka et al., 2000). Immunoelectron microscopy studies have shown that N-cadherin localizes to sites of synaptic vesicle exocytosis, which suggests a role in vesicle trafficking (Uchida et al., 1996). Indeed, presynaptic N-cadherin has been implicated in the regulation of synaptic vesicle recruitment and recycling (Stan et al., 2010). In unstimulated neurons, N-cadherin is distributed uniformly along the synaptic cleft, and evidence suggests that upon synaptic stimulation, N-cadherin redistributes at the synapse (Yam et al., 2013). However, during KCl-induced depolarization, less N-cadherin is found in the region where synaptic vesicle exocytosis takes place, and a protein redistributes to the periphery of the synaptic cleft. After KCl-induced depolarization, N-cadherin redistributes again more uniformly. Thus, the localization of N-cadherin is directly correlated to synaptic activity. These findings possibly indicate that N-cadherin–free regions must be created at the region to which the synaptic vesicle fuses.

CONSERVED PRINCIPLES AND PLAYERS IN SYNAPTIC VESICLE FUSION

During exocytosis, filled vesicles fuse with the plasma membrane to release their contents into the extracellular space. This intracellular membrane fusion has been studied extensively during synaptic vesicle exocytosis (McMahon et al., 2010; Jahn & Fasshauer, 2012; Wu et al., 2013). The release of neurotransmitters from synaptic vesicles follows a core principle of intracellular fusion that involves tethering, docking and priming of vesicles (James & Martin, 2013). These events lead to the zippering of soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs) that catalyse the fusion process and possess fusogenic characteristics. Fusogens are proteins that drive membrane fusion and destabilize the lipid bilayers by inducing local membrane stress (Oren-Suissa & Podbilewicz, 2007). One of the main criteria for

a fusogen is that it should be expressed at the ultimate site and time of fusion. Furthermore, it should be sufficient to fuse cells that normally do not fuse. In an elegant study, Hu et al. (2003) demonstrated that expression of the neuronal vesicle (v-)SNARE Synaptobrevin (Figure 3A) and the neuronal target-membrane (t-)SNAREs Syntaxin-1 and SNAP-25 (Figure 3A) on the cell surface is able to induce the heterologous fusion of Chinese hamster ovary (CHO) and fibroblast cells.

During vesicle fusion, the N-terminal regions of the SNARE proteins, where the SNARE motif is localized, assemble into a trans-SNARE complex (Südhof & Rizo, 2011). Through SNARE assembly, the distance of the apposing membranes is reduced to 3–4 nm, and membranes are destabilized (Chen et al., 2006). However, the time needed for trans-SNARE complex assembly *in vitro* is much lower than that *in vivo*, which suggests the involvement of assisting proteins (Fasshauer et al., 2002). A set of proteins that regulate SNARE-mediated membrane fusion has been identified and some of them are discussed next.

Synaptogyrin and Synaptophysin are abundantly expressed on synaptic vesicles and belong to the MARVEL domain family (Rehm et al., 1986; Stenius et al., 1995; Table 1). Both proteins have been implicated in playing a role in the biogenesis of synaptic vesicles (Thiele et al., 2000; Stevens et al., 2012), and it has been suggested that Synaptophysin promotes the formation of highly curved membranes (Thiele et al., 2000). Furthermore, Synaptophysin is able to bind to the v-SNARE Synaptobrevin. However, not much is known about the function of either protein during synaptic vesicle release and recycling. The genetic analysis of Synaptogyrin and Synaptophysin in mammals is complicated as different homologs exist that possibly behave in a compensatory manner (Janz et al., 1999; Spiwoks-Becker et al., 2001). *Drosophila* lacks Synaptophysin, and produces Synaptogyrin (Table 1). *Drosophila* Synaptogyrin regulates neurotransmitter release and short-term synaptic plasticity (Stevens et al., 2012). Besides Synaptogyrin, the *Drosophila* genome encodes two other MARVEL-domain–containing proteins: the Sing protein relevant for myoblast fusion and CMTM4 (Stevens et al., 2012).

Tethering factors mediate the initial interaction between an intracellular vesicle and its target membrane (Barlowe, 1997; Cao et al., 1998). These factors are either large coiled-coil proteins or multi-subunit tethering complexes. The complexes have a homologous tertiary structure, termed complex associated with tethering containing helical rods (CATCHR; Yu et al., 2010). Munc13 and Munc18 are regulatory proteins that prepare the SNAREs for complex assembly (Figure 3A). Munc13 shows structural similarities to the CATCHR region of Sec6, a subunit of the exocyst tethering complex (Li et al., 2011). Munc18 conducts Syntaxin-1 to the target membrane and stabilizes a closed form of Sytaxin-1 (Hata et al., 1993; Misura et al.,
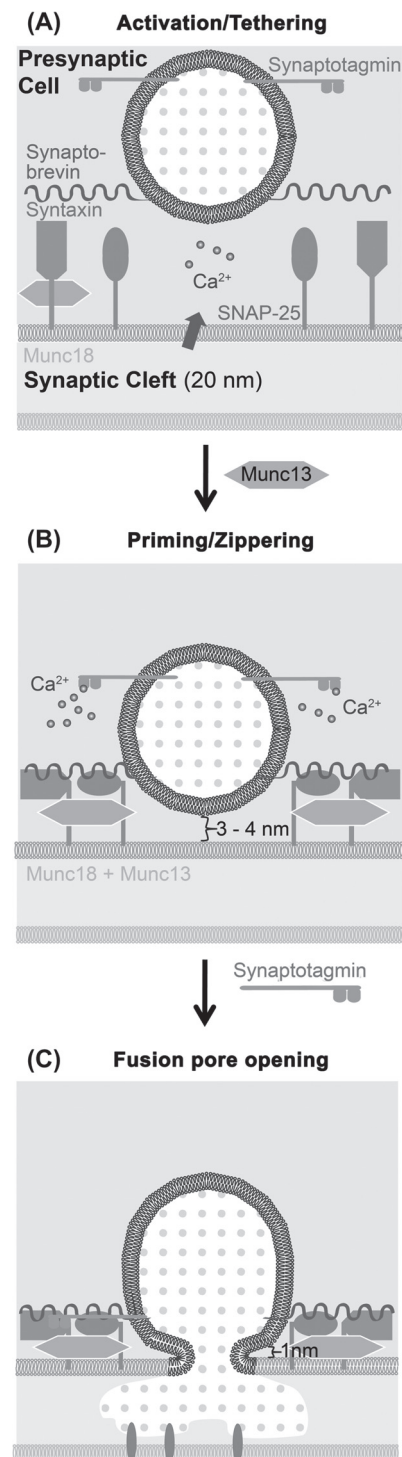

Figure 3. Steps in synaptic vesicle exocytosis. Ca^{2+} influx activates the assembly of the SNARE complex and is required during various steps of vesicle fusion. (A) The vesicle is tethered to the plasma membrane. This involves Munc13 of the CATCHR family and the SM protein Munc18. (B) The zippering of the neuronal SNAREs Syntaxin, SNAP-25 and Synaptobrevin brings the apposing membranes into proximity at 3–4 nm. (C) Fusion pore initiation involves the function of the Ca^{2+}-dependent C2 domain protein Synaptotagmin. After a fusion pore has formed, the content of the vesicle is released into the synaptic cleft.

2000; Dulubova et al., 2003). It has been proposed that Munc13 opens Syntaxin-1 and induces SNARE complex assembly (for detailed review, see Rizo & Südhof, 2012).

The progression of SNARE assembly and zippering involves the activity of Synaptotagmin (Figure 3B) and thereby leads to the opening of a fusion pore (Figure 3C). Synaptotagmin-1 is located to synaptic vesicles and contains two cytoplasmic C2 domains. The C2A domain shows interactions with t-SNAREs in a Ca^{2+}-dependent manner (Chapman et al., 1995; Gerona et al., 2000), but the C2B domain shows interactions with t-SNAREs in a Ca^{2+}-dependent manner (Rickman & Davletov, 2003). Different models have been proposed as to how Synaptotagmin initiates fusion pore formation. One model also suggests that Synaptotagmin-1 serves as a Ca^{2+}-dependent fusogen (Martens & McMahon, 2008).

Do SNARE-like fusogens exist in myoblast fusion or are they involved in the exocytosis of a myoblast-specific fusogen?

SNAREs and Synaptotagmin-1 are specialized proteins that induce membrane curvature during vesicle fusion (Martens et al., 2007; Marsden et al., 2011) and partially fulfil the criteria of a fusogen. A comparable fusogen in *Drosophila* myoblast fusion has not yet been identified. However, the Ig domains of the IRM proteins that are involved in myoblast adhesion are structurally related to the C2 domains of Synaptotagmin-1 (Martens & McMahon, 2008). Heterologous expression of Duf/Kirre and Sns, or Rst/IrreC and Sns in *Drosophila* non-muscle S2 or in murine C2C12 cells cannot induce cell–cell fusion (Galletta et al., 2004; Kesper et al., 2007). Thus, although these proteins are expressed at the ultimate site and time of fusion, they are not sufficient to fuse cells that normally do not fuse.

In pioneering work on the ultrastructural analysis of *Drosophila* myoblast fusion, Doberstein et al. (1997) proposed several possible functions of the electron-dense structures present at apposing membranes. One possibility was that the electron-dense vesicles fuse to the plasma membrane and generate the electron-dense plaques (Figure 4A). However, prefusion complexes are seen more often than electron-dense plaques, and sometimes electron-dense plaques and vesicles are observed together (Figure 2D). Another possibility is that the electron-dense vesicles deliver fusion-relevant components or even a fusogen to the apposing membranes. The identification of *blow* and *sing* mutants and their ultrastructural phenotype substantiate the important role of electron-dense vesicles during fusion. Furthermore, Sing, like Synaptogyrin, contains a MARVEL domain and possibly mediates electron-dense vesicle exocytosis. As Sing and the Ca^{2+}-binding protein DSwip-1 seem to act in concert, the exocytosis of

these vesicles could be triggered by Ca^{2+} influx. However, the measurement of Ca^{2+} influx during fusion is difficult, because myoblast fusion occurs asynchronously and several FCMs can adhere and fuse to an FC/growing myotube at the same time.

The FuRMAS that forms at cell–cell contact points is reminiscent of other cell communication structures, such as invadopodia or the immunological synapse (Önel & Renkawitz-Pohl, 2009; Sens et al., 2010). It has been proposed that the formation of protrusion-like structures in FCMs induces fusion pore formation (Sens et al., 2010).

Recent evidence pointing towards the existence of a fusogen during myoblast fusion comes from studies of mammalian myoblast fusion. The newly identified transmembrane protein Myomaker is essential for myoblast fusion in mice, and forced expression of Myomaker in fibroblasts promotes the fusion of these cells (Millay et al., 2013). Consequently, the hunt for a fusogen involved in *Drosophila* myoblast fusion continues.

FUTURE DIRECTIONS

Cell–cell fusion is fundamental for animal development. In mammals, only gametes, trophoblasts, osteoclasts, macrophages and myoblasts are able to fuse. In all cell types, transmembrane proteins bring the cells that are designated to fuse into contact, and most of the transmembrane proteins identified contain Ig domains. But IgSF proteins on their own are not able to induce local membrane stress, a prerequisite to destabilize the lipid bilayers of the plasma membranes. The fusogens Syncytin-1 and Syncytin-2 involved in trophoblast fusion, which is essential for placenta formation, are retroviral and have been adopted during evolution (Blond et al., 2000; Mi et al., 2000). One hypothesis is that viral-like or SNARE-like fusogens are involved in mediating cell–cell and myoblast fusion, respectively (Oren-Suissa & Podbilewicz, 2007). Another proposal is that F-actin formation in *Drosophila* triggers membrane fusion by inducing actin-propelled invasive membrane protrusions (Sens et al., 2010). However, the identification of Myomaker as a possible fusogen in mouse myoblasts has placed doubt on this proposal. Nevertheless, the existence of a fusogen does not exclude that F-actin formation is a driving force in myoblast fusion and assists the process (Shilagardi et al., 2013).

Studying the ultrastructural features of F-actin formation has lost sight of other features that accompany myoblast fusion, for example, electron-dense vesicles and plaques. We believe that integrating these structures into current models of myoblast fusion will increase our understanding. We suggest that the electron-dense plaques represent cellular junctions that contain the IgSF proteins and N-cadherin (Figure 4B, step 1). The displacement of N-cadherin from the ultimate site of fusion possibly
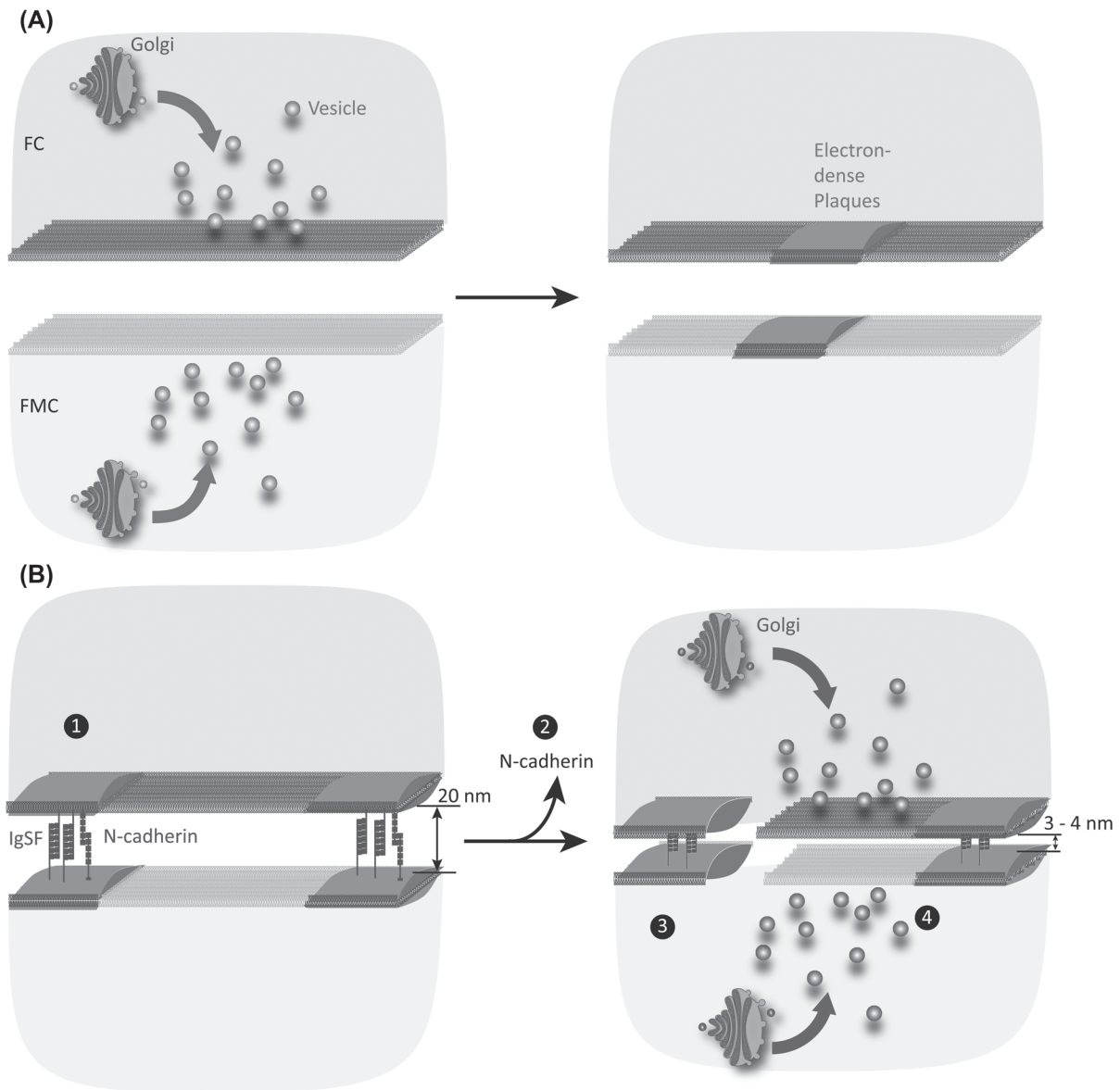
Figure 4. Models for the function of electron-dense vesicle and plaque formation. (A) Based on ultrastructural analyses of various myoblast fusion mutants, Doberstein *et al*. (1997) suggested that the electron-dense vesicles of the prefusion complex fuse to the plasma membranes and generate electron-dense plaques. They hypothesized that the plaques are an intermediate between fusion of the vesicles with the plasma membranes and the formation of pores between the apposed plasma membranes. (B) The striking similarity of electron-dense plaques to Cadherin-containing cellular junctions suggests that the plaques represent a cell-adhesion structure (step 1). Interestingly, the distance of the apposing plasma membranes within the plaques differs from the plasma membrane distance where membrane breakdown starts (see Figure 1C). This could be due to the displacement of N-cadherin from the ultimate site of fusion (step 2). The IgSF proteins keep the membranes in proximity (at least at 3–4 nm; step 3). The role of electron-dense vesicles during myoblast fusion is still hypothetical. Studies by Kim *et al*. (2007) suggest that they arise from the Golgi (step 4). Some myoblast fusion mutants stop the process after prefusion complex accumulation, which indicates that the prefusion complex plays an essential role during myoblast fusion, possibly in delivering a fusogen to the ultimate site of fusion, as suggested by Doberstein *et al*. (1997).

brings the membranes into proximity (step 2). The signalling cascade triggered by cell-adhesion molecules leads to the release of the electron-dense vesicle from the Golgi to the ultimate site of fusion (step 3), which is now free of N-cadherin. This could explain why fusion pore formation is observed next to the electron-dense plaques.

Future challenges will be: (i) to determine which factors lead to electron-dense vesicle release and whether this process is Ca^{2+}-dependent, and (ii) to determine how the electron-dense vesicles are targeted to the plasma membrane and whether F-actin (Kim et al., 2007) and tethering factors known from SNARE-mediated vesicle

fusion are involved. The exocyst multi-subunit tethering complex has been shown to tether post-Golgi secretory vesicles for SNARE-induced membrane fusion. However, protein interactions of the exocyst have demonstrated that it also plays a pivotal role, for example, in invadopodia formation (Sakurai-Yageta et al., 2008). Nevertheless, also proteins containing a CATCHR region, for example, Munc13, could be involved in the targeting of the vesicles. Another challenge will be (iii) to determine whether the vesicles are exocytosed and what they contain.

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REFERENCES

Abmayr, S. M., & Pavlath, G. K. (2012). Myoblast fusion: lessons from flies and mice. *Development,* 139, 641–656.

Artero, R. D., Castanon, I., & Baylies, M. K. (2001). The immunoglobulin-like protein Hibris functions as a dose dependent regulator of myobl ast fusion and is differentially controlled by Ras and Notch signaling. *Development,* 128, 4251–4264.

Aravamudan, B., Fergestad, T., Davis, W. S., Rodesch, C. K., & Broadie, K. (1999). *Drosophila* UNC-13 is essential for synaptic transmission. *Nat Neurosci,* 2, 965–971.

Barlowe, C. (1997). Coupled ER to Golgi transport reconstituted with purified cytosolic components. *J. Cell Biol,* 139, 1097–1108.

Bate, M. (1990). The embryonic-development of larval muscles in *Drosophila*. *Development,* 110, 791–804.

Bao, H., Berlanga, M. L., Xue, M., Hapip, S. M., Daniels, R. W., Mendenhall, J. M., et al. (2007). The atypical cadherin flamingo regulates synaptogenesis and helps prevent axonal and synaptic degeneration in *Drosophila*. *Mol Cell Neurosci,* 34, 662–678.

Beckett, K., & Baylies, M. K. (2007). 3D analysis of founder cell and fusion competent myoblast arrangements outlines a new model of myoblast fusion. *Dev Biol,* 309, 113–125.

Berger, S., Schäfer, G., Kesper, D. A., Holz, A., Eriksson, T., Palmer, R. H., et al. (2008). WASP and SCAR have distinct roles in activating the Arp2/3 complex during myoblast fusion. *J Cell Sci,* 121, 1303–1313.

Blond, J. L., Lavillette, D., Cheynet, V., Bouton, O., Oriol, G., Chapel-Fernandes, S., et al. (2000). An envelope glycoprotein of the human endogenous HERV-W is expressed in the human placenta and fuses cells expressing the type D mammalian retrovirus receptor. *J Virol,* 74, 3321–3329.

Bonn, B. R., Rudolf, A., Hornbruch-Freitag, C., Daum, G., Kuckwa, J., Kastl, L., et al. (2013). Myosin heavy chain-like localizes at cell contact sites during *Drosophila* myoblast fusion and interacts in vitro with Rolling pebbles 7. *Exp Cell Res,* 319, 402–416.

Bour, B. A., Chakravarti, M., West, J. M., & Abmayr, S. M. (2000). *Drosophila* SNS, a member of the immunoglobulin superfamily that is essential for myoblast fusion. *Genes Dev,* 14, 1498–1511.

Brose, N., Hofmann, K., Hata, Y., & Südhof, T. C. (1995). Mammalian homologues of *Caenorhabditis elegans* unc-13 gene definde novel family of C2-domain proteins. *J Biol Chem,* 270, 25273–25280.

Bulchand, S., Menon, S. D., George, S. E., & Chia W. (2010). The intracellular domain of Dumbfounded affects myoblast fusion efficiency and interacts with Rolling pebbles and Loner. *PLoS One,* 5, e9374.

Cao, X., Ballew N., & Barlowe C. 1998. Initial docking of ER-derived vesicles requires Uso1p and Ypt1p but is independent of SNARE proteins. *EMBO J,* 17, 2156–2165.

Chapman, E. R., Hanson, P. I., An, S., & Jahn, R. (1995). Ca2 + regulates the interaction between synaptotagmin and syntaxin 1. *J Biol Chem,* 270, 23667–23671.

Charlton, C. A., Mohler, W. A., Radice, G. L., Hynes, R. O., & Blau, H. M. (1997). Fusion competence of myoblasts rendered genetically null for N-cadherin in culture. *J. Cell Biol,* 138, 331–336.

Chen, X., Araç, D., Wang, T. M., Gilpin, C. J., Zimmerberg, J., & Rizo, J. (2006). SNARE-mediated lipid mixing depends on the physical state of the vesicles. *Biophys J,* 90, 2062–2074.

DiAntonio, A., Parfitt, K. D., & Schwarz, T. L. (1993). Synaptic transmission persists in synaptotagmin mutants of *Drosophila*. *Cell,* 73, 1281–1290.

Doberstein, S. K., Fetter, R. D., Mehta, A. Y., & Goodman, C. S. (1997). Genetic analysis of myoblast fusion: Blown fuse is required for progression beyond the prefusion complex. *J Cell Biol,* 136, 1249–1261.

Dottermusch-Heidel, C., Groth, V., Beck, L., & Önel, S. F. (2012). The Arf-GEF Schizo/Loner regulates N-cadherin to induce fusion competence of *Drosophila* myoblasts. *Dev Biol,* 368, 18–27.

Duan, H, Skeath, J. B., & Nguyen, H. T. (2001). *Drosophila* Lame duck, a novel member of the Gli superfamily, acts as a key regulator of myogenesis by controlling fusion-competent myoblast development. *Development,* 128, 4489–4500.

Dulubova, I., Yamaguchi, T., Arac, D., Li, H., Huryeva, I., Min, S. W., et al. (2003). Convergence and divergence in the mechanism of SNARE binding by Sec1/Munc18-like proteins. *Proc Natl Acad Sci U S A,* 100, 32–37.

Durcan, P. J., Al-Shanti, N., & Stewart, C. E. (2013). Identification and characterization of novel Kirrel isoform during myogenesis. *Physiol Rep,* 1, e00044.

Dworak, H. A., Charles, M. A., Pellerano, L. B., & Sink, H. (2001). Characterization of *Drosophila hibris*, a gene related to human nephrin. *Development,* 128, 4265–4276.

Estrada, B., Maeland, A. D., Gisselbrecht, S. S., Bloor, J. W., Brown, N. H., & Michelson, A. M. (2007). The MARVEL domain protein, Singles Bar, is required for progression past the pre-fusion complex stage of myoblast fusion. *Dev Biol,* 307, 328–339.

Fannon, A. M., & Colman, D. R. (1996). A model for central synaptic junctional complex formation based on the differential adhesive specificities of the cadherin. *Neuron,* 17, 423–434.

Fasshauer, D., Antonin, W., Subramaniam, V., & Jahn, R. (2002). SNARE assembly and disassembly exhibit a pronounced hysteresis. *Nat Struct Biol,* 9, 144–151.

Fischbach, K. F., Linneweber, G. A., Andlauer, T. F., Hertenstein, A., Bonengel, B., & Chaudhary, K. (2009). The irre cell recognition module (IRM) proteins. *J Neurogenet, 23,* 48–67.

Galletta, B. J., Chakravarti, M., Banerjee, R., & Abmayr, S. M. (2004). SNS: Adhesive properties, localization requirements and ectodomain dependence in S2 cells and embryonic myoblasts. *Mech Dev,* 121, 1455–1468.

Gerona, R. R., Larsen, E. C., Kowalchyk, J. A., & Martin, T. F. (2000). The C terminus of SNAP25 is essential for Ca(2+)-dependent binding of synaptotagmin to SNARE complexes. *J Biol Chem,* 275, 6328–6336.

Giagtzoglou, N., Ly, C. V., & Bellen, H. J. (2009). Cell adhesion, the backbone of the synapse: "vertebrate" and "invertebrate" perspectives. *Cold Spring Harb Perspect Biol,* 1, a003079.

Gildor, B., Massarwa, R., Shilo, B. Z., & Schejter, E. D. (2009). The SCAR and WASp nucleation-promoting factors act sequentially to mediate Drosophila myoblast fusion. *EMBO Rep,* 10, 1043–1050.

Hansen, S. M., Berezin, V., & Bock, E. (2008). Signaling mechanisms of neurite outgrowth induced by the cell adhesion molecules NCAM and N-cadherin. *Cell Mol Life Sci,* 65, 3809–3821.

Haralalka, S., Shelton, C., Cartwright, H. N., Katzfey, E., Janzen, E., & Abmayr, S. M. (2011). Asymmetric Mbc, active Rac1 and F-actin foci in the fusion-competentmyoblasts during myoblast fusion in *Drosophila. Development,* 138, 1551–1562.

Hata, Y., Slaughter, C. A., & Südhof, T. C. (1993). Synaptic vesicle fusion complex contains unc-18 homologue bound to syntaxin. *Nature,* 366, 347–351.

Hornbruch-Freitag, C., Griemert, B., Buttgereit, D., & Renkawitz-Pohl, R. (2011). *Drosophila* Swiprosin-1/EFHD2 accumulates at the prefusion complex stage during *Drosophila* myoblast fusion. *J Cell Sci,* 124, 3266–3278.

Hu, C., Ahmed, M., Melia, T. J., Söllner, T. H., Mayer, T., & Rothman, J. E. (2003). Fusion of cells by flipped SNAREs. *Science,* 300, 1745–1749.

Jahn, R., Schiebler, W., Ouimet, C., & Greengard P. (1985). A 38,000-dalton membrane protein (p38) present in synaptic vesicles. *Proc Natl Acad Sci U S A,* 82, 4137–4141.

Jahn, R., & Fasshauer, D. (2012). Molecular machines governing exocytosis of synaptic vesicles. *Nature,* 490, 201–207.

James, D. J., & Martin, T. F. (2013). CAPS and Munc13: CATCHRs that SNARE vesicles. *Front Endocrinol,* 4, 187.

Janz, R., Südhof, T. C., Hammer, R. E., Unni, V., Siegelbaum, S. A., Bolshakov, V. Y. (1999). Essential roles in synaptic plasticity for synaptogyrin I and synaptophysin I. *Neuron,* 24, 687–700.

Jin, P., Duan, R., Luo, F., Zhang, G., Hong, S. N., Chen, E. H. (2011). Competition between blown fuse and WASP for WIP binding regulates the dynamics of WASP-dependent actin polymerization in vivo. *Dev Cell,* 20, 623–638.

Kaipa, B. R., Shao, H., Schaefer, G., Trinkewitz, T., Groth, V., Liu, J., et al. (2013). Dock mediates Scar- and WASp-dependent actin polymerization through interaction with cell adhesion molecules in founder cells and fusion-competent myoblasts. *J Cell Sci,* 126, 360–372.

Kesper, D. A., Stute, C., Buttgereit, D., Kreiskother, N., Vishnu, S., Fischbach, K. F., & Renkawitz-Pohl, R. (2007). Myoblast fusion in Drosophila melanogaster is mediated through a fusion-restricted myogenic-adhesive structure (FuRMAS). *Dev Dyn,* 236, 404–415.

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

Kreisköther, N., Reichert, N., Buttgereit, D., Hertenstein, A., Fischbach, K. F., & Renkawitz-Pohl, R. (2006). *Drosophila* Rolling pebbles colocalises and putatively interacts with alpha-Actinin and the Sls isoform Zormin in the Z-discs of the sarcomere and with Dumbfounded/Kirre, alpha-Actinin and Zormin in the terminal Z-discs. *J Muscle Res Cell Motil,* 27, 93–106.

Komori, T., Gyobu, H., Ueno, H., Kitamura, T., Senba, E., & Morikawa, Y. J. (2008). Expression of kin of irregular chiasm-like 3/mKirre in proprioceptive neurons of the dorsal root ganglia and its interaction with nephrin in muscle spindles. *Comp Neurol,* 11, 92–108.

Kozlovsky, Y., Efrat, A., Siegel, D. P., & Kozlov, M. M. (2004). Stalk phase formation: effects of dehydration and saddle splay modulus. *Biophys J,* 87, 2508–2521.

Knaus, P., Marquèze-Pouey, B., Scherer, H., & Betz, H. (1990). Synaptoporin, a novel putative channel protein of synaptic vesicles. *Neuron,* 5, 453–462.

Krauss, R. S. (2010). Regulation and promyogenic signal transduction by cell-cell contact and adhesion. *Exp. Cell Res,* 316, 3042–3049.

Li, W., Ma, C., Guan, R., Xu, Y., Tomchick, D. R., & Rizo, J. (2011). The crystal structure of a Munc13 C-terminal module exhibits a remarkable similarity to vesicle tethering factors. *Structure,* 19, 1443–1455.

Marsden, H. R., Tomatsu, I., & Kros, A. (2011). Model systems for membrane fusion. *Chem Soc Rev,* 40, 1572–1585.

Martens, S., Kozlov, M. M., & McMahon, H. T. (2007). How synaptotagmin promotes membrane fusion. *Science,* 316, 1205–1208.

Martens, S., & McMahon, H. T. (2008). Mechanisms of membrane fusion: disparate players and common principles. *Nat Rev Mol Cell Biol,* 9, 543–556.

Maruyama, I. N., & Brenner, S. (1991). A phorbol ester/diacylglycerol-binding protein encoded by the unc-13 gene of *Caenorhabditis elegans. Proc Natl Acad Sci U S A,* 88, 5729–5733.

Massarwa, R., Carmon, S., Shilo, B. Z., & Schejter, E. D. (2007). WIP/WASp-based actin-polymerization machinery

is essential for myoblast fusion in *Drosophila*. *Dev Cell,* 12, 557–569.

McMahon, H. T., Kozlov, M. M., & Martens, S. (2010). Membrane curvature in synaptic vesicle fusion and beyond. *Cell,* 140, 601–605.

Mi, S., Lee, X., Li, X., Veldman, G. M., Finnerty, H., Racie, L., et al. (2000). Syncytin is a captive retroviral envelope protein involved in human placental morphogenesis. *Nature,* 403, 785–789.

Mielenz, D., Vettermann, C., Hampel, M., Lang, C., Avramidou, A., Karas, M., & Jäck, H. M. (2005). Lipid rafts associate with intracellular B cell receptors and exhibit a B cell stage-specific protein composition. *J Immunol,* 174, 3508–3517.

Millay, D. P., O'Rourke, J. R., Sutherland, L. B, Bezprozvannaya, S., Shelton, J. M., Bassel-Duby, R., & Olson, E. N. (2013). Myomaker is a membrane activator of myoblast fusion and muscle formation. *Nature,* 499, 301–305.

Missler, M., Südhof, T. C., & Biederer, T. (2012). Synaptic cell adhesion. *Cold Spring Harb Perspect Biol,* 4, a005694.

Misura, K. M., Scheller, R. H., & Weis, W. I. (2000). Three-dimensional structure of the neuronal-Sec1-syntaxin 1a complex. *Nature,* 404, 355–362.

Nagar, B., Overduin, M., Ikura, M., & Rini, J. M. (1996). Structural basis of calcium-induced E-cadherin rigidification and dimerization. *Nature,* 380, 360–364.

Najarro, E. H., Wong, L., Zhen, M., Carpio, E. P., Goncharov, A., Garriga, G., et al. (2012). *Caenorhabditis elegans* flamingo cadherin fmi-1 regulates GABAergic neuronal development. *J Neurosci,* 32, 4196–211.

Nonet, M. L., Grundahl, K., Meyer, B. J., & Rand, J. B. (1993). Synaptic function is impaired but not eliminated in *C. elegans* mutants lacking synaptotagmin. *Cell,* 73, 1291–1305.

Nonet, M. L., Holgado, A. M., Brewer, F., Serpe, C. J., Norbeck, B. A., Holleran, J., et al. (1999). UNC-11, a *Caenorhabditis elegans* AP180 homologuue, regulates the size and protein composition of synaptic vesicles. *Mol Biol Cell,* 10, 2343–1360.

Oren-Suissa, M., & Podbilewicz, B. (2007). Cell fusion during development. *Trends Cell Biol,* 17, 537–546.

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

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

Perin, M. S., Brose, N., Jahn, R., & Südhof, T. C. (1991). Domain structure of synaptotagmin (p65). *J Biol Chem,* 266, 623–629.

Prakash, S., Caldwell, J. C., Eberl, D. F, & Clandinin, T. R. (2005). *Drosophila* N-cadherin mediates an attractive interaction between photoreceptor axons and their targets. *Nat Neurosci,* 8, 443–450.

Pollard, T. D. (2007). Regulation of actin filament assembly by Arp2/3 complex and formins. *Annu Rev Biophys Biomol Struct,* 36, 451–477.

Rehm, H., Wiedenmann, B., & Betz, H. (1986). Molecular characterization of synaptophysin, a major calcium-binding protein of the synaptic vesicle membrane. *EMBO J,* 5, 535–541.

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

Rickman, C., & Davletov, B. (2003) Mechanism of calcium-dependent synaptotagmin binding to target SNAREs. J Biol Chem, 278, 5501–5504.

Rizo, J., & Südhof, T. C. (2012). The membrane fusion enigma: SNAREs, Sec1/Munc18 proteins, and their accomplices–guilty as charged? *Annu Rev Cell Dev Biol,* 28, 279–308.

Rochlin, K., Yu, S., Roy, S., & Baylies, M. K. (2010). Myoblast fusion: when it takes more to make one. *Dev Biol,* 341, 66–83.

Rotty, J. D., Wu, C., & Bear, J. E. (2013). New insights into the regulation and cellular functions of the Arp2/3 complex. *Nat Rev Mol Cell Biol,* 14, 7–12.

Ruiz-Gómez M. (1998). Muscle patterning and specification in *Drosophila*. *Int J Dev Biol,* 42, 283–290.

Ruiz-Gómez, M., Coutts, N., Price, A., Taylor, M. V., & Bate, M. (2000). *Drosophila* dumbfounded: A myoblast attractant essential for fusion. *Cell,* 102, 189–198.

Sanchez-Pulido, L., Martin-Belmonte, F., Valencia, A., & Alonso, M. A. (2002). MARVEL: a conserved domain involved in membrane apposition events. *Trends Biochem Sci,* 27, 599–601.

Sakurai-Yageta, M., Recchi, C., Le Dez, G., Sibarita, J. B., Daviet, L., Camonis, J. et al. (2008). The interaction of IQGAP1 with the exocyst complex is required for tumor cell invasion downstream of Cdc42 and RhoA. *J Cell Biol,* 181, 985–998.

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

Sens, K. L., Zhang, S. L., Jin, P., Duan, R., Zhang, G. F., Luo, F. B., et al. (2010). An invasive podosome-like structure promotes fusion pore formation during myoblast fusion. *J Cell Biol,* 191, 1013–1027.

Shelton, C., Kocherlakota, K. S., Zhuang, S. F., & Abmayr, S. M. (2009). The immunoglobulin superfamily member Hbs functions redundantly with Sns in interactions between founder and fusion-competent myoblasts. *Development,* 136, 1159–1168.

Shen, K., Fetter, R. D., & Bargmann, C. I. (2004). Synaptic specificity is generated by the synaptic guidepost protein SYG-2 and its receptor, SYG-1. *Cell,* 116, 869–881.

Shen, K., & Bargmann, C. I. (2003). The immunoglobulin superfamily protein SYG-1 determines the location of specific synapses in *C. elegans*. *Cell,* 112, 619–630.

Sheng, L., Leshchyns'ka, I., & Sytnyk, V. (2013). Cell adhesion and intracellular calcium signaling in neurons. *Cell Commun Signal,* 11, 94.

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

Sohn, R. L., Huang, P., Kawahara, G., Mitchell, M., Guyon, J., Kalluri, R., et al. (2009). A role for nephrin, a renal protein,

in vertebrate skeletal muscle cell fusion. *Proc Natl Acad Sci U S A 106*, 9274–9279.

Spiwoks-Becker, I., Vollrath, L., Seeliger, M. W., Jaissle, G., Eshkind, L. G., & Leube, R. E. (2001). Synaptic vesicle alterations in rod photoreceptors of synaptophysin-deficient mice. *Neuroscience,* 107, 127–142.

Srinivas, B. P., Woo, J., Leong, W. Y., & Roy, S. (2007). A conserved molecular pathway mediates myoblast fusion in insects and vertebrates. *Nat Genet,* 39, 781–786.

Stan, A., Pielarski, K. N., Brigadski, T., Wittenmayer, N., Fedorchenko, O., Gohla, A., et al. (2010). Essential cooperation of N-cadherin and neuroligin-1 in the transsynaptic control of vesicle accumulation. *Proc Natl Acad Sci USA,* 107, 11116–11121.

Stenius, K., Janz, R., Südhof, T. C., & Jahn, R. (1995). Structure of synaptogyrin (p29) defines novel synaptic vesicle protein. *J Cell Biol,* 131, 1801–1809.

Stevens, R. J., Akbergenova, Y., Jorquera, R. A., & Littleton, J. T. (2012). Abnormal synaptic vesicle biogenesis in *Drosophila synaptogyrin* mutants. *J Neurosci,* 32, 18054–18067.

Strünkelnberg, M., Bonengel, B., Moda, L. M., Hertenstein, A., de Couet, H. G., Ramos, R. G. P., & Fischbach, K. F. (2001). rst and its paralogue kirre act redundantly during embryonic muscle development in Drosophila. *Development,* 128, 4229–4239.

Südhof, T. C. & Rizo, J. (2011). Synaptic vesicle exocytosis. *Cold Spring Harb Perspect Biol.,* 3(12), pii: a005637.

Sugie, A., Umetsu, D., Yasugi, T., Fischbach, K. F., & Tabata, T. (2010). Recognition of pre- and postsynaptic neurons via nephrin/NEPH1 homologs is a basis for the formation of the *Drosophila* retinotopic map. *Development,* 137, 3303–3313.

Takamori, S., Holt, M., Stenius, K., Lemke, E. A., Grønborg, M., Riedel, D., et al. (2006). Molecular anatomy of a trafficking organelle. *Cell,* 127, 831–846.

Takenawa, T., & Suetsugu, S. (2007). The WASP-WAVE protein network: connecting the membrane to the cytoskeleton. *Nat Rev Mol Cell Biol,* 8, 37–48.

Tanaka, H., Shan, W., Phillips, G. R., Arndt, K., Bozdagi, O., Shapiro, L., et al. (2000). Molecular modification of N-cadherin in response to synaptic activity. *Neuron,* 25, 93–107.

Thalhammer, A., & Cingolani, L. A. (2014). Cell adhesion and homeostatic synaptic plasticity. *Neuropharmacology,* 78, 23–30.

Thiele, C., Hannah, M. J., Fahrenholz, F., & Huttner, W. B. (2000). Cholesterol binds to synaptophysin and is required for biogenesis of synaptic vesicles. *Nat Cell Biol,* 2, 42–49.

Togashi H, Sakisaka T, & Takai Y. (2009). Cell adhesion molecules in the central nervous system. *Cell Adh Migr,* 3, 29–35.

Uchida, N., Honjo, Y., Johnson, K. R., Wheelock, M. J., & Takeichi, M. (1996). The catenin/cadherin adhesion system is localized in synaptic junctions bordering transmitter release zones. *J Cell Biol,* 135, 767–779.

Völker, L. A., Petry, M., Abdelsabour-Khalaf, M., Schweizer, H., Yusuf, F., Busch, T., et al. (2012). Comparative analysis of Neph gene expression in mouse and chicken development. *Histochem Cell Biol,* 137, 355–366.

Vuadens, F, Rufer, N., Kress, A., Corthésy, P., Schneider, P., & Tissot, J. D. (2004). Identification of swiprosin 1 in human lymphocytes. *Proteomics,* 4, 2216–2220.

Wiedenmann, B., & Franke, W. W. (1985). Identification and localization of synaptophysin, an integral membrane glycoprotein of Mr 38,000 characteristic of presynaptic vesicles. *Cell,* 41, 1017–1028.

Wu, L. G., Hamid, E., Shin, W., & Chiang, H. C. (2013). Exocytosis and Endocytosis: Modes, function, and coupling mechanisms. *Annu Rev Physiol,* [Epub ahead of print]

Yam, P. T., Pincus, Z., Gupta, G. D., Bashkurov, M., Charron, F., Pelletier, L., and Colman, D. R. (2013). N-cadherin relocalizes from the periphery tp the center of the synapse after transient synaptic stimulation in hippocampal neurons. *PLoS One,* 8, e79679.

Yu, I. M., & Hughson, F. M. (2010). Tethering factors as organizers of intracellular vesicular traffic. *Annu Rev Cell Dev Biol,* 26, 137–156.